Sustainable scenarios for alkaline protein extraction from leafy biomass using green tea residue as a model material

Zhang, C., Slegers, P. M., Wisse, J., Sanders, J. P. M., & Bruins, M. E.

Zhang, C., Slegers, P. M., Wisse, J., Sanders, J. P. M., & Bruins, M. E. (2018). Sustainable scenarios for alkaline protein extraction from leafy biomass using green tea residue as a model material. Biofuels, Bioproducts and Biorefining, 12(4), 586-599. https://doi.org/10.1002/bbb.1870

Modeling and Analysis

Biofpr Biofuels Bioproducts & Biorefining

Sustainable scenarios for alkaline protein extraction from leafy biomass using green tea residue as a model material

Chen Zhang,† Institute of Food Science and Technology, Fuzhou University, Fuzhou, China and Biobased Chemistry and Technology, Wageningen University and Research, The Netherlands
Petronella M. Slegers† **and Jacobus Wisse,** Biobased Chemistry and Technology, Wageningen University and Research, The Netherlands
Johan P.M. Sanders, Biobased Chemistry and Technology, Wageningen University and Research, The Netherlands and Wageningen Food & Biobased Research, Wageningen University and Research, The Netherlands
Marieke E. Bruins, Wageningen Food & Biobased Research, Wageningen University and Research, The Netherlands

Received September 30, 2017; revised January 17, 2018; accepted January 26, 2018
View online at March 5, 2018 Wiley Online Library (wileyonlinelibrary.com);
DOI: 10.1002/bbb.1870; *Biofuels, Bioprod. Bioref.* 12:586–599 (2018)

Abstract: Leaf protein can be extracted cost-efficiently using 0.1 mol dm^{-3} NaOH, but this process is less sustainable due to the generation of large amounts of sodium salts. KOH or $Ca(OH)_2$ are considered as replacements for NaOH, as these salts can be reused. This work evaluates the economic and environmental sustainability of weak alkaline pectin extraction followed by KOH enhanced protein extraction, and Viscozyme® L-aided pectin extraction followed by $Ca(OH)_2$ enhanced protein extraction. The evaluations are made for green tea residue and are compared to related processes using NaOH. The predicted profits using KOH are comparable to those using NaOH. Environmental sustainability improves for all impact categories in the case of KOH extraction. Further environmental benefits are obtained by substituting conventional K fertilizer with the K-rich salty waste water from the extraction process. The profits of the process using $Ca(OH)_2$ are highly dependent on the extraction yield of the protein product. Protein extraction yields using $Ca(OH)_2$ need to be higher than 70% to be more profitable than the same process with NaOH. The environmental benefits of $Ca(OH)_2$ extraction include the absence of salty waste water and the net production of heat. This is accompanied by increased electricity consumption. Thus, the impact categories of climate change, fossil and water depletion, and particulate matter formation worsen. Photochemical oxidant formations remain the same, while the other impacts improve. This work has shown the potential and bottlenecks of NaOH, KOH and

Correspondence to: Chen Zhang, Institute of Food Science and Technology, Fuzhou University, Fuzhou 350108, China.
E-mail: zhangchenfj@sina.com, Office.BCT@wur.nl
† Both authors contributed equally

$Ca(OH)_2$ protein extraction on different types of biomass in terms of environmental and economic sustainability.

Supporting information may be found in the online version of this article.

Keywords: tea residue; micro-algae; biorefinery; protein extraction; cost; LCA

Introduction

Since the 1960s, leaf proteins have been considered as an alternative source of protein.[1,2] Some leaf proteins, such as vegetables, are commonly used for food[3] and animal feed.[4] However, the wide application of leaf protein has so far been limited due to the low cost-efficiency of leaf protein production.[5] Recently, the cost-efficiency of leaf protein extraction was improved by redefining alkaline extraction conditions[6] and by process integration with pretreatments using Viscozyme® L and ethanol.[7,8] With these improvements, leaf biorefining becomes economically feasible. However, the extraction process with NaOH is still not environmentally sustainable due to the generation of large quantities of sodium salts in the extraction and the subsequent acidic precipitation step. A suitable alternative extraction approach should be found to improve overall sustainability of alkaline protein extraction.

Other alkali sources can be used instead of NaOH. KOH has similar chemical characteristics and therefore a similar high protein yield can be expected. The potassium salts generated can be recycled or the residual salty water can be used as fertilizer.[9] The higher price of KOH compared to NaOH can be compensated by the value of the potassium fertilizer generated and by the reduction of wastewater management fees. Ideally, all the watery potassium residues are used directly as fertilizer on local fields. This approach reduces the need for additional K-fertilizer and reduces transportation costs. The chemical similarity of KOH will also allow for easy integration with pretreatments like Viscozyme® L-aided pectin extraction and weak-alkaline pectin extraction, which have already been cost-effectively integrated with protein extraction.[7,8]

Alternatively, leaf protein can be extracted using $Ca(OH)_2$. The residual water with the calcium salts can be recycled on site. Calcium salts can be precipitated as $CaCO_3$ by bubbling CO_2 through the remaining liquid after protein extraction. This leads to reduced alkali use in protein production. No waste salts are generated and less acid is needed for further protein purification.[10] In addition, the $Ca(OH)_2$ protein extraction can benefit from the lower price of $Ca(OH)_2$ compared to KOH and NaOH. However, $Ca(OH)_2$ extraction leads to relatively low protein yields.[11,12] This low yield might be due to the low solubility of $Ca(OH)_2$ in water,[13] which results in a less alkaline solution compared to NaOH and KOH. Besides, calcium atoms contain two valence electrons through which calcium cations can function as bridges between different chelating molecules.[14,15] This chelating effect of calcium cations is widely applied in the coagulation of polymers.[16,17] In protein extraction, the calcium ions are hypothesized to cause coagulation of leaf components, including polyphenol,[18] pectin,[14] lignin,[19] and even protein.[19,20] The latter case will reduce protein extraction yields.

This work was intended to investigate the economic and environmental sustainability of these alkaline extraction scenarios. The focus was on leaf protein extraction with green tea residue (GTR) as model material. Protein extraction yields obtained by weak alkaline extraction with subsequent KOH extraction and Viscozyme-assisted extraction with $Ca(OH)_2$ were determined. Extraction using NaOH was considered as a control. The economics and environmental impacts of these two scenarios were analyzed for several integrated processes of pectin extraction with protein extraction.[7,8] The scenario of $Ca(OH)_2$ protein extraction was further tested using microalgae to confirm the applicability of the proposed integrated process chains to other materials.

Approach

Biomass sources

Green-tea residue (GTR) was a gift from Damin Foodstuff (Zhangzhou) Co., Ltd., Fujian Province, China. The green tea leaves were collected from Camellia sinensis trees in Zhejiang province, China, in 2014. This residue was collected after producing tea lemonade. In this process, the green-tea leaves were soaked in water at 85 °C for 45 min. After collection, the GTR was sun dried and ready for further processing.

Microalgae (*Nannochloropsis* sp., CCAP 211/78) were obtained from AlgaePARC, Wageningen University and Research (Wageningen, the Netherlands). The micro-algae

were grown in natural seawater in a turbidostat set-up and collected by self-cleaning disc separators (SSD 6-06-007, GEA Westfalia Separator, Germany). The dry matter of the microalgae sample was 11% with a protein content of 37% in dry weight. In this study, microalgae were used as controls.

Protein yields

In previous work the protein extraction yields were determined using NaOH as an alkali source.[6,7] Pretreatments using weak alkaline or Viscozyme® were also included in those studies. For the current study, the protein extraction yields were determined using KOH and $Ca(OH)_2$ in a similar approach.[7] Protein yields were quantified via Dumas analysis and the results are presented in Fig. 1. As expected, KOH and NaOH showed similar protein extraction efficiencies. Addition of 1 mmol KOH or NaOH to the GTR resulted in approximately 50% protein extraction after 3 h. Protein yields obtained with $Ca(OH)_2$ were significantly lower.

Pretreatment with Viscozyme® L improved protein yields to 80% when using either KOH or NaOH, but had no influence when $Ca(OH)_2$ was applied. Extraction yields with NaOH and KOH using weak alkaline pretreatment were similar to those using Viscozyme® L-assisted pretreatment (not shown). Our previous work showed that Viscozyme® L removed almost all cellulose and pectin, as well as 50% of polyphenol and hemi-cellulose.[7,8] This pretreatment had no positive influence on the subsequent $Ca(OH)_2$-assisted protein extraction. The low protein yield may have resulted from the chelator effect of calcium ions with lignin, which remained in the matrix after Viscozyme® L-assisted pretreatment. With a longer extraction time, protein can be hydrolyzed and released. As shown in Fig. 1, extended extraction time with $Ca(OH)_2$ increased the GTR protein extraction yield, even without applying a pretreatment.

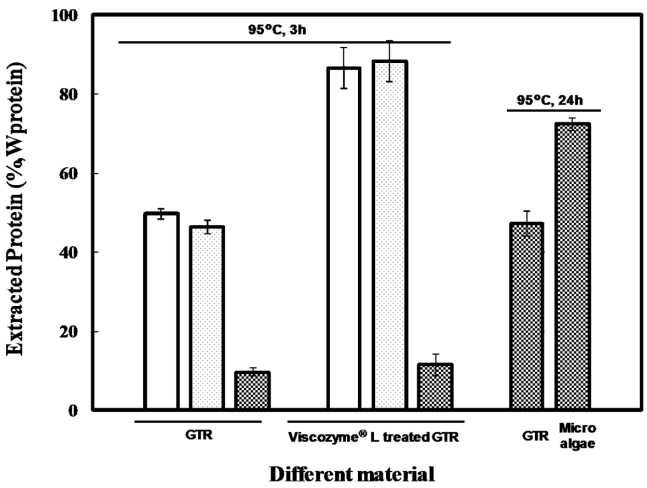


Figure 1. Protein yield (%, $W_{protein}$) after adding different amounts and types of alkali to 0.5 g (pretreated) GTR or microalgae (on dry weight basis) in 20 ml H_2O. White: 1 mmol NaOH; dot: 1 mmol KOH; grid: 1 mmol $Ca(OH)_2$. Results are also shown for Viscozyme(R) L pretreated GTR. Extractions with NaOH and KOH using weak alkaline pretreatment showed similar results to those using Viscozyme® L-assisted pre-treatment (results not shown).

For feedstocks without lignin, such as micro-algae, even higher protein yields are obtained. At long extraction times, the extraction yield with microalgae reached 75%. This relatively high protein yield obtained by $Ca(OH)_2$ extraction in the absence of lignin has also been shown for other non-lignin-containing biomass such as dehulled soy bean.[21,22]

With optimal heating and mixing, protein yield will not be affected by the scale of the process.[8] The protein extraction yields reported above were therefore used for the economic and environmental analysis.

Process descriptions

Production scale

The process evaluation was carried out for a processing scale of 5000 ton GTR per year. This represents a medium-to-large-scale tea lemonade factory. This 5000 ton GTR originates from processing 8375 ton tea leaves (personal communication with Damin Company). To process 5000 ton GTR within 300 days each year the processing capacity should be higher than 17 ton GTR per day. The processing capacity of the reactor for protein extraction is assumed to be about 2 ton GTR per batch, thus requiring three parallel reactors, which each process three batches per day. The calculations are based on continuous processing of the GTR. We assume that the chosen reactor design will allow for the same extraction yields as under laboratory conditions.

Reference: single-step NaOH protein extraction

The reference scenario is a single-step NaOH protein extraction. This reference process was designed based on previous work[6] and is shown in Fig. 2. For each reactor, 2000 kg GTR is mixed with 6400 mol NaOH and 20 m^3 water. The reaction is carried out at 95 °C for 3 h with regular mixing. The mixture is then separated into a solid and a liquid fraction by pressing. Energy efficiency has been a major focus point for the Chinese tea industry in the past year.[23] In our designs, therefore, the residues after protein extraction are sundried and combusted to generate heat.

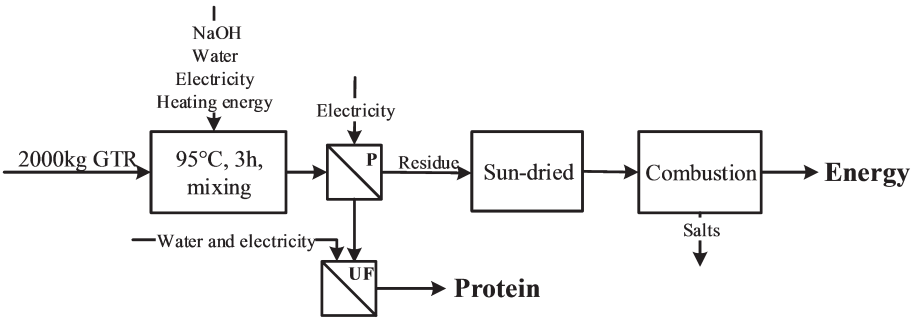


Figure 2. Process of single-step NaOH protein extraction.

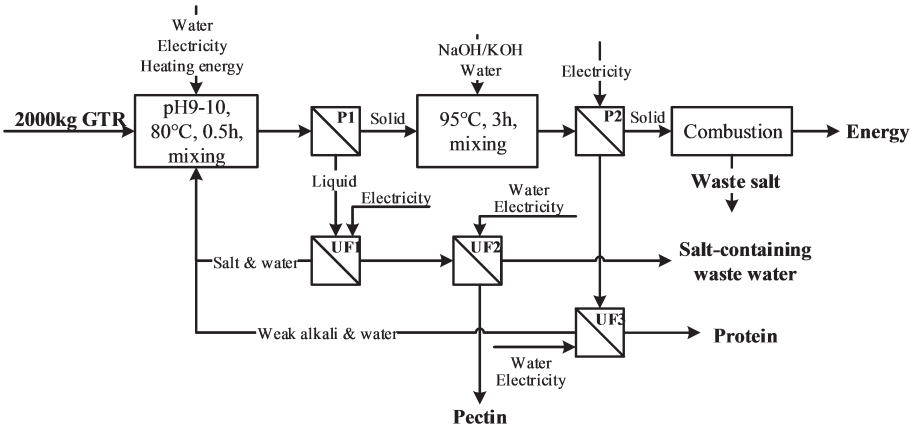


Figure 3. Integrated process design of weak alkaline pretreatment for pectin and alkaline protein extraction using NaOH or KOH. P: press; UF: ultra-filtration.

To apply protein concentrates in animal feed, an additional purification step using ultrafiltration is needed to remove sodium salts. The protein concentrates contain 880 kg GTR components, of which 460 kg are protein.[6] The process mass flows are listed in Table A1 of the Appendix.

Weak alkaline pretreatment with alkaline extraction (using NaOH or KOH)

The economic feasibility of an integrated process with weak alkaline pretreatment for pectin recovery (represented by the amount of galacturonic acid) extraction followed by NaOH protein extraction was already assessed previously.[7] Here, we take the process design a step further by including (1) further purification of the pectin fraction through ultrafiltration and (2) energy generation by combustion of the remaining solids. The integrated process design for NaOH and KOH is presented in Fig. 3. Weak alkaline pretreatment is not feasible for $Ca(OH)_2$ as pectin precipitates with calcium ions at a pH close to neutral conditions.[8]

In the process, 2000 kg GTR is mixed with recycled water from the ultrafiltration steps of both pectin and protein products. Then the pH of the mixture is adjusted to pH 9–10. Pretreatment for pectin extraction is carried out at 80 °C for 0.5 h while mixing. After pressing, the solid fraction is used for the alkaline protein extraction step using NaOH or KOH. The liquid fraction, which has a high pectin content, is purified by two ultrafiltration steps. The first ultrafiltration step is to concentrate pectin four times, by which 75% salt-containing water is removed. The permeate fraction obtained in this first ultrafiltration is recycled to the pretreatment. The pectin fraction is then further purified in the second ultrafiltration step in which 4.8 m^3 water is added, after which a pectin fraction is obtained and 4.8 m^3 salt-containing waste water is generated. The alkaline protein extraction is carried out at 95 °C for 3 h while mixing. This is followed by pressing, drying, and combustion of the solids (740 kg) similar to the reference scenario. The only difference is that the waste water from the protein ultrafiltration is recycled to the pretreatment.

In this integrated process, 690 kg of protein concentrate is formed, of which 400 kg is protein. The difference between the scenarios of NaOH and KOH is in the application of the salt-containing waste water. In the former sodium salts are formed, which can only be treated as waste. In the latter, potassium salts are formed. These can be used as fertilizer. The salty water can be transported back to the tea farms or applied at nearby other farms. Both scenarios avoid the use of conventional potassium fertilizer, but the first requires more transportation. The potential reduction in environmental impacts due to reuse of the potassium-rich waste water and avoiding conventional fertilizer was roughly estimated as follows. The amount of K-fertilizer potentially avoided was calculated from the amount of potassium present in the waste stream. This was multiplied with the environmental impacts associated with conventional potassium fertilizer. This is the maximum amount that can be avoided by substituting the conventional fertilizer with the potassium-rich waste water, and these impacts were deducted from the system impacts. The mass flows of this process are listed in Table A2 in the Appendix.

Viscozyme® L pretreatment with alkaline extraction (using NaOH or $Ca(OH)_2$)

Combining Viscozyme® L pretreatment of GTR to obtain pectin with NaOH protein extraction leads to lower costs and slightly higher revenues.[7] In this work, we further improve this process by including combustion of the remaining solids for heat generation. This scenario is evaluated for both NaOH and $Ca(OH)_2$ as alkali sources. The integrated process is presented in Fig. 4a and Fig. 4b. Here, KOH was not explicitly included as the protein extraction results of KOH are similar to the NaOH results.

During pretreatment, enzymes with 24 million U activity and 20 m^3 water are added to 2000 kg GTR, and incubated at 30 °C for 3 h. In previous work[7] it was shown that incubation at 30 °C for 3 h was sufficient to yield more than 90% pectin. After filtration, 140 kg pectin, 220 kg glucose, 80 kg other sugars, and 80 kg polyphenols can be obtained together with 9 m^3 water in the liquid fraction, while about 1260 kg GTR press cake is used for subsequent protein extraction. In the protein-extraction

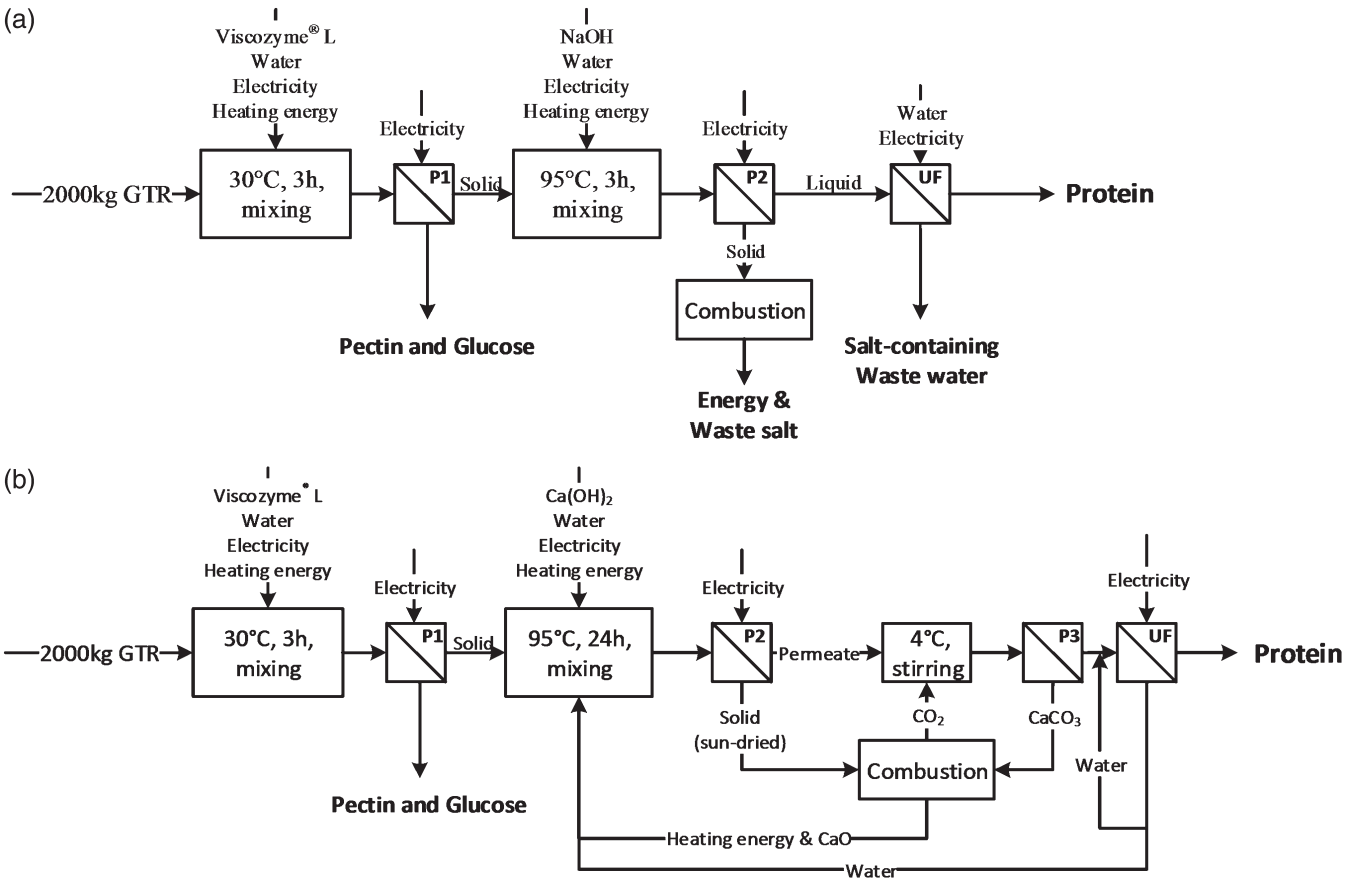


Figure 4. Integrated process of pretreatment with Viscozyme® L and alkaline protein extraction using a, NaOH or b, Ca(OH)2. P: press; UF: ultra-filtration.

step, there is a difference between the scenarios using NaOH or $Ca(OH)_2$. Using NaOH, the process design is similar to the single-step NaOH extraction. Based on these results (as also presented in Fig. 1) the final protein product is 720 kg, of which 460 kg is protein. For the scenario with $Ca(OH)_2$ a different process is used, which is illustrated in Fig. 4b. After separation of the protein, the remaining solids, including $CaCO_3$ and residual GTR, are sundried and combusted to CaO and CO_2. The generated heat and CaO can be recycled to the protein extraction step. The CO_2 produced can be reused for either calcium precipitation or as a carbon source in greenhouses. The calcium ion recycling will improve the efficiency of chemical use. As already shown in Fig. 1, the protein yields from extraction with $Ca(OH)_2$ are highly dependent on the composition of the biomass. For GTR, only 360 kg protein product containing 230 kg protein can be obtained. With micro-algae as feedstock, more than 70% of the proteins can be recovered. The effect of biomass choice is included in the evaluation. The mass flows of this process are listed in Tables A3a and A3b in the Appendix.

Summary of five scenarios

- Scenario 1: single-step NaOH (reference).
- Scenario 2: weak alkaline pectin extraction integrated with NaOH protein extraction.
- Scenario 3: weak alkaline pectin extraction integrated with KOH protein extraction.
- Scenario 4: Viscozyme® L-assisted pectin extraction integrated with NaOH protein extraction.
- Scenario 5: Viscozyme® L-assisted pectin extraction integrated with $Ca(OH)_2$ extraction.

Economic calculations

The economics of processing one batch of GTR was evaluated for the five scenarios that are mentioned in the previous subsection.

The economics of all scenarios were calculated in US dollar ($) per ton. For each of the scenarios the costs were calculated based on the prices and specifics of related subjects as listed in Table A4a in the Appendix, including investment costs of equipment, material costs for chemicals and enzymes, utility costs for electricity, and heat from coal,[23] other operational and waste treatment costs, and labor. The heating costs were calculated based on the price of coal and the combustion energy of coal. Combustion values are based on composition and reported heating values.[24] Other heat sources could also be used. The outputs and associated revenues are listed in Table A4 b of the Appendix.

Each reactor requires one heating element, and their depreciation rates are both assumed at 10%. The costs of presses and ultrafiltration units are based on their capacities,[25, 26] also with 10% depreciation. The number of workers required is based on the number of unit operations required in each scenario and the number of unit operations one worker can handle. The results are listed in Table A4c of the Appendix.

The product amounts were calculated for each scenario based on the extraction and separation yields of protein, glucose, pectin, and the amount of lignocellulose that can be used for heating after combustion (see Appendix, Tables A1–A3). Currently, coal is the major energy source in China. We therefore consider that the utilities are produced from coal. Coal is a major cause of fossil-fuel-related CO_2 emissions. The potential revenue of the generated heat is estimated by calculating the same amount of heat that can be produced by coal. In the best case, the heat consumption is balanced with the internal heat production. This is the case for single-step NaOH treatment (Scenario 1) as well as for Viscozyme-assisted $Ca(OH)_2$ extraction (Scenario 5). In the other scenarios the internal heat supply is not sufficient and additional heat is bought. Pectin can be used indirectly for food applications[27] and sugar can be used directly for the production of alcohols or organic acids by fermentation.[28] Further purification is needed for applying polyphenols as anti-oxidants in food. Only the potential revenues of pectin, glucose and energy are included in the evaluation.

Environmental calculations

Environmental sustainability was assessed using a gate-to-gate lifecycle analysis (LCA) in which the upstream impacts of the material and energy inputs of the GTR processing factory were included. The upstream impacts from tea farming and the tea lemonade company are excluded, as these are the same for all processing scenarios. In current practice, the GTR is a waste stream that is combusted at the tea lemonade company. These avoided combustion emissions are not accounted for in this work (i.e. the GTR input itself is free from emissions). The goal of the accounting LCA was to compare the environmental performance of the different processing scenarios. To ease comparison between the scenarios, the functional unit is 1 ton GTR from a tea lemonade company, with the geographical scope of China. In this study the system boundaries encompass the GTR entering the factory

(no upstream impacts allocated) and include processing until the production of the unrefined products (pectin, protein, glucose, amounts of waste, heating energy). The type and amount of products varies between the scenarios, i.e. a basket of products is produced. The impacts are therefore not allocated between the different products. The generated heating energy (75% efficiency)[29] is assumed to be consumed by other processes in the factory and is thus deduced from the required heat. The combustion of the process remainders leads to combustion emissions. It is unknown which combustion emissions are produced and in which quantities. These emissions are therefore excluded in the evaluation. To summarize, this work accounts for the fossil emissions and environmental impacts associated with processing and biogenic CO_2 were not discounted (i.e. these are set to zero). The detailed flow schemes, including the internal recycles of water, salts, heat, and biomass, are shown in the Appendix (Tables A1–A3). The evaluation is based on the processing scale of a commercial factory, for which the experimental data were projected using engineering rules (see Figs 2–4). All input/output data are given in the Appendix.

The lifecycle impact assessment was performed with SimaPro, Version 8.0.5.13 software (Pre Consultants, Amersfoort, the Netherlands) using the ReCiPe Midpoint (H) V1.09 / World Recipe H method and the EcoInvent 3.0 database.[30] Enzyme production impacts were derived from literature[31] and were adjusted to the ReCiPe characterization factors for the categories 'Climate Change,' 'Terrestrial acidification,' 'Freshwater eutrophication,' 'Photochemical oxidant formation,' and 'Fossil depletion.'

The LCA results are quantified in detail for the most relevant impact categories for China[32] and tea production,[33] i.e. climate change, ozone depletion, terrestrial acidification, eutrophication, photochemical oxidant formation, particulate matter formation, water depletion, natural land transformation, and fossil depletion.

Sustainability evaluation

The economics of the GTR biorefinery using single-step alkaline extraction with NaOH (Scenario 1) was compared to the scenario including pretreatment with weak alkaline and to pretreatment with Viscozyme® L in previous work.[8] The profit of single-step protein extraction was only \$84/ton GTR, and this had the lowest profit potential due to the relatively low product amounts and the large amount of salts generated. The environmental impacts for single-step extraction with NaOH are summarized in Table 1.

Table 1. Absolute LCA impact results for single-step NaOH protein extraction (Scenario 1).

Selected categories	Unit (per ton of GTR)	
Climate change	kg CO_2 equivalent	381.48
Ozone depletion	kg CFC-11 equivalent [a]	9.55E-05
Terrestrial acidification	kg SO_2 equivalent	2.89
Freshwater eutrophication	kg P equivalent	0.11
Marine eutrophication	kg N equivalent	0.08
Photochemical oxidant formation	kg NMVOC[b]	1.39
Particulate matter formation	kg PM10 equivalent	0.98
Natural land transformation	m^2	0.03
Water depletion	m^3	1428.74
Fossil depletion	kg oil equivalent	84.18

[a] CFC-11 = trichlorofluoromethane.
[b] NMVOC = non-methane volatile organic compounds, PM10 = particular matter of 10 μm.

The impacts of the production of the NaOH and electricity used are responsible for more than 95% of total impacts in this scenario. Environmental sustainability can thus be improved by more efficient use of NaOH and electricity or by using clean electricity from sources other than coal.

In the evaluation below, we focus on comparing two scenarios for weak alkaline pretreatment integrated with protein extraction. A key point is the recycling of the large amounts of generated salts in the case of NaOH or the application of KOH salts as fertilizer. The integrated process designs with Viscozyme® L pretreatments are discussed below. These lead to more products and possibly to a higher profit. A critical issue in those scenarios are the cost estimate for Viscozyme® L, which requires further development of current Viscozyme® L production. The present results should therefore be compared pairwise – by comparing the results of weak alkaline pretreatment scenarios (NaOH versus KOH), and by comparing the two scenarios with Viscozyme® L pretreatment (NaOH versus $Ca(OH)_2$).

Economic sustainability

NaOH versus KOH (Scenario 2 versus Scenario 3)

As illustrated in Table 2, the profits of both integrated processes are similar and are two times higher than using single-step protein extraction (Scenario 1). Using KOH, the costs of chemicals increased from 24 to 84 \$/ton GTR due to the higher price of KOH. The ultrafiltration costs also

Table 2. Cost, revenue, and profit of integrated weak alkaline pectin extraction with alkaline protein extraction using NaOH (Scenario 2) or KOH[a] (Scenario 3) (based on $/ton feedstock).

Input (cost)	NaOH	KOH	Output (revenue)	NaOH	KOH
GTR	16	16	Pectin	240	240
Chemical	24	84	Protein	163	163
Water	18	18	Energy[b]	4	4
Electricity	21	21	Fertilizer		55
Heating	5	5			
Waste water	4	0.4			
Press filtration	5	5			
Ultrafiltration	15	15			
Capital	14	14			
Labor	16	16			
Subtotal	138	194	**Subtotal**	407	462
			Profit	269	268

[a] Calculation based on data in the Appendix, Table A2.
[b] Energy derived from the combustion.[41]

Table 3. Cost, revenue, and profit of integrated Viscozyme® L pectin extraction with alkaline protein extraction using NaOH (Scenario 4) or $Ca(OH)_2$ [a] (Scenario 5) (based on $/ton feedstock).

Input (cost)	NaOH	$Ca(OH)_2$	Output (Revenue)	NaOH	$Ca(OH)_2$
GTR	16	16	Pectin	240	240
Enzyme	60	60	Protein	171	94 (141)[c]
Chemical	24	2	Glucose	55	55
Water	18	10	Energy[b]	3	10
Electricity	14	24			
Heating	5	6			
Waste water	4	0.2			
Press Filtration	3	4			
Ultrafiltration	2	2			
Capital	14	20			
Labor	5	6			
Subtotal	165	150	**Subtotal**	469	399 (446)
			Profit	304	249 (296)

[a] Calculation based on data presented in Appendix, Tables 3a and 3b.
[b] Energy derived from the combustion.[41]
[c] Protein revenue was calculated as 70% protein was extracted from GTR.

increased due to the relatively large processing volume for the isolation of potassium salts from the pectin extract. The economic benefits in the KOH scenario are a result of the reduced cost for waste-water management and the increased revenue on fertilizer. Compared to NaOH, less than 10% of waste water is generated in the KOH scenario, and thus the costs for waste-water management reduces to only 0.4$/ton GTR. By using the potassium salts as fertilizer, an extra revenue of 55$/ton GTR is expected.[34] Overall, a similar profit is expected for both the KOH scenario and the NaOH scenario.

NaOH versus $Ca(OH)_2$ (Scenario 4 versus Scenario 5)

The processing scenario for $Ca(OH)_2$ has relatively low costs, as most calcium ions and water can be recycled and reused (Fig. 4b). Generally, the total cost for processing 1 ton GTR using calcium hydroxide was $15/ton GTR less than the scenario using NaOH. A biomass boiler is needed in the $Ca(OH)_2$ scenario and expenses for capital and labor therefore increased. Heat cost in the $Ca(OH)_2$ scenario was slightly increased, due to the need for 178 kJ to convert 1 mol $CaCO_3$ into CaO and CO_2.[35] The price of $Ca(OH)_2$ is only $70/ton (see Appendix, Table A4a), and, with a recycling recovery of about 85%, the estimated chemical cost for $Ca(OH)_2$ protein extraction was only $2/ton GTR (Table 3). After CO_2 treatment, the supernatant of the protein extract can be treated further by ultrafiltration to obtain protein product and about 8 m^3 water. Approximately 7 m^3 water can be reused in the protein extraction step while 1 m^3 water can be reused in ultrafiltration. Because of the recycling, costs of water and waste-water management fees were both reduced.

The revenues of these scenarios depend on the protein extraction efficiency, as the pretreatment step yields the same amount of pectin in both scenarios. The revenue is mainly determined by the total yield of protein because protein prices for feed are almost linearly related to the protein content regardless of its original sources.[36] Processing 1 ton GTR with NaOH produced 360 kg protein product with a protein content of 64%, whereas $Ca(OH)_2$ treatment produces 180 kg protein product with protein content of 64%. The lower protein yield when applying $Ca(OH)_2$ thus results in a lower total profit ($249/ton GTR) compared to the process with NaOH ($304/ton GTR). The profit of $Ca(OH)_2$ extraction (296$/ton) can be comparable to the process using NaOH only for protein yields higher than 70%.

Environmental sustainability

NaOH versus KOH (Scenario 2 versus Scenario 3)

The weak alkaline pretreatments have more processing steps than the reference scenario of a single-step NaOH extraction (Scenario 1). As a result, electricity consumption is higher when this weak alkaline pectin extraction is applied. Despite the reduction in alkali requirement, this increases the environmental impacts (Appendix A5) compared to the single-step extraction (Table 1). The normalized LCA results for the weak alkaline scenarios are shown in Fig. 5. The results confirm that the replacement of NaOH by KOH in the integrated design indeed improves the environmental sustainability for all impact categories assessed. The reduced impacts are all a direct result of the alkali source. In this weak alkaline design, the other inputs such as water, electricity and heat are the same for NaOH and KOH protein extraction. The use of KOH also offers the potential to reduce the demand of commercial muriate of potassium.[34] This further improves the environmental sustainability (see Fig. 5) by avoiding conventional potassium fertilizer production from parent rock materials.[9] The results in Fig. 5 indicate the potential reduction in environmental impacts, provided that conventional potassium fertilizer is avoided. Residual water with potassium is best used on nearby fields to avoid long-distance transportation.[37] Returning potassium-rich waste water to tea farms that are located in the mountains requires transportation (not accounted for in Fig. 5). Instead, residual water with potassium can be used in rice fields located near to tea factories. Moreover, rice fields have far larger requirements for potassium than tea farms. Finally, seasonality has to be taken into account. Production of protein product can be year round, while fertilization may only be needed at certain times, thus requiring storage and/or concentration of the waste water.

Little is published on the environmental impacts of tea cultivation and processing.[33] The CO_2 equivalent emissions for tea cultivation and harvesting under Kenyan conditions are estimated around 0.111–0.133 kg CO_2 equivalent/kg fresh tea leaves.[38] For GTR, an impact of 185.6–222.7 kg CO_2 equivalent/ton GTR is thus expected. The climate change impact from processing GTR into protein and pectin is about three times higher (Appendix, Table A5). Cleaner electricity production can be applied to reduce this impact.

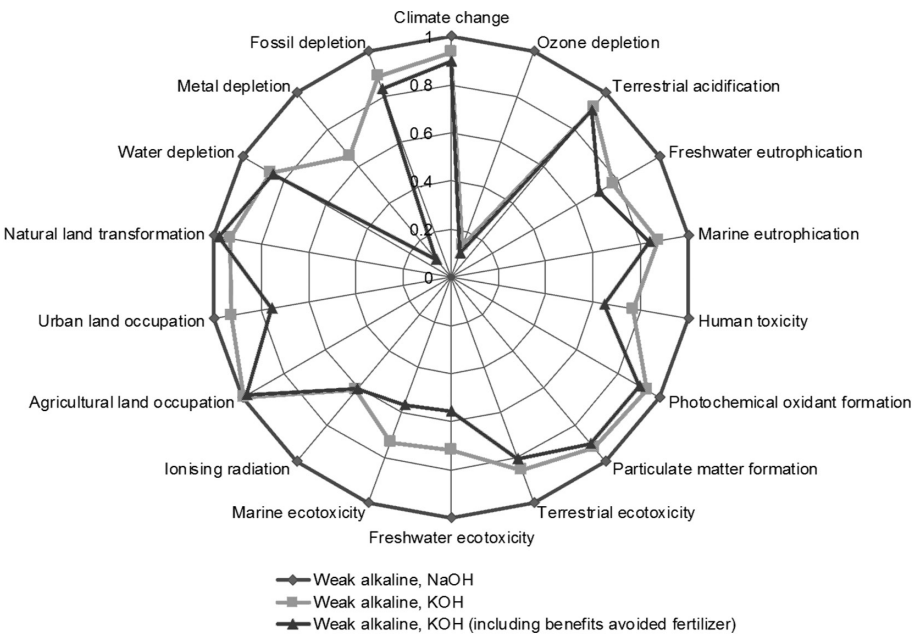


Figure 5. Normalized LCA results for weak alkaline pretreatment and protein extraction using NaOH (diamonds), KOH (squares), and KOH where the production of conventional K fertilizer is included (triangles). Results were normalized to the outcomes of weak alkaline pretreatment with NaOH extraction. The contribution analysis can be found in the Appendix.

The water consumption has been studied in detail.[39] Based on those results a water depletion of 7187 m^3/ton GTR can be expected under Chinese conditions. The water depletion for GTR processing is thus significant (Appendix, Table A5) but still less than for cultivation. Reducing the water depletion is not straightforward as it is a result of upstream cultivation practices, energy generation, and alkali production. The salty waste water from NaOH extraction is negligible in terms of water depletion (14.6 m^3/ton GTR). More data is required to assess the impact of this waste stream on the environment.

Viscozyme® L-assisted pectin extraction integrated processes (NaOH versus $Ca(OH)_2$)

For the Viscozyme® L scenarios, more processing steps are also needed compared to the reference scenario (Scenario 1). Overall, the electricity consumption is thus also higher. Despite the reduction in the alkali requirement, this increases the environmental impacts (Appendix, Table A6) compared to the single-step extraction (Table 1). The increase in impacts is less than for the weak alkaline treatments. In the Viscozyme® L scenarios the advantage of using $Ca(OH)_2$ instead of NaOH is that sufficient heat is produced for the process and there is no salty waste-water stream. In the normalized LCA results this leads to the improvement of some LCA impact categories, such as ozone depletion, eutrophication, toxicity, and land use (Fig. 6). However, categories like fossil depletion, climate change, acidification, particulate matter formation, and water depletion are negatively affected. This is a result of the increased electricity consumption in the protein extraction step. The stakeholders involved will thus need to decide which impact categories are most important. Furthermore, cleaner electricity production can improve the environmental sustainability. The results are similar for the extraction with micro-algae, as the chemical and utility consumption was not affected.

In these scenarios, too, the climate change impact is also higher than that of cultivation. Based on tea cultivation and harvesting under Kenyan conditions, for GTR, an impact of 185.6–222.7 kg CO_2 equivalent/ton GTR is expected. The climate-change impact from processing GTR into protein and pectin is about three to four times higher (Appendix, Table A5). The water depletion is similar to the scenarios with weak alkaline pretreatment, and again much lower than for cultivation (7187 m^3/ton GTR[39,40]). Still, the extraction with NaOH results in an almost 25% lower water depletion compared to $Ca(OH)_2$, while it produces 8.44 m^3 salty waste water/ton GTR. This amount is negligible in terms of water depletion and

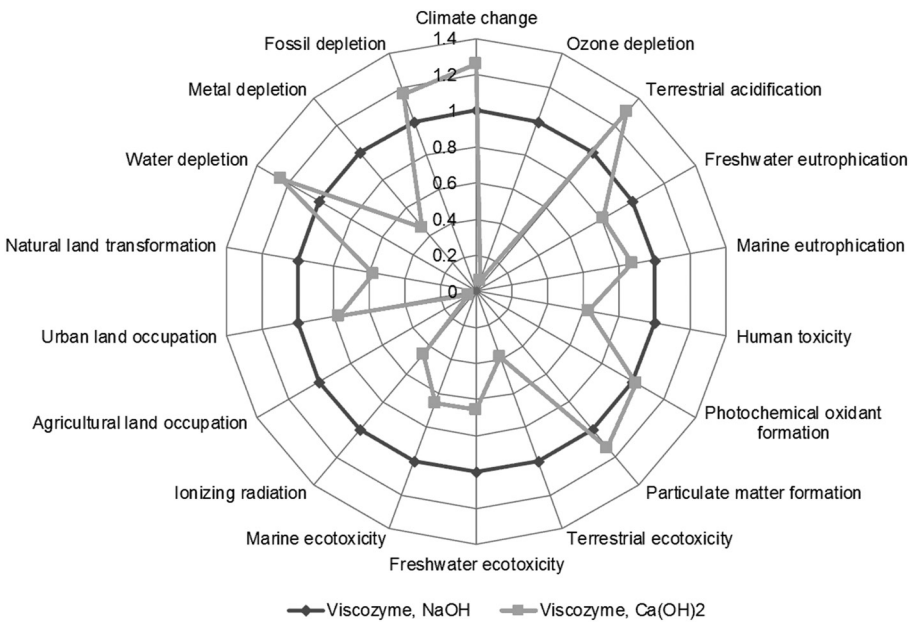


Figure 6. Normalized LCA results for Viscozyme® L pretreatment and protein extraction using NaOH (diamonds) and $Ca(OH)^2$. Results were normalized to the outcomes of Viscozyme pretreatment with NaOH extraction. The contribution analysis can be found in the Appendix.

an important next step is to quantify the environmental impact of the salty water.

Discussion

Currently GTR is considered as waste at the tea lemonade company and is burned. In this work, this GTR is used as a resource to produce a variety of products in various processing scenarios. In the assessment the focus is on comparing the performance of the different options for processing the GTR into valuable products. Outside the system boundaries were tea farming and the processing of tea into tea lemonade, and the performance of the processing scenarios is not compared to the current practice of burning the tea residue. We have chosen not to discount for the combustion emissions of GTR (not for the biogenic CO_2 or for the fossil emissions of combustion), and the GTR therefore enters our factory without any emissions attached to it. The main reason for this is that we wanted to compare the performance of the different options for processing the GTR into valuable products, which are all on basis of processing 1 ton of GTR. Due to lack of data it was also not possible to compare the performance of our scenarios to the current situation of burning the GTR. Similar to the lack of data on combustion emissions for the GTR, the emissions from the combustion of the downstream residues within our factory could also not be quantified. These emissions would be mostly biogenic CO_2, but NO_x can also be generated.

Because of these choices and data limitations, we have not accounted for the biogenic CO_2 in any way in the LCA and the climate change impact category is only affected by the process emissions. In general, the environmental impacts are mostly influenced by the process utilities (see Appendix, Figs A1–A4). The climate change impact is mostly a result of the electricity use. The fossil emissions caused by heat use are limited due to the heat production on site. In the scenario Viscozyme pretreatment with $Ca(OH)_2$ extraction the released (biogenic) CO_2 is used internally. We have not discounted for the alternative of using fossil CO_2, not have we given credits for this recycling. One limitation of the current LCA is thus that the impact of generated combustion emissions of the residues is not included. However, similarly, the (avoided) combustion emissions of GTR at the tea lemonade company have also not been discounted, thereby avoiding artificial upgrading or greenwashing of the LCA results.

To assess the performance of the complete system from tea farming until GTR products it is necessary to: (1) expand the system boundaries so that they also account for the impacts of tea farming, tea lemonade production, and the application of potassium waste water as fertilizer, (2) quantify and include the combustion emissions, (3) complete the lifecycle inventory assessment of enzyme production.

Sustainability of environment / economics

The above analysis illustrates that more processing effort results in more products and that the increased demand of inputs results in a larger environmental impact. However, these additional processing efforts also change the economic performance. Figure 7 illustrates the relative sustainability performance of the five processing scenarios using a proxy. For each scenario the environmental performance of the basket of products produced is divided by the profit generated in that specific scenario. The results are expressed relative to the performance of the single-step NaOH scenario (Scenario 1). As shown, the single-step NaOH protein extraction has the relative highest sustainability proxy. This means that this scenario has the highest environmental impacts compared to the profit generated, which is due to the relatively low profit generated in this scenario.

For the four other scenarios, the proxy of the sustainability performance is at least 20% lower and up to 98% lower than for the single-step reference. According to Fig. 7, using Viscozyme® L in the pretreatment (Scenarios 4 and 5) results in an overall better proxy for sustainability compared to the scenarios using weak alkaline (Scenarios 2 and 3). Note that the comparison between the weak alkaline and Viscozyme scenarios should be done only for 'climate change,' 'terrestrial acidification,' 'freshwater eutrophication,' 'photochemical oxidant formation,' and 'fossil depletion' because the other impact categories were not quantified for the production of the enzyme itself. Using KOH (Scenario 3) to replace NaOH (Scenario 2) improves the proxy for all categories except for natural land transformation and ozone deletion, which are the same. A similar result may be expected when substituting NaOH with KOH in the Viscozyme® L-aided pretreatments. For the scenarios with Viscozyme (Scenarios 4 and 5), the proxy results vary for the different categories. The natural land transformation has the best proxy for the $Ca(OH)_2$ related process (Scenario 5) due to the lower amount of chemical use. However, compared to the scenario using NaOH (Scenario 4), this scenario results in similar proxies (ozone depletion, freshwater and marine eutrophication), or higher ones (all other categories), which is due to the low overall profit resulting from the low protein extraction yield. This scenario can only be competitive when protein yield increases to at least 70%.

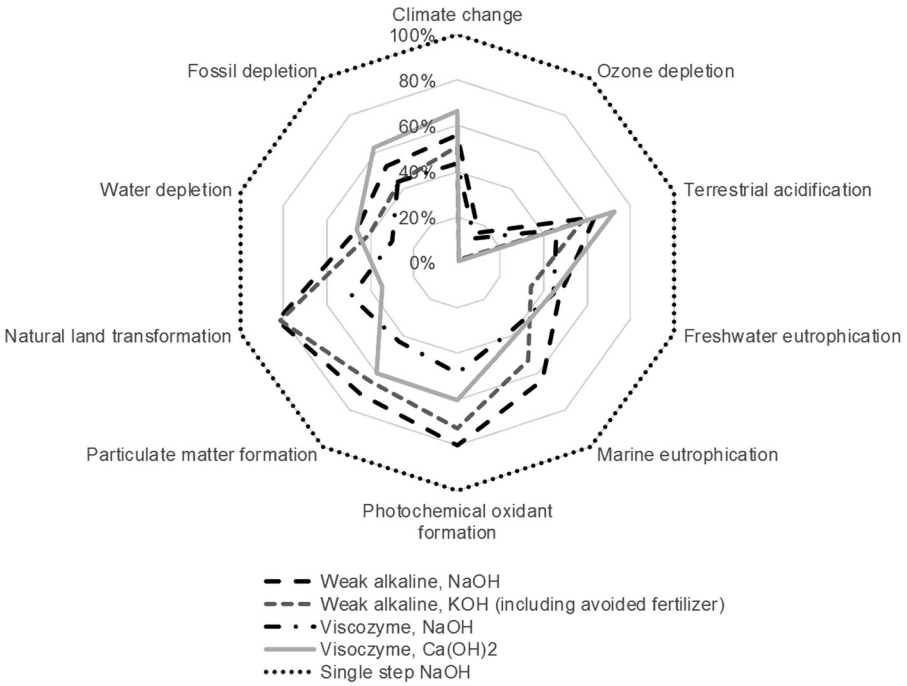


Figure 7. LCA impacts divided by the corresponding profit for each of the five scenarios. Results are expressed relative to the performance of the single-step NaOH scenario (Scenario 1).

Each of the scenarios results in a different basket of products. It goes beyond the scope of this study to compare the environmental and economic sustainability of each of these products (from GTR) with the alternative production chains. For example, this study does not evaluate whether pectin from GTR is economically competitive with or more environmentally friendly than the current practice of producing pectin from citrus. A next step would be to perform such a product-orientated study, which requires expanding the system boundaries to include tea farming and adjusting the functional unit to make comparisons on a product basis.

Conclusion

Compared to single-step NaOH protein extraction (Scenario 1), integrated processes produce more products and higher profits. Waste salts from alkaline protein extraction can be either reused as fertilizer or recycled if KOH or $Ca(OH)_2$ are used instead of NaOH. As KOH has similar functionality to NaOH and can extract more than 90% protein from GTR, the profit of the KOH-aided protein extraction process (Scenario 3) is similar to that using NaOH (Scenario 2). The environmental sustainability improves for all impact categories in the case of KOH extraction.

The use of $Ca(OH)_2$ results in a relatively low protein yield in GTR protein extraction, which reduces its profit. Although $Ca(OH)_2$ (Scenario 5) can be recycled, reducing the impact of natural land transformation, its relatively environmental impacts are therefore generally higher than those using NaOH (Scenario 4). Applying the $Ca(OH)_2$ scenario to biomass without lignin can lead to protein extraction yields above 70%. This is beneficial for both economic and environmental impacts.

To conclude, the application of KOH or $Ca(OH)_2$ for protein extraction enables commercialization of yet untapped protein resources, which can be economically attractive. The environmental impacts are mostly caused by the utilities. The environmental benefits are the avoided waste and pollution.

Acknowledgement

This research was supported by the Dutch Technology Foundation (STW), which is part of the Netherlands Organization for Scientific Research (NWO), and is partly funded by the Ministry of Economic Affairs. This research was also supported by NSFC No.31701649. Special thanks to Damin Company (China), which donated the green tea residues.

References

1. Akeson WR and Stahmann MA, Leaf proteins as foodstuffs, nutritive value of leaf protein concentrate, an *in vitro* degestion study. *J Agric Food Chem* **13**(2):145–148 (1965).
2. Gerloff ED, Lima IH and Stahmann MA, Leaf proteins as foodstuffs, amino acid composition of leaf protein concentrates. *J Agric Food Chem* **13**(2):139–143 (1965).
3. Ghaly AE and Alkoaik FN, Extraction of protein from common plant leaves for use as human food. *Am J Appl Sci* **7**(3):331–342 (2010).
4. Kondo M, Kita K and Yokota H, Feeding value to goats of whole-crop oat ensiled with green tea waste. *Anim Feed Sci Technol* **113**(1–4):71–81 (2004).
5. Bals B and Dale BE, Economic comparison of multiple techniques for recovering leaf protein in biomass processing. *Biotechnol Bioeng* **108**(3):530–537 (2011).
6. Zhang C, Sanders JPM and Bruins ME, Critical parameters in cost-effective alkaline extraction for high protein yield from leaves. *Biomass Bioenergy* **67**:466–472 (2014).
7. Zhang C, Bozileva E, van de Klis F, Dong Y, Sanders JPM and Bruins ME, Integration of galacturonic acid extraction with alkaline protein extraction from green tea leaf residue. *Ind Crops Prod* **89**:95–102 (2016).
8. Zhang C, van Krimpen MM, Sanders JPM and Bruins ME, Improving yield and composition of protein concentrates from green tea residue in an agri-food supply chain: effect of pretreatment. *Food Bioprod Process* **100**:92–101 (2016).
9. Hasler K, Bröring S, Omta SWF and Olfs HW, Life cycle assessment (LCA) of different fertilizer product types. *Eur J Agron* **69**:41–51 (2015).
10. Baraniak B and Baraniak A, Application of polyelectrolytes to fractionation of alfalfa juice protein. *Food / Nahrung* **31**(4):341–343 (1987).
11. Urribarrí L, Ferrer A and Colina A, Leaf protein from ammonia-treated dwarf elephant grass (Pennisetum purpureum Schum cv. Mott). *Appl Biochem Biotechnol* **122**(1):721–730 (2005).
12. Holtzapple MT, Davison R, Granda C and Noyes GP, Method and system for solubilizing protein. US Patent 7705116B2 (2005).
13. Bates RG, Bower VE and Smith ER, Calcium hydroxide as a highly alkaline pH standard. *J Res Natl Stand* **56**(6):305–312 (1956).
14. Ropers MH and Leroy T, Calcium bridges in fatty acid/pectin assemblies. *Food Hydrocoll* **22**(7):1396–1403 (2008).
15. Fontana A, Vita C, Boccu E and Veronese FM, A fluorimetric study of the role of calcium ions in the stability of thermolysin. *Biochem J* **165**(3):539–545 (1977).
16. Chen J, Liang R-H, Liu W, Luo S-J, Liu C-M, Wu S-S *et al.,* Extraction of pectin from *Premna microphylla* turcz leaves and its physicochemical properties. *Carbohydr Polym* **102**:376–384 (2014).
17. Li J-L, Cheng Y-Q, Wang P, Zhao W-T, Yin L-J and Saito M, A novel improvement in whey protein isolate emulsion stability: generation of an enzymatically cross-linked beet pectin layer using horseradish peroxidase. *Food Hydrocoll* **26**(2):448–455 (2012).
18. Boukhoubza F, Jail A, Korchi F, Idrissi LL, Hannache H, Duarte JC *et al.,* Application of lime and calcium hypochlorite in the dephenolisation and discolouration of olive mill wastewater. *J Environ Manage* **91**(1):124–132 (2009).
19. Zahrim AY, Nasimah A and Hilal N, Coagulation/flocculation of lignin aqueous solution in single stage mixing tank system: modeling and optimization by response surface methodology. *J Environ Eng* **3**(3):2145–2154 (2015).
20. Marfo EK and Oke OL, Effect of sodium chloride, calcium chloride and sodium hydroxide on Denolix regia protein solubility. *Food Chem* **31**(2):117–127 (1989).
21. Smith AK and Circle SJ, Peptization of soybean proteins extraction of nitrogenous constituents from oil-bases with and without added slats. *Ind Eng Chem* **30**(12):1414–1418 (1938).
22. Cogan U, Yaron A, Berk Z and Mizrahi S, Isolation of soybean protein: effect of processing conditions on yields and purity. *J Am Oil Chem Soc* **44**(5):321–324 (1967).
23. Ji Qin N and Hang Z, Energy consumption and conservation in tea processing in China. *Renewable Energy* **2**(1):1–5 (1992).
24. Gani A and Naruse I, Effect of cellulose and lignin content on pyrolysis and combustion characteristics for several types of biomass. *Renewable Energy* **32**(4):649–661 (2007).
25. Tripathi AK, Iyer PVR and Kandpal TC, A techno-economic evaluation of biomass briquetting in India. *Biomass Bioenergy* **14**(5–6):479–488 (1998).
26. Drouiche M, Lounici H, Belhocine D, Grib H, Piron D and Mameri N, Economic study of the treatment of surface water by small ultrafiltration units. *Water SA* **27**(2):199–204 (2001).
27. Ciriminna R, Fidalgo A, Delisi R, Ilharco LM and Pagliaro M, Pectin production and global market. *Ago Food Industry Hi-Tech* **27**(5):17–20 (2016).
28. Grohmann K, Baldwin EA, Buslig BS and Ingram LON, Fermentation of galacturonic acid and other sugars in orange peel hydrolysates by the ethanologenic strain of Escherichia coli. *Biotechnol Lett* **16**(3):281–286 (1994).
29. Dornburg V and Faaij APC, Efficiency and economy of wood-fired biomass energy systems in relation to scale regarding heat and power generation using combustion and gasification technologies. *Biomass Bioenergy* **21**(2):91–108 (2001).
30. Wernet G, Bauer C, Steubing B, Reinhard J, Moreno-Ruiz E and Weidema B, The ecoinvent database version 3 (part I): overview and methodology. *Int J Life Cycle Assess* **21**(9):13 (2016).
31. Liptow C, Tillman A-M, Janssen M, Wallberg O and Taylor GA, Ethylene based on woody biomass—what are environmental key issues of a possible future Swedish production on industrial scale. *Int J Life Cycle Assess* **18**(5):1071–1081 (2013).
32. Yang JX and Nielsen PH, Chinese life cycle impact assessment factors. *J Environ Sci (China)* **13**(2):205–209 (2001).
33. Taulo JL and Sebitosi AB, Material and energy flow analysis of the Malawian tea industry. *Renew Sustain Energ Rev* **56**:1337–1350 (2016).
34. Silva G, Fertilizer prices (2015). [Online]. Michigan State University Extension. Available at: http://msue.anr.msu.edu/news/fertilizer_prices_in_2015/ [February 15, 2018].
35. Lin S, Kiga T, Wang Y and Nakayama K, Energy analysis of $CaCO_3$ calcination with CO_2 capture. *Energy Procedia* **4**:356–361 (2011).
36. Teekens AM, Bruins ME, van Kasteren JMN, Hendriks WH and Sanders JPM, Synergy between bio-based industry and the feed industry through biorefinery. *J Sci Food Agric* **96**(8):2603–2612 (2016).
37. Bruins ME and Sanders JPM, Small-scale processing of biomass for biorefinery. *Biofuels Bioprod Biorefin* **6**(2):135–145 (2012).

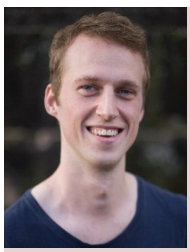
38. Azapagic A, Bore J, Cheserek B, Kamunya S and Elbehri A, The global warming potential of production and consumption of Kenyan tea. *J Cleaner Prod* **112**(5):4031–4040 (2016).
39. Jefferies D, Muñoz I, Hodges J, King VJ, Aldaya M, Ercin AE *et al.,* Water footprint and life cycle assessment as approaches to assess potential impacts of products on water consumption. Key learning points from pilot studies on tea and margarine. *J Cleaner Prod* **33**:155–166 (2012).
40. Chapagain AK and Hoekstra AY, The water footprint of coffee and tea consumption in the Netherlands. *Ecol Econ* **64**(1):109–118 (2007).
41. Robak J, Janusz M and Sobolewski A, Preparation of lignocellulosic waste for combustion process. *Chemik* **66**(5):436–440 (2012).

Chen Zhang

Chen Zhang is associate professor at the Institute of Food Science and Technology, Fuzhou University, where he develops technologies to obtain valuable products from bio-waste produced at food factories. He obtained his PhD at the BCT group of Wageningen University (2010–2016), during which he was working on the biorefinery of leafy biomass.

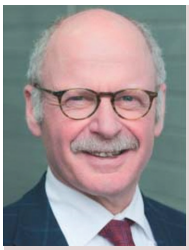
Petronella M. Slegers

Petronella M. Slegers is an assistant professor at the operations research and logistics group of Wageningen University. Her research is on sustainable biobased and circular chains, particularly focusing on optimizing sustainability performance and quantitatively assessing sustainability at early stages.



Jacobus Wisse

Jacobus (Ko) Wisse has been a researcher on reservoir microbiology at Shell Global Solutions since 2014. He is working at the Department of Projects and Technologies, where he investigates microbial issues related to oil recovery processes. He graduated with a BSc and MSc in biotechnology at the university of Wageningen (2008–2013).



Johan P.M. Sanders

Johan Sanders is Em Professor of the VPP Chair in Wageningen University. His work focuses on enabling optimal application of all plant components. He holds a PhD in molecular biology from Amsterdam University. He worked at Gist Brocades (1977–1993) as associate director of food research. From 1993–2001 he worked at AVEBE as R&D director.

Marieke Bruins

Marieke Bruins is senior researcher mild separation at Wageningen Food & Biobased Research. She worked as an assistant professor at BCT group on small-scale processing and protein biorefinery. She holds a PhD in biotechnology (2003) and worked as a post-doc (2004–2008) at Wageningen University on a personal VENI grant on enzymatic processes at extreme conditions.

