Opportunities for the refinement of grass - FrieslandCampina

Final report

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Preface

This project was commissioned by FrieslandCampina and conducted by students from the 'Tesla minor'. This is a five month course at the University of Amsterdam that aims to integrate science into business and society. During this time, two to three beta-master students take up an interdisciplinary project from an external client, which was in our case FrieslandCampina. Besides this, there are weekly lectures from inspiring professionals and the students follow trainings that are aimed at the development of soft skills. In 2014, fourteen students participated in the Tesla minor, divided into five groups. More information about the Tesla minor and the projects can be found at http://gss.uva.nl/future-msc-students/tesla-minor.html or at http://www.teslaminor.nl/.

Samenvatting

Gedurende dit project hebben we onderzocht of het winnen van eiwitten uit gras door middel van bioraffinage een mogelijkheid is voor FrieslandCampina. Om dit complexe project uit te voeren hebben we het opgedeeld in verschillende secties. Zo hebben we een overzicht gemaakt van de beschikbaarheid en eigenschappen van gras. Daarnaast hebben we de technische mogelijkheden van grasraffinage onderzocht en de mogelijke toepassingen van de producten die bij grasraffinage ontstaan in kaart gebracht. Met deze kennis hebben we drie scenario's ontwikkeld die uiteenlopende opties tot raffinage representeren. Vervolgens zijn deze scenario's met behulp van een kosten-baten analyse op financiële haalbaarheid getest. Deze kosten-baten analyse is uitgevoerd in een Excel bestand die samen met dit verslag het door ons afgeleverde product voor FrieslandCampina vormt. In het eerste scenario wordt een voeder-kwaliteit niet-functioneel eiwitproduct geproduceerd in een kleine mobiele installatie. Het tweede scenario betreft een voedsel-kwaliteit non-functioneel eiwitproduct dat geproduceerd wordt in een grote centrale raffinaderij. In het derde scenario wordt een puur functioneel voedsel-kwaliteit eiwitproduct gewonnen, ook in een grote centrale raffinaderij. Uit de kosten-baten analyse is gebleken dat met onze conservatieve assumpties deze drie scenario's op dit moment financieel niet rendabel zijn. Echter, een van de scenario's (het derde scenario, waarin functioneel eiwit gewonnen wordt in een centrale raffinaderij) laat veel potentie zien. Kleine verschuivingen van de door ons gedane aannames leidt tot een positief scenario. Dit in tegenstelling tot de andere twee scenario's. Het eerste scenario bleek onrendabel te zijn en toonde weinig ruimte voor verbetering. Het tweede scenario bleek zeer onrendabel en toonde vrijwel geen ruimte voor verbetering. Om deze reden adviseren wij FrieslandCampina om verder onderzoek te doen naar de winning van functionele eiwitten uit gras op grote schaal.

Summary

During this project, we investigated whether FrieslandCampina should engage and invest in the refinement of grass. In order to do this we divided the project into different sections. First, we reviewed the availability and properties of grass. Next, the technical possibilities of biorefinement and the products that are produced were defined. After this, possible applications for these products were determined. With this knowledge, we designed three scenarios that represent different approaches to grass refinement. These scenarios were subsequently tested for financial viability with a cost-benefit analysis. The costs-benefit analysis was executed in an Excel file, which -along with this report- comprises our deliverables to FrieslandCampina. The first scenario entails the refinement of a feed-grade non-functional protein product in a small mobile refinery. In the second scenario, food-grade non-functional protein is produced in a large centralized refinery. The third scenario also uses a large central refinery, but here a pure, food-grade functional protein is produced. The cost-benefit analysis shows that with our conservative assumptions all three scenarios are not financially viable. However, one of the scenarios (scenario 3, in which functional protein is extracted in a central refinery) shows great potential. Small changes in our assumptions lead to a profitable business case. This in contrast to the other two scenarios. The first scenario was not financially viable and there was little room for improvement. The second scenario turned out to be very unprofitable and did not show any room for improvement. For this reason, we recommend FrieslandCampina to further investigate the production of functional protein from grass.

List of abbreviations

AAS	Amino Acid Score
ARR	Average Rate of Return
CP	Crude Protein
COGS	Costs Of Goods Sold
DCF	Discounted Cash Flow
DM	Dry Matter
EBIT	Earnings Before Interest and Taxes
EBITDA	Earnings Before Interest Taxes Depreciation and Amortization
EU	European Union
FM	Fresh Matter
FTE	Full-Time Equivalent
IRR	Internal Rate of Return
N	Nitrogen
NDF	Neutral Detergent Fibre
NFR	Novel Food Regulation
NPV	Net Present Value
P	Phosphate
PPO	Polyphenol Oxidase
OPEX	Operating Expenses
ROI	Return on Investment
WPC	Whey Protein Concentrate
WUR	Wageningen University & Research centre

Contents

1 Introduction

In the last decade, biorefinement has become an interesting and widely discussed topic. Biorefinement is the process of refining multiple products from green biomass, which is similar to the refining of oil to produce different fuels and chemicals. The growing popularity of biorefinement is illustrated by the number of biorefinement initiatives that have started in the past years and the growing number of (non-)scientific publications. However, the commercial exploitation of a biorefinery remains a financial challenge. Due to the small margins and the experimental technologies required for biorefinement, companies are hesitant to invest in biorefinery plants. This might change in the coming years as more biorefinement initiatives succeed in running their daily business without structural governmental subsidies. Especially the refinement of grass is becoming interesting due to the high prevalence of grass and the technical knowledge gained from grass refinement initiatives. This project was commissioned by FrieslandCampina in response to that growing popularity of grass refinement. As a major player in the agricultural sector, FrieslandCampina has the ability to change the field of biorefinement. However, this does require a financial dedication that is not without risk. Therefore, the aim of this project was to investigate whether grass refinement would be a worthwhile investment for FrieslandCampina.

1.1 FrieslandCampina

FrieslandCampina emerged end 2008 as a result of the merger of Royal Friesland Foods and Campina[1]. In 2013, FrieslandCampina was the fifth largest dairy company in the world with an annual revenue of € 11.4 billion[2]. It is owned by Zuivelcoöperatie FrieslandCampina U.A., which has 19,244 member dairy farmers in the Netherlands, Germany and Belgium. In the Netherlands, approximately 50% of all farmland is managed by farmers of FrieslandCampina, showing the importance and size of FrieslandCampina in the Netherlands (online interview with T. Kingma[3] and personal communication, Y. de Vries). The activities of FrieslandCampina are divided into four different business groups. One of these groups is 'Ingredients', which encompasses several different brands. Among others, it produces protein ingredients for infant nutrition and medical nutrition, demonstrating the involvement of FrieslandCampina in both business-to-business and business-to-consumer activities. In the future, grass refinement could be part of the Ingredients group.

1.2 Relevance of the project

This project based upon two trends. The first one is that in our world, roughly 850 million people are chronically malnourished[4]. Especially protein is often problematic[4] as this is a relatively expensive resource. Furthermore, it is estimated that our current agricultural protein output is not sufficient to feed the expected world population in 2050 if we continue with our modern food pattern[5]. Thus, the current world's protein production will not be sufficient to feed the world's population in the future. The second reason why this project is relevant is the low efficiency of milk production from grassland. Due to the strong structure of grass, not all protein can be effectively used by the cow. The level of protein that ultimately ends up in the milk can be measured by means of the total nitrogen content in milk (as explained in section 2.3). One publication reports that in cows approximately 72% of the consumed nitrogen is excreted in faeces and urine[6], indicating that only 28% of the protein is used for milk and meat production. A later study showed that on average 30% of the consumed nitrogen is excreted in the milk[7]. Thus, of all the protein in grass, approximately 2/3 is not effectively used in the dairy chain. This means that there is room for improvement of the protein production chain from grassland. By using biorefinement, the usable protein production per hectare of grassland can be increased. Estimates show that 45-90% of the protein in grass can be extracted[8–10], meaning that the usable protein production per hectare would be doubled at the least.

1.3 Goal of the project

The main deliverable of this project is to provide FrieslandCampina with a conclusive analysis on whether and how they should invest in the refinement of grass. To reach this main deliverable, several sub deliverables are formulated:

- An overview of grass production in the Netherlands
- An overview of the grass refinement process and the different (side-)products of that process
- An overview of the applications of the (side-)products
- A cost-benefit analysis of grass refinement

1.4 Methods

The structure of this reports is based on the sub deliverables described in section 1.3. In chapter 2, the background information on the grass production in the Netherlands is discussed. Chapter 3 elaborates on the process of biorefinement. In order to do so, three scenarios with different biorefinement processes are designed. For each of those scenarios a detailed process is described and substantiated. Next, in chapter 4 the possible applications of the biorefinement (side-)products are addressed. In chapter 5 a cost-benefit analysis (CBA) is performed for each of the three scenarios. All financial aspects of grass refinement are analysed in this chapter. In the following chapter the results of the CBA are discussed and summarised. Challenges that could not be taken into account in the CBA are explained in chapter 7 and this is followed by the final recommendation for FrieslandCampina and suggestions for future research.

This report was written based on available literature and contact with experts. As grass refinement is a relatively new and practical field, scientific literature is limited. As such, ‘grey literature’ (e.g. websites, presentations) was also used to gather information. In all chapters, part of the information is based on conversations with experts. Information about these experts can be found in appendix I. Especially chapter 3 heavily relies on discussions with experts. For chapters 3 and 5 a more detailed description about the used method is included in the text of that specific chapter. Besides literature and experts, Microsoft Excel 2010 was essential for chapter 5, the cost-benefit analysis. This report is accompanied by an extensive Excel file in which the cost-benefit analysis is performed. The Excel file is publically available and can be adjusted if new developments occur.

2 Grass in the Netherlands

This chapter discusses the details of grass production in the Netherlands. Specifically: 1) information about the grass landscape in the Netherlands; 2) the chemical composition of grass; 3) costs and methods associated with grass processing and 4) possible diseases.

2.1 Yearly grass production

Grass is the most abundant crop in the Netherlands. In 2012, there were 938.000 hectares of grassland of which 746.000 hectares were permanent grassland[11]. The highest concentration of grassland is in the provinces Friesland, Gelderland and Overijssel. Together, these provinces account for approximately 50% of all grassland[11]. The high prevalence of grassland is due to two reasons. First, land such as peat is only suited for grass. The second reason is that grass is relatively simple and cheap to produce while containing relatively high amounts of protein. Like most crops in the Netherlands, fresh grass is only available in a limited period. In general, grass starts growing when temperatures reach 10° C (personal communication, E. Ensing). This is usually somewhere in April, although it differs for each year. When grass starts growing in April, the first cut of grass can be collected in May. Roughly, the grass season ends on the 1st of October[12], which means that fresh grass is available from May until the end of September.

It is important to note the difference between cultured grassland and natural grassland. Cultured grassland is focussed on producing as much high-quality grass as possible, where natural grassland has no production focus. In general, the yield in terms of protein and dry matter (DM) are higher for cultured grassland than for nature grassland. In the period 1998-2002, the average production was estimated to be 10,400 kg matter DM/hectare for cultured grassland in the Netherlands[13]. For comparison, the average yield of several prominent crops is displayed in table 1.

Table 1 Yield per hectare of some common crops in the Netherlands.

Crop	Yield	Source
Soybeans	2,310 kg/hectare	Masuda *et al*. (2009), world average 2005-2007[14].
Grass	10,400 kg DM/hectare	Aarts (2005), average for the Netherlands in the period 1998-2002 with the exclusion of data from 2000.
Maize	15,675 kg DM/hectare	LEI, average for the Netherlands in the period 2006-2012.

2.2 Grass species

The majority of the cultured grassland consists of perennial ryegrass (*Lolium perenne*), which is often complemented with other grass species or clover[15–17]. Among others, plants that are used in combination with perennial ryegrass are timothy (*Phleum pratense*), tall fescue (*Festuca arundinacea*) and meadow fescue (*Festuca pratensis*)[12,18]. Perennial ryegrass is broadly used in the Netherlands due to its positive characteristics for animal husbandry. It has a high nutritional value, responds quickly to nitrogen fertilization and is relatively tasty for cattle[16,19]. Tall fescue (*F. arundinacea*) is a grass species that has a higher yield in terms of DM/hectare compared to perennial ryegrass[12]. With good grassland management, soft leaved tall fescue has a similar nutritional value to perennial ryegrass when it is harvested. However, it is not suited for grazing as the strong structure of the plant hinders uptake of nutrients (personal communication, E. Ensing). The suitability of tall fescue for biorefinery is unknown. To assess this, experiments with an established biorefinery are required.

2.3 Grass composition

Grass consists mainly of water. Approximately 10-20% of the grass is dry matter (DM), with a yearly average of 16,3% according to the 'Centraal Veevoeder Bureau' (CVB)[20]. This dry matter mainly consists of protein, amino acids, carbohydrates, minerals and fats (Table 2). The quantity of these components is often expressed in grams/kg DM, as this generalizes the contents of grass into a common standard for different types of grass. For example, the average crude protein content is 23% of the dry matter[20]. With an annual production of 10,400 kg DM per hectare, the theoretical amount of protein would amount to 2400 kg per hectare on average. Of all proteins present in grass, the most abundant protein is called *ribulose-1,5-bisphosphate carboxylase oxygenase*, or simply Rubisco. This protein is present in all green plant material as it is involved in the photosynthesis. Therefore, Rubisco is also said to be the most prevalent protein in the world[21].

Instead of measuring the actual protein level in grass, it is easier to measure the level of nitrogen (N) in a grass sample using either the Kjeldahl or Dumas method[22]. These methods assume that for each kg of N, approximately 6.25 kg of protein is present, although this differs between crops[22,23]. Thus, a nitrogen content of 32 g/kg DM would amount to 200 g protein/kg DM (32 x 6.25 = 200). This calculated protein quantity is termed *crude protein* (CP) content and can be viewed as an estimate of the actual protein content. The percentage of crude protein in DM changes during the season. In general, protein levels are lowest in spring and highest in autumn, ranging from 150 to 250 grams/kg DM[12,19]. This is a challenge for grass refinement as the process has to be adapted to the composition of the grass and the profitability largely depends on the protein content as will be discussed later in this report.

Another important factor to consider is the presence of polyphenol oxidase (PPO) and proteases in grass. Proteases are enzymes that break down other proteins, which is undesired if protein is to be extracted from the grass. PPO is an enzyme that will oxidize any phenolic substances, which causes a browning reaction that diminishes the quality of the grass and complicates the refinement process. After cutting the grass, this reaction will start immediately, making it essential that after cutting, the grass is either processed rapidly or preserved well enough to prevent degradation. For the development of the scenarios in chapter 3 these factors were taken into account.

The largest component of DM of grass is its fibres. The fibre fraction is not made up of a single uniform material which makes determining the fibre content of grass less straightforward. In general, the fibre fraction is measured as neutral detergent fibre (NDF)[24]. The NDF includes cellulose, hemicellulose and lignin molecules, which make up part of the cell wall. They not only contribute to the rigidity of the plant, but also impede the digestion of grass cells. If grass is to be refined into its individual contents, the lignocellulose structure has to be disrupted first. According to a grass seed company, the optimal NDF content of grass for cattle feed is roughly 475 g/kg DM[18]. From literature, we found a mean NDF content of 448 in a range of 348-548[25–27]. The amount of NDF increases with age or length of the grass[28].

Table 2 Average annual composition of fresh perennial ryegrass in g/kg dry matter (DM) and g/kg fresh matter (FM)[a]

Composition of grass	Dry matter	Ash	Crude protein	Fat	Carbo-hydrates	Sugars	Phos-phate	Potas-sium
g/kg DM $^{-1}$	1000	106	227	44	228	96	4,1	35,1
g/kg FM $^{-1}$	163	17	37	7	37	16	0,7	5,7

a) Adapted from Tabellenboek Veevoeding (2012)

2.4 Grass price

For the cost-benefit analysis, it is important to have an overview of the grass price. It is difficult to give an exact price as there is no direct market for fresh grass. Most farmers do not sell their grass, but use it within their own farming business. If they are nevertheless in need of grass, they will buy from a neighbouring farmer (personal communication, N. den Besten). Therefore, Wageningen UR Livestock Research calculates prices for grass based on its nutritional value[29]. In 2013, the price was € 0,24/ kg DM for excellent summer grass[30]. It is important to note that the market price for grass will probably differ for each region, depending on supply and demand. Besides this, grass prices based on the nutritional value are usually overestimated. Farmers generally assume that roughage such as grass costs € 0,15/kg DM (personal communication, N. den Besten and J. van de Ven). This indicates that a grass price of € 0,24/ kg DM is an overestimation. However, in the near future grass prices might increase to this price level if the demand for high-quality grass keeps increasing. This is likely as it is estimated that the dairy industry will grow when the milk quota is abolished in 2015[31], which in turn would increase the demand for roughage such as grass. Another factor that needs to be taken into account is the price of other feed-ingredients. The diet of animals consists of roughage, concentrated feed and, if necessary, supplements. Concentrated feeds have a high density of nutrients, are very protein rich and more costly. Roughage such as grass, hay and maize has a lower nutritional value but is generally cheaper. If the price of other roughage rises, farmers will increase the percentage of grass in the cows' diet. This in turn could lead to increased grass prices (personal communication, G. Remmelink).

2.5 Storage of grass

As fresh grass is only available from May until the end of September, excess grass needs to be conserved to be used in the remaining period. There are several processes for conserving grass, but most traditional methods rely on the drying of grass, which increases the percentage of DM per kilogram of grass. Grass can be either dried naturally on the land or artificially in a factory. After drying, the grass can be conserved in a silage, grass bales or processed to pelleted grass[32]. Drying grass artificially and making grass pellets costs 10 times more energy than conserving grass in a silage and is therefore more expensive and unsustainable. An advantage however is that artificially drying is not subject to weather conditions and loss of the nutritional value of the grass is minimized[33]. In the case of biorefinement, conserving grass in a silage is more suitable as the process requires a relatively low dry matter content (personal communication, B. Koopmans). Therefore, only this method will be discussed in this report.

There are two main processes for obtaining a good silage. One is to reach a low pH by lactic acid fermentation as soon as possible. The other is maintaining anaerobic conditions. In an anaerobic environment lactic acid bacteria convert carbohydrates (sugars) from grass to lactic acid which leads to a decrease in pH. A lower pH preserves the grass and inhibits protein degradation by bacteria. For grass silage, two different methods of conservation can be distinguished. The most common one is *dry silage*, in which grass is dried on the land to a dry matter percentage of 35-45%[34,35]. During the drying of the grass and the aerobic phase, proteins are converted into amino acids by plant proteases. This decrease in protein content is not desirable for grass refinement and the dry matter percentage is too high for biorefinement. So in the case of grass refinement, dry silage is unwanted. In contrast, *wet silage* can be used to achieve a silage with a lower DM percentage. In this method, the grass is immediately transported from the land to a special silage were formic acid is added to conserve the grass. For silages with a DM content of <25%, a pH of 3.8-4.2 is optimal[19]. For drier silages, the values in figure 1 can be used as a guide.

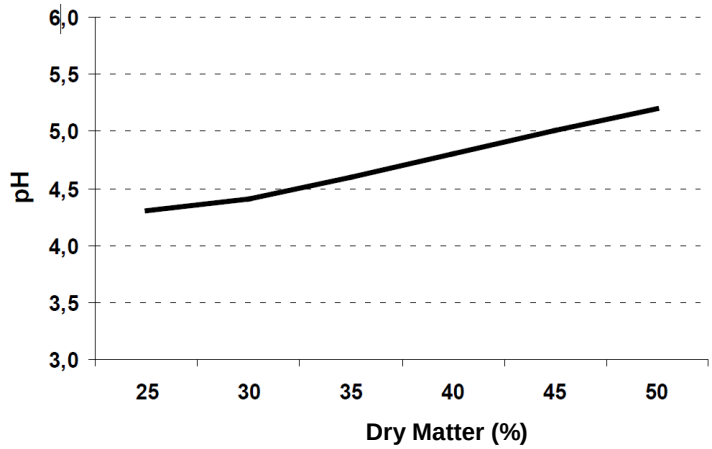

Figure 1 | Optimal pH for grass silage with different dry matter percentages. Adapted from Handboek melkveehouderij[19].

2.6 Grass diseases

Perennial ryegrass can be affected by several pathogens. A brief overview of these pathogens and diseases is depicted in table 3. The most common threat is crown rust, which is caused by *Puccinia coronata Corda*[36]. The occurrence of crown rust is partly influenced by the availability of nutrients. For example, crown rust is more likely to invade perennial ryegrass when nitrogen levels are low[37]. This is important as there is a tendency in the EU to lower N fertilisation levels, which indirectly increases the chance of crown rust outbreaks[38].

Crown rust can be treated with chemicals, but this is often not desirable both from an ecological and economical perspective. The best method to prevent crown rust is by using resistant perennial ryegrass species and good grassland management[37,39]. As such, a high resistance against crown rust is an important aspect for ryegrass breeding programs[38]. Unfortunately, this resistance is negatively correlated with DM yield[38,40]. In conclusion, grass diseases might pose a threat to grass refinement. This is especially the case if infection with certain pathogens leads to a contaminated refinement product. However, the pathogens discussed in table 3 are well recognisable and major outbreaks are not common. For this reason grass diseases are not further discussed in this report.

Table 3 Bacterial and fungal diseases affecting perennial ryegrass[a]

Disease	Pathogen
Crown rust	*Puccinia coronata Corda*
Stem rust	*Puccinia graminis,* subsp. *Graminicola*
Brown rust	*Puccinia loliina*
Leaf spot	*Drechslera (Helminthosporium)* spp.
Bacterial wilt	*Xanthomonas campestris* pv. *Graminis*

a) adapted from Muylle (2003)[38]

3 The process of biorefinement

There have been a number of different initiatives that successfully developed processes for refinement of grass or other green leaf material. At this moment, many of these processes are not yet financially viable. Nevertheless, there are currently running initiatives such as Grassa!, HarvestaGG and TNO that claim to have developed a viable process, with a business case that should not require any structural governmental subsidy (personal communication, B. Koopmans, S. Snaas and M. Burgering). From the differences between the biorefinement processes of those initiatives, it becomes apparent how much variation is possible in designing a process for the refinement of grass. Depending on what is desired, techniques of refinement can produce a wide range of products, ranging from feed-grade protein to pure food-grade protein as well as biofuels, fertilizer, sugar, dry fibres and compounds for the chemical industry such as amino acids and lactic acid[41,42]. It is important to consider both the costs of the process itself and the products that will be produced in this process. Because the costs of refining differ strongly from one set of products to another and maximum profit margins are reported to be relatively low (personal communication, J. Sanders), figuring out how to optimize the process is extremely important. In order to determine the viability of grass refinement, it is important to consider multiple options within the field of possibilities. To this end, concrete scenarios can be designed to explore the costs and benefits of grass refinement in more detail.

3.1 Scenario's

Based on literature research and consultation with experts, three scenarios were developed to illustrate the range of possibilities for refinement of grass (Figure 2). The design of these scenarios started with the observation that refinement of grass can be roughly classified based on two variables. The first variable is the *degree of technological complexity* of the refinement process. This means that a process can either depend on relatively low-tech simple methods to keep process costs as low as possible, or depend on high-tech intricate methods to maximize the value of the products that are produced by the process. In this report, low-tech methods produce non-functional or denatured protein, while the high-tech process produces a functional protein concentrate. A functional protein has certain properties, which for example allow it to form gels, foams, or to be soluble in water. Similar to what happens when boiling an egg, Rubisco and other proteins that are present in grass will change structure and lose these functional properties when heated above a certain temperature for a certain time period. In general, functional protein has a higher value than non-functional protein (see section 4.1)

The second variable is the *scale* of the process. Implications of this variable are that on a smaller scale, it is preferred to have a process that is mobile so transport costs of the grass can be limited. On a larger scale, the benefit of centralized and more efficient processing might outweigh the higher transportation costs. These two variables are not completely independent; a small and mobile refinery is not realistically capable of using the same high-tech methods as a large centralized refinery, so refining grass locally into high-grade functional protein is most probably not a viable option. Furthermore, the production of food protein on small scale is probably not interesting for FrieslandCampina as large quantities of protein are needed, which cannot be achieved with small, mobile refineries. Refinement of feed-grade denatured protein on a large scale in a centralized facility was also deemed not interesting as this scenario would overlap too much with a centralized facility for non-functional food protein. This is because it is likely that a food-grade protein product will be more profitable on this scale, while the difference between the processes for both products is quite similar. The three scenarios that were developed during this project encompass the three remaining combinations of the two variables:

Scenario 1 - Small scale, mobile plant that produces feed-grade denatured protein
Scenario 2 - Large scale, central plant that produces food-grade denatured protein
Scenario 3 - Large scale, central plant that produces food-grade functional protein

Scenarios 1 and 3 are strongly based on the processes used by Grassa! and TNO respectively. It is also important to note that in the design of each of these scenarios, the protein fraction is the most important product. Before going into detail about these scenarios, an overview of the possible techniques has been comprised in order to clarify the different options available for refinement of grass. A general biorefinement process outline is displayed in figure 3.

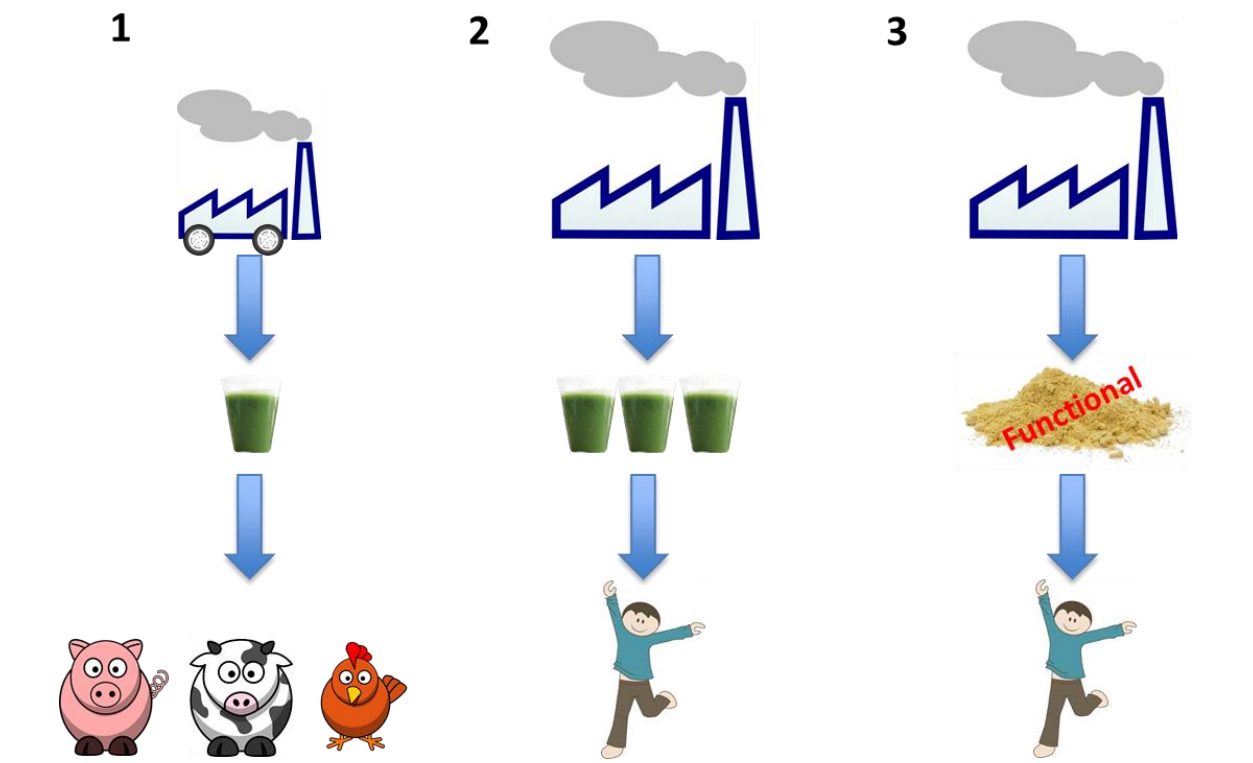

Figure 2 | The three different scenarios that are used in this report. 1) Scenario 1 in which a small mobile refinery produces a fluid protein product for animal consumption. **2)** In scenario 2, a central refinery extracts protein juice from grass for human consumption. **3)** Scenario 3 differs from scenario 2 in its process so that the protein product retains its functional properties. Besides this, the product has a higher protein concentration and is available as powder.

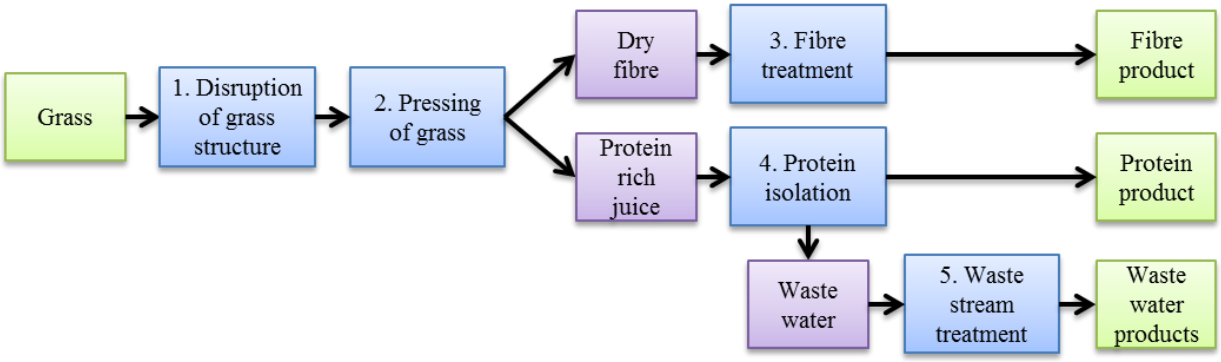

Figure 3 | General overview of the biorefinement process with protein as the main product. Boxes in green indicate products, blue certain processes and purple intermediates.

3.2 Pre-processing

Though there are multiple options for the process of grass refinement, there are also some common requirements. The most important of those requirements is that the grass must be separated into a liquid grass juice fraction and a solid fibre fraction by pressing of the grass. In order to efficiently separate the two fractions, the cellular structure of the grass has to be disrupted prior to pressing. These methods are termed pre-processing steps. Some steps are optional while other steps are essential. Different steps for pre-processing are as follows:

- **Refining**[43] This technique is used to disrupt the cellular structure of the grass in order to ease the pressing of grass into dry and liquid fractions. The grass is bruised between two rotating disks, and the machine that is used for this process is called a *refiner*. Refiners have their origin in the paper-making industry, for the effect that they have on cellulose fibres.

  1. Disruption of grass structure

- **Extrusion**[44] This pre-processing step has a similar purpose as refining. The difference is that instead of just bruising the grass, the shear forces exerted onto the grass result in a more extensive disruption of the grass structure. This allows for a more complete separation of dry fibre and grass juice. The downside is that this process costs more energy than refining. It is expected that the higher energy costs of extrusion do not outweigh the benefits of the more complete cellular disruption in comparison to refining. Therefore, it is less suitable for low-tech options. The machine used for extrusion is called an *extruder*, which uses a screw in order to pressure the grass.

  1. Disruption of grass structure

- **Enzymatic degradation**[45,46] An optional experimental pre-processing step before pressing is to follow refining or extrusion by treatment with the enzyme *cellulase*, to break down cellulose into sugars. Not only will this ease pressing of the grass, but there will also be more sugars available in the grass juice. These sugars can be isolated later on with nanofiltration or fermented in a digester for biofuel production.

  1. Disruption of grass structure

- **Pressing**[47] This step separates the fibre fraction from the grass juice. As mentioned before, regardless of which refinement methods are used, this step is essential. There might be some variation in the type of press that is used, given that the wrong type of press will not separate the grass properly or wear out too fast. The type of press that is used in grass refinement is called a *screw-press*.

  2. Pressing of grass

In general there are two approaches that can be considered for the process. One is refining before pressing (which is more suitable for low-tech processes), and another is extrusion before pressing (which is more suitable for high-tech processes). Enzymatic degradation can theoretically be included in both approaches, although either fermentation or nanofiltration is required later on in the process in order for this method to be useful. With pre-processing as the foundation of the refinement process, the next step is to process the two fractions that are obtained by pressing.

3.3 Processing of fibre fraction

3. Fibre treatment

As explained earlier, the products that can be obtained from the grass juice still need some degree of purification and/or post-processing in order to make it suitable for further use. The same is true for the fibre fraction. However, for the fibre fraction it is less evident how it should be processed after pressing, as opposed to the products from the grass juice. Because the potential for use of the fibre fraction is so dependent on the exact properties of the obtained fibre, further processing details of the fibre fraction are not being discussed here. Besides this, the process

of the fibre fraction is often not publically available, in contrast to the processing of the liquid fraction. Different applications for the fibre fraction are discussed in section 4.2 of this report.

3.4 Processing of liquid fraction

The pre-processing steps strongly influence later steps in the process. However, the highest variation between different refinement processes is found in the steps after pressing as these steps determine which products are produced. Even more then for pre-processing, it is difficult to find the right combination of these options as not every combination is technically or financially viable. Besides this, some techniques require or exclude each other. Though the following list of possible techniques is non-conclusive, it does provide an overview of the most important options for valorisation of grass juice:

- **Heating (> 50° C)**[48] If proteins are heated to a certain temperature for a particular period of time, their structure is irreversibly changed in a process called denaturation. This change in structure will strongly decrease the solubility of the proteins in water, causing them to aggregate in the grass juice. Aggregation of protein due to denaturation is called coagulation. Due to this coagulation, the protein can be separated from the liquid grass juice. This type of heating is conducted with heat-exchangers which use left-over heat from earlier processing steps to optimize efficiency. Note that beside coagulation of protein, other contents of the grass juice will also coagulate, leading to impurities in the protein product.

  4. Protein isolation

- **Heating (< 50° C)** If the purpose is to preserve the functional properties of the grass juice protein that is dissolved in the grass juice, the temperature of the protein fraction may not exceed a certain temperature for a certain amount of time. Above this temperature the protein will denature, thus losing its structure and functional properties. One study reported that 50% of all Rubisco protein is denatured when the protein fraction is heated for 1 hour at 55.2° C[49]. Heating of the grass juice to a temperature just below 50° C for 20 minutes and cooling it down afterwards will keep the proteins intact. In contrast, small solids in the grass juice such as chlorophyll, cell-membranes and cellulose will coagulate[50]. This makes it easier to remove these solids by centrifugation later on in the process.

  4. Protein isolation

- **Centrifugation**[51,52] As the grass juice still contains some solid material after pressing, an additional separation step is required. Most processes, whether they are high- or low-tech, use centrifugation to accomplish this. However, the purpose of centrifugation varies from process to process. The machine used for continuous centrifugation is called a *decanter centrifuge*, and depending on whether functional protein is desired or not, a decanter can serve two purposes. For non-functional coagulated protein, a decanter is used to separate the protein product from the grass juice, thereby obtaining a protein product. For functional protein, a decanter is used to get rid of solid impurities in the protein-rich grass juice. Another type of centrifuge is called *plate centrifuge*, which is mainly used as a filter for any further solid impurities. The main difference between these two types of centrifuges is that a decanter is more useful for liquids that have a relatively high amount of suspended solids, while a plate centrifuge gives a more complete separation into a solid and liquid fraction. A plate centrifuge is less suited for liquids with high amounts of suspended solids because it has to be cleared of solids after a certain amount of time. Thus, the higher the amount of suspended solids in the liquid, the more inefficient a plate centrifuge is. For this reason, it is usually used in conjunction with a decanter when refining functional protein. In such a setting, the decanter isolates the majority of solids, after which the plate centrifuge further purifies the grass juice.

  4. Protein isolation

- **Membrane filtration**[53,54] This process encompasses techniques in which filtration happens with semi-permeable membranes. Depending on the size of the pores in the membrane, this is termed *ultrafiltration (10-100 nm)*, *nanofiltration (1-10 nm)* or *reverse osmosis (< 1 nm)*. With ultrafiltration, the pores are impermeable to proteins, while smaller molecules can pass. Ultrafiltration is therefore used to isolate protein from a solution, for instance in the dairy industry to concentrate whey-proteins. The result is a concentrated protein fraction.

  4. Protein isolation

  Nanofiltration and reverse osmosis operate on the same principle, but the pore-sizes are smaller. With nanofiltration it is possible to isolate small molecules such as sugars, while with reverse osmosis even ions such as potassium can be concentrated to a certain degree. An important prerequisite for membrane filtration is that the filtrated liquid is clear of solids as any solids will foul the membrane. Therefore, membrane filtration is usually preceded by centrifugation to ensure the absence of solids. The fraction that passes the membrane during membrane filtration is called the 'permeate' and the fraction that cannot pass the membrane is called the 'retentate'.

- **Column chromatography**[55] This technique separates substances on basis of solubility of the liquid phase and affinity for the solid phase. Some larger contaminants such as chlorophyll are difficult to isolate in earlier steps. Column chromatography, using *resins*, can be used to remove these contaminants through adsorption. These resins have a hydrophobic layer to which the chlorophyll can bind. Upon saturation of the resins, they can be re-used by rinsing them with a non-polar solvent, such as ethanol, to remove the chlorophyll.

  4. Protein isolation

- **Spray drying**[56] This is a very fast method to dry products that still contain water. By using hot gas, the product will turn into a dry powder. The downside to this process is that it very energy consuming and is thus relatively expensive.

  4. Protein isolation

- **Phosphate precipitation**[57–61] After extraction of protein from the grass juice, the left-over grass juice can be discarded as waste. However, it might be required to extract certain elements such as phosphate from the waste stream to meet certain environmental criteria in the Netherlands. According to article 3.5e of 'activiteitenbesluit milieubeheer', the allowed concentration of phosphate in urban waste water is 1-2 mg/l, depending on the size of the waste stream. By adding magnesium to a phosphate holding solution in a struvite reactor, the phosphate will precipitate in the form of $MgNH_4PO_4$, also called struvite. During the process sodium hydroxide has to be added to maintain a stable pH of >8[60]. The struvite that is collected can be used as fertiliser.

  5. Waste stream treatment

- **Fermentation**[62,63] One of the most commonly used techniques for the valorisation of biomass is fermentation into biofuels (with aerobic fermentation), or lactic acid (with anaerobic fermentation). Fermentation is a process during which yeasts or bacteria metabolize sugars into gases, acids, or alcohol. Although fermentation is an interesting method of valorising the fibre fraction and the grass juice after protein extraction, no scenarios were developed that include fermentation as this technique was considered to be out of scope. However, fermentation is an option that should be seriously considered for future research as a way of valorising the dry fibre fraction and the sugar rich grass juice that remains after protein extraction. A combination of cellulase treatment during pre-processing and fermentation could also be interesting, as this ensures a higher sugar content that can be used for fermentation.

  5. Waste stream treatment

As mentioned before in this section, not each technique is necessary and not every combination of these techniques is viable. This is important to consider in the design of any refinement process, as some machines will break if the processed material does not meet certain requirements. Therefore, design of the scenarios was based on successions of techniques as observed in literature and in other refinement initiatives.

3.5 Scenario 1 - Small scale, feed-grade protein

In this scenario grass is processed in a mobile low-tech refinery (Figure 4). The general idea here is that fresh grass from a radius around the installation can be processed before moving to another location. This keeps transportation costs as low as possible. Furthermore, only fresh grass is used. Due to this, the production plant is only operational for approximately 5 months each year. The process itself is as low-cost as possible, with the emphasis on fast coagulation and separation of protein from the grass juice.

For scenario 1, refining was selected as the pre-processing technique before pressing. As mentioned in section 3.2, extrusion is probably too expensive for a simple and low-tech process as in this scenario. After the grass is bruised by refining it is pressed. Although it is not included in this scenario, it might be beneficial to add water to the dry fibre fraction after pressing in order to press a second time. This could obtain leftover protein or nutrients that were not extracted after the first pressing.
The protein that is present in the grass juice is coagulated by heating the grass juice above 50° C with a heat exchanger. Following heating of the grass juice, the coagulated protein is separated from the grass juice by centrifugation. For this, a decanter centrifuge is used. Centrifugation will yield a product of coagulated protein and other solids that were present in the grass juice such as cellulose, cell membranes and chlorophyll. The leftover waste water still contains dissolved nutrients and substances that are valuable when isolated. However, a mobile plant is restricted in size, and valorisation of waste water is likely to be unprofitable when executed on a smaller scale. Therefore, in this scenario the remaining grass juice will be spread out over the land as a source of nutrients for new grass.

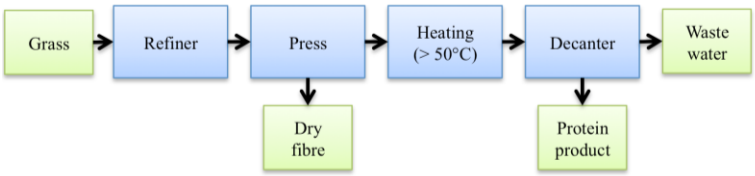

Figure 4 | Scenario 1 consists of a small, mobile plant which produces feed-grade protein.

This type of plant can process 8,800 tonnes of fresh grass/year, based on 5 operating months per year. The expected protein yield is 45% of crude protein content, based on previous reports[10]. The protein product consists of 11% protein, 4% other dry material and 85% H_2O (Table 3). For the fibre fraction, 58% is H_2O 34% is fibre and 8% is other dry material.

3.6 Scenario 2 - Large scale, food-grade protein

In this scenario, grass is transported to a central plant for processing into food-grade denatured protein. This process is similar to the process in scenario 1, with the main differences being a larger scale and more processing steps (Figure 5). In order to obtain a protein product of higher quality, the grass juice is first heated to a temperature just under protein denaturation temperature to coagulate any unwanted solids in the grass juice. These are subsequently removed by centrifugation with a decanter. Then, in a second heating step, protein is coagulated from the grass juice by heating to a higher temperature, similar to the heating step in scenario 1. A second centrifugation step will then separate the protein from the grass juice, yielding a product of higher purity than in scenario 1 (Table 3).

Due to the relative absence of restrictions in size and scale of the process, the remaining grass juice can be valorised after protein extraction in this scenario. However, techniques to isolate individual amino acids are not yet firmly established. Furthermore, another possible product could be sugars from the waste water. However, due to the low concentration of sugars it is uncertain whether this additional step would be financially viable. Lastly, phosphate can be isolated from the grass juice to comply with environmental regulations. This technique has been incorporated in the scenario outline as phosphate extraction is likely a requirement if the waste water is to be discharged.

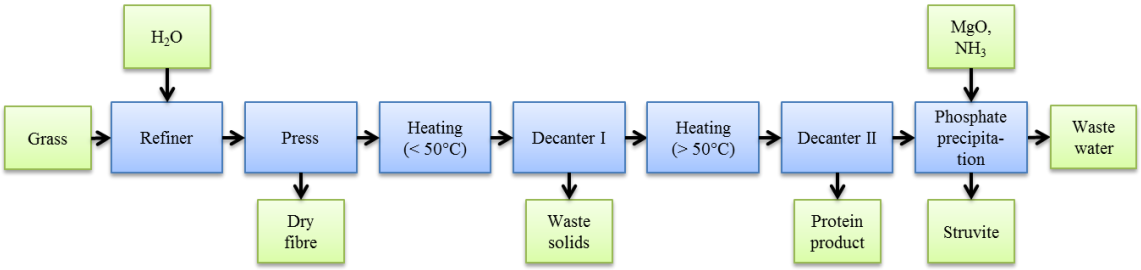

Figure 5 | Scenario 2 consists of a large, central plant that produces non-functional food-grade protein.

This type of plant can process 259,210 tonnes of ensilaged grass/year. The expected protein yield is 45% of crude protein content, based on previous reports[10]. The protein product consists of 20% protein, 1% other dry material and 79% H_2O (Table 3). For the fibre fraction, 58% is H_2O, 34% is fibre and 8% is other dry material.

3.7 Scenario 3 - Large scale, functional food-grade protein

In this scenario, emphasis lies on the highest possible valorisation of protein. Therefore, unlike in the two previous scenarios, an extruder is used to achieve a better disruption of the cellular structure of the fresh grass (Figure 6). After pressing, any present solids are coagulated by heating to a temperature just under protein denaturation temperature, and removed by centrifugation with a decanter similar to scenario 2. To ensure the complete removal of any solids still present in the grass juice, a second centrifugation step with a plate centrifuge is used. After centrifugation, ultrafiltration of the grass juice yields a *retentate* which contains a high concentration of protein and a *permeate*, consisting of water and smaller dissolved substances. From this permeate, phosphate will be extracted by precipitating it as struvite. From the retentate, the protein concentrate is purified by column chromatography from large hydrophobic impurities such as chlorophyll. Finally, the protein concentrate is spray dried, yielding a pure and functional protein powder. Although this scenario yields the highest quality protein product, it is also the most costly to execute. Therefore it might be worth considering to further valorise the ultrafiltration permeate as this still contains valuable substances like amino acids and sugars.

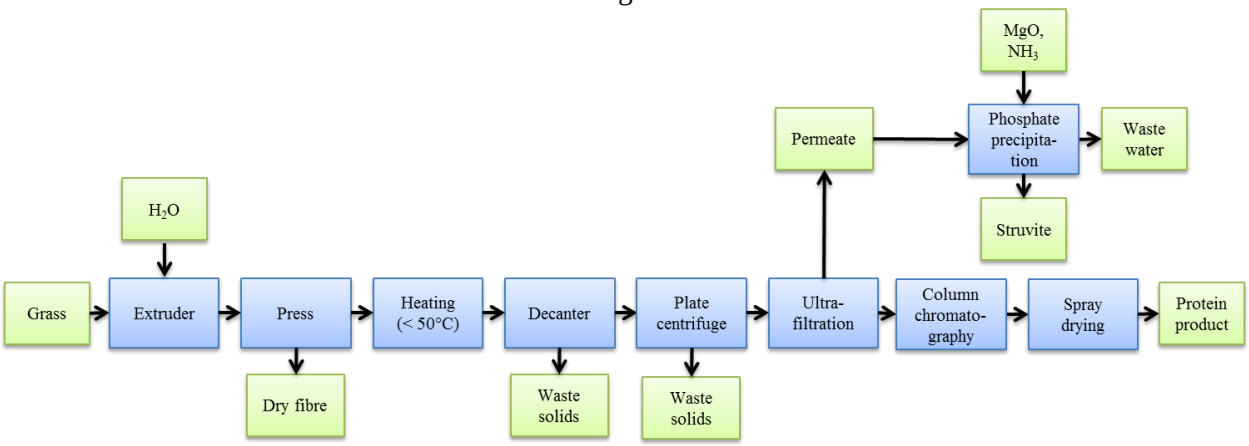

Figure 6 | Scenario 3 consists of a large, central plant that produces functional food-grade protein.

This type of plant can process 259,210 tonnes of ensilaged grass/year. The expected protein yield is 59% of crude protein content, based on previous reports[10]. The protein product consists of 90% protein, 9% H_2O and 1% other dry material (Table 3). For the fibre fraction, 58% is H_2O, 34% is fibre and 8% is other dry material.

Table 3: Composition of protein and fibre fraction for each scenario.

	Protein fraction			Fibre fraction		
	% H2O	% DM	% Protein*	% H2O	% DM	% Fibre*
Scenario 1	85	15	11	58	42	34
Scenario 2	79	21	20	58	42	34
Scenario 3	9	91	90	58	42	34

* percentage of total fraction

The techniques in this chapter are described in a way that fits the vision of refinement gained during this project. This is not necessarily the only way of looking at the possible technical processes for refinement of grass. Besides this, personal communication with a number of experts was essential in developing an outlook of the possibilities. Aside from detailed knowledge about their respective fields, these conversations revealed fundamental disagreements between experts in the same field concerning viability of each other's proposed processes. Finally, although literature research and consultation of experts did result in a broad overview of possibilities, we do not assume to have covered all possible biorefinement techniques. For an extensive list of experts that were consulted, see appendix I. Furthermore, for individuals that are interested in biorefinement a list with recommended reading was composed (see appendix III).

4 Application of (side-)products

Most business cases require that besides their main product, the side-products are as well valorised. Previous biorefinement initiatives have shown that this is also the case for grass refinement (personal communication, M. Burgering). In this report, the protein fraction was stated as the main product with the fibre fraction and the waste stream as side-products. It is highly unlikely that grass refinement is profitable without finding a suitable application for the fibre fraction and waste stream. This chapter focusses on different applications for the (side-)products and their expected benefit.

4.1 Protein product

The use of grass as a protein source for human consumption has its advantages and disadvantages when compared to traditional animal protein sources. In general, animal-based protein has a higher nutritional value, better digestibility[64] and an optimized refinement process, which is illustrated by the dairy sector. On the other hand, plant protein has a lower allergenicity and is more efficiently produced, which increases land-usage efficiency and makes it a more sustainable protein source[65].

The suitability of the extracted protein for human consumption is mostly dependent on the *quality* of the protein product, its *nutritional value* and its *functional properties*. The more functional properties (e.g. gelling, foaming, solubility), the better the protein can be processed into different products. As discussed in chapter 3, whether or not these functional properties are present in a grass protein product strongly depends on the refinement process[48]. The nutritional value of grass protein is determined by its digestibility and amino acid composition. For food purposes, the protein should be easy to digest and contain amino acids concentrations without high deviations. Especially the concentration of essential amino acids is important for the protein's nutritional value as essential amino acids cannot be synthesised by the human body and need to come from external sources.

Table 4 Amount of essential amino acids in different products and their recommended intake (in mg/g protein).

Essential amino acids	FAO/WHO infant (<0,5yr)[a]	FAO/WHO adult (>18 yr)[a]	Rubisco[b]	WPC 80%[c]	Soybean[d]
Isoleucine	32	30	49	50	54
Leucine	66	59	94	107	91
Lysine	57	45	65	88	74
Cysteine + methionine	27	22	34	80	33
Phenylalanine +tyrosine	52	38	128	58	100
Threonine	31	23	53	69	48
Tryptophan	8,5	6,0	27	17	16
Valine	43	39	67	18	56

a) From Dietary protein quality evaluation in human nutrition; report of an FAO Expert Consultation (2013)[66]; b) van de Velde *et al.* (2005)[67]; c) Sindayikengera & Xia (2006)[68]; d) United States Department of Agriculture and authors calculations.

The protein Rubisco comprises up to 50% of the soluble protein[21], or 20-30% of the total amount of nitrogen[69] in grass. The other 50% consists of all other soluble proteins, which are present in much smaller numbers. Because Rubisco is the only protein that is present in such a high concentration, it is the most influential factor in determining the nutritional value of potential grass protein products. Rubisco functions as an important enzyme for photosynthesis and is a key-player in the carbon dioxide fixation. Furthermore, it has a high nutritional value with an AAS (*Amino Acid Score)* of 87%[70], which is a measure of the concentration of essential amino acids (Table 4). As comparison, wheat protein only scores 51% on this scale[70]. Furthermore, Rubisco from spinach was easily digested in a simulated gastric digestion experiment[71]. Besides the high nutritional value, Rubisco also has several useful functional properties. Functional Rubisco powder is highly soluble, has excellent foaming capacity and stability, and is able to form into a gel[65,72,73]. Due to these characteristics, functional Rubisco is suited to be used in the food industry.

A non-functional protein isolate of grass protein could possibly be used in existing food products, although its application will be highly restricted when compared to a functional protein isolate (personal communication, M. Burgering). This is mainly due to restrictions such as reduced solubility. Finally, an additional factor to consider is the allergenicity of proteins. In general, leaf protein is quickly degraded in the human body after consumption, which prevents the development of allergies[74].

To summarise, a grass protein extract appears to be of sufficient nutritional value for use as a source of both feed and food and is applicable with or without any functional properties. However, as the general quality of the product has a substantial influence on its possible applications in practice, it is important to consider what impurities are expected in the protein products and how these impurities affect the product. Depending on which process is chosen, the end product could be a feed-grade protein product (scenario 1), a food-grade protein product (scenario 2) or a functional food-grade protein powder (scenario 3) (Table 5).

- **Feed-grade protein product (scenario 1)** The low-tech extraction method of coagulation and centrifugation of the protein directly after pressing results in a relatively high amount of solid impurities in the product. These impurities consist of small non-toxic particles such as cellulose, lignine and chlorophyll, which give the product a green colour and a slightly bitter taste. Therefore, this protein product is mostly suitable for animal feed. Due to this, the price estimate was based on Brazilian soybean meal. Feed concentrate for ruminants and non-ruminants is very precisely put together and tuned to specific needs of the animals (personal communication, A. Hamminga). This protein product might be used in the production of this concentrate, thereby reducing the need for other protein sources such as imported soybean meal.

Table 5 The different protein fractions from the three scenarios

Application	Value (€/ton)	Note	Reference
Feed-grade protein	375	Based on Brazilian soybean meal 48%*. Prices from 2010-2013.	LEI
Food-grade protein Non-functional	375	Based on Brazilian soybean meal 48%*. Prices from 2010-2013.	LEI
Food-grade protein Functional	4500-6000	Authors estimate based on personal communication and chickpea concentrate.	USDA AMS Dairy Market News Personal communication M. Burgering and B. Mersbergen

***** Protein concentration in product

- **Food-grade protein product (scenario 2)** The protein product that is obtained by the refinement process in this scenario is similar to the protein product in scenario 1 and is therefore also comparable in price. The relevant differences are the additional heating (to less than 50°C) and the centrifugation steps prior to coagulation of the protein. These steps should theoretically remove most of the solid contaminants, although this still needs to be tested in practice. A higher purity would make the product more suitable for further processing, such as drying. However, the absence of functional properties of this product limits the amount of possible applications in the food industry. Furthermore, application of the protein product is further complicated by the high water content of the protein product (79% water). Spray drying of the product could improve handling of this product, although it remains unsure whether the increased energy costs are worth this benefit. Applications of spray dried, non-functional protein powder are as protein enhancer, for example in protein shakes.

- **Functional feed-grade protein product (scenario 3)** The content of the pure functional protein powder will mainly be Rubisco. As described above, this protein is highly nutritional and suitable for human consumption. Therefore, the protein is comparable to whey protein concentrate. Because of the high purity of the product, as well as its functional properties, it could be used as an ingredient in many different products in the food industry such as desserts, beverages, ice creams, etc[65]. NIZO has also developed a technique to form fibrillar structures from Rubisco, potentially making the protein suitable as a meat substitute[65]. Beside use in the food industry, this product could also be used in the cosmetics industry where the functional properties of this protein product are of interest[75].

4.2 Fibre fraction

The valorisation of grass fibres depends greatly on the properties of the grass that is used. For example, grass fed to cows is young and lush, in contrast to grass in nature areas, which is older and more fibre rich[76]. This influences the possible application of the grass fibre fraction. In table 6, an overview of the known possible applications is provided.

- **Feed** According to a patent application, 33-50% of the crude protein, remains in the fibre fraction after pressing[9]. Another patent reports that the crude protein fraction is evenly split between the fibre and fluid fraction[77]. A high protein level in the fibre fraction implicates low protein content in the fluid fraction, meaning that less pure protein is recovered for feed or food usage. However, a fibre fraction with high protein content is more suited as roughage due to its higher energy level.

- **Cardboard/paper** Fibres for paper or cardboard need to be cleansed of protein before usage. Otherwise, the remaining protein can cause excessive foaming during the production process as well as odour problems within the product (personal communication, M. Adriaanse). Due to this, the use of grass fibres for cardboard/paper production might be more expensive than traditional fibres. Besides this, when culture grass is harvested, it is usually too young and lush to be used for the paper industry. This is due to the low fibre content and structure (personal communication, M. Adriaanse). Older grass might be used for refinement, but this will lower protein yield as old grass contains less protein[76]. Another option is to use grass from nature areas which is only harvested once or twice each year and thus contains more and longer fibres. To conclude, the grass that might be used by the farmers of FrieslandCampina is not suited for this application as the fibre fraction does not meet the requirements.

Table 6 Different applications of the fibre fraction.

Application	Value (€/ton)	Note	Reference
Feed (cows)	184	Value depends on nutritional value.	Authors calculations
Cardboard/ paper	100-130	Requires fibre-rich, old grass. Price based on recycled paper with a DM of 90-92%.	Personal communication M. Adriaanse (KCPK) and Eurostat
Insulation material	800	Material is more flammable than other insulation materials[78]. Requirements of grass are unknown.	S. Grass (2004) [79]
Fermentation	75	Not sufficiently supported by reference, additional information needed. Furthermore, reference is several years old and an estimate.	Based on presentation of Courage
Fibre boards	n/a	In several papers, the application of grass fibre for particle board production has been noted[80,81], but no actual applications have been found yet. However, US patent 6,596,209 B2 describes the use of agricultural waste for board production[82].	Kromus *et al*. (2004)[80] McEniry *et al.* (2011)[81]

- **Insulation** In Switzerland, grass-based insulation boards have already been successfully produced by Gramitherm®[83]. Grass insulation is more sustainable compared to other insulators such as polystyrene and also has a high added-value compared to other fibre applications. However, grass insulation boards are also relatively flammable. According to the "European classes of reaction to fire performance for construction products", grass-insulation falls into category E[78], which is the lowest category that is still approved[84] (category F equals uncertified products). As comparison, normal wood products are class D and stone products class A1[78]. Another difficulty is that Gramitherm® is the only company that produces grass insulation boards. Due to this, technical details concerning the process are not known.

- **Fermentation** Another application for grass fibre is fermentation in a digester. In a fermentation process, sugars are converted into other products such as lactic acid or bioethanol that can be used either as fuel or as a resource in the chemical industry. In the light of the food versus fuel discussion, the use of high-quality crops for fuel production is becoming more controversial. Therefore, it would be better to use low-quality crops for fermentation such as roadside grass or grass from nature areas. Currently, HarvestaGG[85], Indugras[86] and NewFoss[87] are investigating grass fermentation in the Netherlands. HarvestaGG plans to have their first production plant operational end 2014 – begin 2015[85].

4.3 Waste stream

When the protein is extracted from the fluid fraction, a solution containing amino acids, minerals, sugars and other small compounds remains. This fraction is difficult to valorise due to many different compounds and the low concentration in which they are present. However, there are several options that might be feasible in the near future.

- **Animal feed** The fluid fraction contains several essential amino acids and minerals. If cows are fed with the fibre fraction, it is possible that their intake of certain minerals is insufficient. It might therefore be interesting to feed cows with the waste fraction containing both energy (sugars) and minerals. However, minerals and sugars in the waste stream are considerably diluted, meaning that

a cow has to drink a lot of this fluid fraction. Besides this, it is not known if this product would be tasty enough. This could make this application for the waste stream difficult.

- **Struvite** If the waste stream is to be dumped in the sewer, the phosphate concentration has to be lowered according to environmental regulations (Activiteitenbesluit milieubeheer, article 3.5e). This can be done by adding magnesium which causes phosphate to precipitate in the form of struvite ($MgNH_4PO_4 \cdot 6H_2O$)[57–60]. Struvite can be used as fertiliser[61], which is especially useful in regions in which manure is limited and fertiliser prices are high. There have been several studies that investigated the financial aspect of struvite extraction with both positive[60] and negative[58] results. In all cases, most of the expenses were due to the addition of magnesium. The process and the technique required are relatively simple and cheap. In the Netherlands, struvite extraction is probably not economically viable due to an excess of phosphate from manure which diminishes struvite selling prices.

- **Amino acids** In theory, amino acids could be extracted from the fluid waste stream. However, the technique to do this is still being developed[88,89] and could be adapted into the refinement process in a later stage. Purified single amino acids have a high value that is comparable or higher than protein concentrates, although this differs for each amino acid.

- **Sugar concentrate** In theory, the sugars present in the fluid fraction can be isolated. This technique is used by TNO. However, the authors of this report have serious doubts about the cost-efficiency of this technique. It might be more beneficial to use the sugar-rich waste stream for fermentation as is described below.

- **Fermentation** Due to the sugars present, the fluid waste can also be used in a fermentation process. The feasibility of this depends on the concentration of sugars present in the waste stream. If there is not sufficient sugar present the fermentation process will not work properly. For more information on fermentation see sections 3.4 and 4.2.

- **Fertiliser** Grassa! plans to return the fluid waste fraction to the grassland. This fertilises the land and is a relatively low-cost solution as the waste fraction does not need to be processed (personal communication, B. Koopmans).

5 Cost-benefit analysis

For this chapter, an extensive Excel file was used to perform the cost-benefit analysis. This file can be requested from the authors. See appendix II for a detailed explanation of the Excel file.

A cost-benefit analysis (CBA) is a financial evaluation method in which the expected costs a business or project is going to incur are weighed against the expected benefits. Typically, multiple scenarios or subjects are considered so that the most beneficial solution can be found. The aim of this project is to identify whether the refinement of grass could be a profitable and interesting business for FrieslandCampina to engage in. For this, a CBA was used as an assessment tool for the financial viability of the bio-refinement of grass. In the analysis, quantifiable financial advantages and disadvantages of the different scenarios are compared.

Costs and benefits are not necessarily of a financial nature and therefore cannot always be expressed in currency. Many social and ecological costs and benefits such as reputation or sustainability cannot be easily quantified. These aspects will be taken into account for the final recommendation, but this chapter will only focus on the financial side. First, the financial model is discussed after which the results are presented. The chapter concludes with an economical background of the biorefinement sector.

5.1 Financial model

For each of the scenarios there are different costs and benefits. The costs can be categorized as investment costs, fixed costs or variable costs. The benefits are generated by the sale of the obtained end products. In the section below the different costs and benefits relevant for this project are discussed. Furthermore, the consideration and assumptions regarding the quantities and prices are addressed. After the costs and benefits, some methods to assess the financial viability are explained. To conclude, a short note on financial statements is given. All above mentioned sections are based on data from different sources and therefore the approach for the data collection is discussed first.

5.1.1 Data collection

To perform a CBA, quantities, energy usage, prices, and other numbers are required. Since this project is relatively novel and unique, not many of these numbers are discussed in the literature and therefore had to be estimated. The processes for the scenarios are put together by consulting many different experts and comparing grey literature such as websites and presentations. Often, for a specific variable an average number was based on different sources. However, some variables were especially difficult to estimate. As no experiments were done for the different processes, the quantities and composition of the products produced are rough estimates. This complicates the price estimation of the products. This was partly mediated by talking to experts in different fields. Furthermore, for prices and the use of machinery and equipment, manufacturers, process engineers and preceding projects were consulted (see appendix I). So even though a lot of data has been collected from many different sources, varying in reliability, it should be taken into account that many numbers are averages or educated guesses.

5.1.2 Investment costs

Investment costs include all costs that have to be made at the beginning (year 0) to put the project into operation. These are one-off costs that serve as a long term investment in the project. In this project the investment costs are all the expenses that have to be made to start be able to start the processing plant.

- **Machinery** For each of the scenarios thorough investigations have been done in order to compose the processes. For every process the prices of the machines are estimated, either by contacting machine manufacturers, process engineers or consulting literature. The machines of scenario 1 have

a lower capacity than the machines in the other two scenarios, because the machines in scenario 2 and 3 have to process significantly more grass per hour. Due to this differences in capacity, the machines in the second and third scenario are bigger and more expensive. The process of the third scenario however consists of high-tech machinery, in contrast to the processes of scenarios 1 and 2. As prices for high-tech machinery are considerably higher than prices for low-tech machinery, the machinery costs of the third scenario are by far the highest.

- **Material, equipment and installation** The machines need to be connected to each other by pipelines and other materials. In addition, pumps and conveyors are required to move and transport the processed material. All products have to be collected and saved in containers and reservoirs. For the installation costs and additional materials 1.5 times the machinery costs are taken (personal communication, A. van der Padt). In addition, in the first scenario 3 second hand sea containers (€ 1250 apiece) are needed to store and transport the mobile machinery from one location to another.

- **Location** The mobile refineries will move from one grassland to another and not require a fixed location. Therefore no extra costs are applicable for the location in this scenario. In the case of the central refinery it is assumed that FrieslandCampina is in possession of a possible location (personal communication, Y. de Vries). The expectation is that the location will always need some reconstruction to make it suitable for the bio-refinement of grass and therefore €100,000 is budgeted.

5.1.3 Fixed costs

The fixed costs are expenses that do not depend on the amount of product that is produced. They are fixed in this sense, but do not have to remain constant forever; they can vary over the years, but are fixed with regard to the quantity produced for the relevant period. In this report, the fixed costs are considered per year.

- **Depreciation** As machines yearly decrease in value and ultimately have to be replaced, a fixed amount of money is saved each year, which is named depreciation. This cost does not contribute to actual cash outflow, but is taken into account as a cost. At FrieslandCampina the general depreciation of machinery is assumed to be seven years (personal communication, A. van der Padt). To calculate the depreciation the straight-line method is used[90]. With this method the depreciation is calculated by dividing the machine's purchase cost by the machine's estimated lifetime. Thus, for each year 14% of the purchase price of the machine is budgeted as fixed cost.

- **Maintenance, research, engineering and development** All machines require some form of maintenance. Experience taught that especially processes with high-tech machinery require a significant amount of maintenance. Parts of machines will have to be replaced or renewed in time. To cover the annual maintenance costs, an amount of 10% of the investment costs is reserved[90]. Additionally, in the first year research, engineering and development costs are expected as the process will have to be optimized. Similar to the maintenance, these costs were also assumed to be 10% of the total investment costs[90].

- **Labour** Another fixed cost is labour. Employees have to be paid, regardless of how much product is processed. Each year an estimate is made on the required number of employees for the year. An average wage of € 54,700 per year for one full-time employee (FTE) is taken[11]. The mobile refinery is assumed to be operational 5 months per year, the period that fresh grass is available. During this time the refinery is expected to run approximately 80 hours per week. This equals an operational time of 1600 hours per year. Provided that 1 FTE equals roughly 1650 hours per year, 1 FTE is

needed to keep the machines running and 1 additional FTE is required for administrative, sales and maintenance tasks. Thus, 2 FTEs are required for a mobile refinery. A central refinery will be processing all year long, excluding 6 weeks for extensive maintenance and repair[91]. Assuming the machinery run 161 hours per week, this comes down to about 7406 hours per year. Thus, 5 FTEs are required to keep the machinery running, 1 FTE is counted for maintenance, 1 FTE for the transport of grass and 1 FTE for sales and administration. In total, 8 FTEs are needed for a central refinery.

5.1.4 Variable costs

The variable costs are dependent on the level of production. Thus, all costs that depend on the amount of grass that is processed are gathered in this category. This includes energy, water and the purchase costs of raw materials.

- **Grass** In chapter 2 the composition and prices of fresh and silage grass are discussed. The mobile refinery only uses freshly harvested grass as raw material. This means that the grass in a radius around the refinery is processed, after which the refinery moves on to the next grassland. Based on different sources (see chapter 2), the price for fresh grass is assumed to be € 150 per ton dry matter. This includes costs for mowing, harvesting and transportation from the land to the place of processing. In the scenarios with a central refinery, raw material has to be available throughout the year. Due to this ensilaged grass has to be used, as fresh grass is only periodically available. The price for silage grass is estimated to be € 250 per ton dry matter, including mowing, harvesting, and ensiling. For each of the scenarios, is considered that farmers will only sell their grass for bio-refinement if this returns more money than cultivating another crop on their land. This is therefore always the minimum price to give the farmers for their grass.

- **Water and energy** For each machine, the energy (kWh) and water (m^3) consumption is estimated. The average standard Dutch prices for energy (€ 0.12/kWh) and water (€ 1.09/m^3) are used. For scenarios 2 and 3, in which silage grass is the raw material, water is added to correct for the higher dry matter percentage of the grass. The silage grass is diluted with water to a dry matter content of 16.3%, similar to that of fresh grass. Energy usage is a large cost in each of the scenarios. In the third scenario however, it is extremely large due to high-energy consuming processes such as a spray drying and column chromatography.

- **Chemicals** In the second and third scenario the phosphate in the waste stream will precipitate as struvite by adding magnesium oxide (MgO) and sodium hydroxide (NaOH). For each ton fresh grass processed, 0.045 kg of MgO should be added and twice this amount (0.09 kg per ton FM grass) of NaOH[60]. The price for MgO is taken to be €146 per ton and € 25 per ton for NaOH[60]. However, because of conflicting studies about the financial viability of phosphate precipitation and conflicting struvite prices, this step is excluded in the CBA. In the future, this step might be included as much research about struvite extraction is being done at the moment.

- **Transport of grass** Transportation on a farm ordinarily takes place with tractors. However, for long distances (more than 6 to 7 km) a truck is cheaper and more appropriate[29]. Besides this, transport costs depend on the scale of operations. For example, a larger biorefinery needs more raw material (grass), which means that transport costs increases. For this report, the average transportation distance and costs are estimated. In the Netherlands, approximately 28% of all land is grassland (CBS and authors calculations). If a biorefinery processes 78,000 ton of DM grass per year, 7500 hectares of grassland is required when the average annual yield per hectare is 10.4 ton DM. As 100 hectares equals 1 km^2, approximately 75 km^2 of grassland is needed each year. When it is assumed that 28% of all land is grassland, enough grass would be produced in a circle of 268

km^2 around the biorefinery. The radius of that circle would be 9.24 kilometres. Thus, the furthest grass transport would be approximately 9.23 kilometres, assuming that there is a straight road from the land to the bio-refinery. Naturally, it is unlikely that all grass produced in that circle is sold for bio-refinement. However, it is also certain that there are areas with a higher concentration of grassland. When the concentration of grassland is doubled (56%), only half of the available grassland in a circle of 268 km^2 would be used for grass refinement. In this project it is assumed that all grass in the second and third scenario is transported by truck for at least 6 km. This averages the grass that comes from close and further away. The price for transport by truck is estimated to be € 2.20 per ton fresh matter for distances over 6 to 7 km[92].

- **Storage of grass, fibre and protein fraction** Before the fibre and protein fractions can be sold they are collected and stored under certain conditions. This storage incurs some extra costs. For the CBA it is assumed that on average products are saved for about 2 months. The storage costs were estimated to be € 10 per ton DM per month for the protein products and € 2.77 per ton DM per month for the fibre products[90].

5.1.5 Benefits

The main product of grass refinement in this project is protein. However, previous projects have taught us that the protein fraction alone will not be enough to make the process profitable (personal communication, M. Burgering). Thus, in order to make a business case for grass refinement it is important to also valorise the side-products. Depending on the scenario, different side-products are produced which all have different applications, as has been discussed in chapter 4. The chosen applications and corresponding prices for the (side)-products are discussed below.

- **Protein product** The three scenarios are primarily based on the different desired protein products. As noted before, the protein product of the first scenario is used for feed. The protein product of the second scenario is a non-functional protein product that is suitable for the food industry and scenario 3 produces a functional protein product. The protein product price for feed is estimated based on the price of Brazilian soybean meal, as it is comparable in quality. The price for soy is € 375 per ton, based on a product containing 48% crude protein (CP)[93]. The level of crude protein in the obtained protein product in the first scenario is estimated to be 11%. This comes down to 4.36 times lesser crude protein. Assuming that the protein price is mainly based on the level of crude protein, the price for the food grade protein product is € 375/4.36 = €86 per ton. The non-functional food-grade protein product from scenario 2 is likewise based on the price of Brazilian soybean meal. This protein product consist for 20% of crude protein, which is 2.4 times less than Brazilian soybean meal, resulting in a price of (€ 375/2.4 =) €156 per ton protein product.

  The functional food-grade protein product in scenario 3 mainly consists of Rubisco. It has a higher quality compared to the abovementioned protein products, which is mainly due to its functional properties and higher protein concentration. TNO estimates a price of € 6000 per ton for a comparable product (personal communication, M. Burgering). However, it is expected that the protein product in scenario 3 is of lesser quality than that of TNO and thus more comparable to other plant proteins such as chickpea isolate. Based on these assumptions, the established price for the functional-grade protein product is the same as chickpea isolate which costs approximately € 4500 per ton (personal communication, B. Mersbergen).

- **Fibre fraction** As discussed in chapter 2, the fibre fraction can be used for a variety of purposes. For scenario 1 it is logical to use the fibre fraction for cattle feed as this reduces the purchase of other roughage. This is not desirable for the other scenarios as the fibres would have to be transported back to the farmer, which would increase transportation costs. With regard to the

nutritional value, the fibre fraction is best comparable to hay. The price for hay is approximately €130 per ton for a product with 85% dry matter. The fibre fraction of the scenarios consists for 48% of dry matter, which is 1.8 times less than hay. Therefore, the price would come down to approximately € 73 per ton. Hay however has the functional property that it stimulates activity of the rumen of ruminants, which is not the case for the fibre fraction as its structure has been weakened. Therefore, the price has been downscaled and is estimated to be € 50 per ton.

For a central refinery, the fibre is used for insulation material. The selling price for this insulation material is estimated between € 800 and € 1200[79,90]. However, the production costs of this material are unknown. Therefore, an assumption was made based on the expected benefit of the fibre for feed purposes. Insulation material was expected to have a benefit that is at least similar to that of fibre for feed (€ 50 per ton). It is likely that a high-quality product such as insulation material has a higher profit margin than cattle feed. In any case, the fibre fraction in scenarios 2 and 3 can always be used as cattle feed.

- **Struvite** Due to conflicting literature studies about struvite extraction viability, it was decided to exclude this step from the CBA. Besides this, the financial overview of struvite extraction greatly depends on the magnesium source that is being used. Before this step can be included, more elaborate research on pricing and magnesium sources is required.

5.1.6 Profitability indicators

An analysis of the costs and benefits alone is not sufficient to assess whether an investment is solid. To predict the financial viability of a project, the investment should be compared to the expected generated cash flow or net profit. This can be done by means of profitability indicators. These indicators are financial methods to determine the profitability of an investment. Three different methods are used and explained below. These are Net Present Value, Internal Rate of Return and Return On Investment.

- **Net present value of an investment (NPV)**[94] With this technique the investment costs are compared to the future cash flow, after this cash flow has been discounted by a specific rate. This rate stands in relation to the value of money. Money has a higher value today, than tomorrow. The reason for this is that with money one can make more money, for example by putting it in the bank and earning interest. If one puts € 100 in the bank today at an interest rate of 10% per year, then in one year the future value of this amounts to € 110. So the present value of €110 next year is € 100 today. The NPV of a time series of cash flows (difference between incoming and outgoing cash) is the sum of the present values of the individual cash flows. Each individual cash flow in year *t* is discounted back to its present value. The formula to calculate the NPV is given by[95]:

$$NPV = \sum_{t=0}^{T} \frac{CF_t}{(1+i)^t}, with$$

T = *Period of operation in years*
CF_t = *Net Cash Flow at time t (€/ year)*
t = *Time in years*
i = *Discount rate (%)*

For this project the period of investment is assumed to be 7 years since this is the time period estimated for depreciation of machinery at FrieslandCampina (personal communication, A. van der Padt). Thus, T=7 and t runs from 0 to 7. At time t=0, the CF_0 equals the investment costs, which is a minus amount, since costs are an outflow of money. The net cash flow amounts for the cash money that is actually earned minus the cash money that is spent. Depreciation for example is a

cost, but it not actually spent and therefore does not contribute to the cash flow. The discount rate indicates the risk that comes with the investment. In case of an unproven and high-risk business, this rate is high. In literature a range of rates was found, but an estimation of 12% was given by Dutch experts for a similar project[90]. Hence, for this project *i*=0.12 is taken. When the NPV is less than zero the project is considered to be not viable and investing is not recommended. On the contrary, when the NPV is greater than zero it suggests that the project is financially attractive and seems a good investment.

- **Internal Rate of Return (IRR)**[94] The IRR is the discount rate at which the NPV equals zero. This rate is used as an indicator for the profitability of the investment. The higher this rate, the more economically attractive the project. In case the IRR is lower than the selected discount rate *i* (the estimated risk of the investment) the project is assumed to be an unwise investment. Thus when the IRR value is greater than the discount rate *i*, the NPV must be greater than zero, which means that the project is considered to be a wise investment.

- **Return on Investment (ROI)**[94] The ROI is a way to measure the effectiveness of the spent investment to generate profit. This means that it compares the profit of the project to the investment in the project. As with NPV and IRR, the higher the return, the better. The difference between profit and ROI is that profit measures the performance of the project. ROI on the other hand measures the money that is invested in the project and the return that is realised with that investment, based on the net profit of the project. ROI is expressed in a percentage and can be calculated in different ways. The following calculation is used for the CBA:

$$ROI = \frac{Net\ profit}{Investment} * 100\%$$

5.1.7 Financial statements

In order to analyse the financial status of a project, it is useful to forecast the expected profit and loss and cash flow of the project. A profit and loss statement, also known as an income statement, gives an overview of all expenses (fixed and variable costs) and revenues over a certain period. It can be seen as a receipt, where at the top the gross revenue is stated and at the bottom the net profit, after subtracting all costs[94]. Again, the time period is taken to be seven years. The profit and loss statement also takes interest and corporate taxes into account as cost. For this project it was assumed that no loan from the bank was required, since FrieslandCampina has enough resources to finance such a project. The corporate taxes in the Netherlands are 20% for all amounts up to € 200,000 and after that the tax rate is 25%. No tax was calculated in case of a negative EBIT. The amount of money before the interest and corporate taxes are subtracted is referred to as EBIT (Earnings Before Interest and Taxes). This equals benefits minus all fixed and variable costs. The EBIT at year 1 will be given for each of the scenarios.

Next to the profit and loss statement a cash flow statement is generated. The cash flow statement displays all cash flow that is generated for year 0 and the next seven years. In year 0, the only cash outflow is the investment. The cash flow statement is similar to the profit and loss statement, but does include investment cost and excludes depreciation. The cash flow statement is used to calculate the profitability indicators described above.

5.2 Results

For all three scenarios the costs and benefits are weighed against each other. For the outcomes of the scenarios not only the prices are of importance, but also the quantities of the products produced. In table 7 an overview can be found with the important quantities processed, the costs and benefits, the financials results and the profitability indicators, for each of the scenarios.

Table 7 General overview of the cost-benefit analysis of the different scenarios

Category	Item	Scenario 1	Scenario 2	Scenario 3
Quantities (ton/year)				
	Grass (FM in tonnes)	8.800	259.210	259.210
	Protein product (in tonnes)	1.338	30.269	8.819
	Fibre fraction (in tonnes)	2.112	104.956	104.956
Investment (€)				
	Machinery	€ 360.000-	€ 590.000-	€ 4.430.000-
	Material & Equipment	€ 543.750-	€ 885.000-	€ 6.645.000-
	Location		€ 100.000-	€ 100.000-
Fixed costs (€/year)				
	Depreciation	€ 50.400-	€ 82.600-	€ 620.200-
	Maintenance	€ 90.375-	€ 157.500-	€ 1.117.500-
	Research, engineering and development costs	€ 90.375-	€ 157.500-	€ 1.117.500-
	Labour	€ 109.400-	€ 437.600-	€ 437.600-
Variable costs (€/year)				
	Grass	€ 215.160-	€ 19.440.750-	€ 19.440.750-
	Chemicals	€ -	€ -	€ -
	Water	€ -	€ 237.471-	€ 237.471-
	Energy	€ 167.106-	€ 9.702.687-	€ 20.863.716-
	Transport grass	€ -	€ 565.078-	€ 565.078-
	Storage (protein, fibre)	€ -	€ 1.186.842-	€ 757.841-
Benefits (€/year)				
	Protein product	€ 115.034	€ 4.722.003	€ 39.686.347
	Dry fibre fraction	€ 105.600	€ 5.247.810	€ 5.247.810
	Struvite		€ -	€ -
Financial results				
	Investment (year 0)	€ 903.750-	€ 1.575.000-	€ 11.175.000-
	EBIT (year 1)	€ 502.182-	€ 21.998.216-	€ 223.500-
	Net Profit (year 1)	€ 502.182-	€ 21.998.216-	€ 223.500-
Profitability indicators				
	NPV	€ 2.633.816-	€ 101.014.369-	€ 6.046.064-
	IRR	ND*	ND*	-7%
	ROI	-56%	-1397%	-2%

***** Not determined. Value was too low for IRR calculation

For this cost-benefit analysis, a conservative approach was chosen to be on the safe side. By means of this table, the scenarios can easily be compared. Besides the investment costs, the largest cost for each of the scenarios is the purchase of the grass. The other major cost is energy due to the high energy consumption of the machines. One could investigate the idea of producing biogas and using this partly for the supply of energy, but this is left for future research (see section 8.2). The highest benefits are gained by the sales of the protein products. As can be seen in table 7, none of the scenarios has a financially viable outcome with the used numbers. However, this cost-benefit analysis used a conservative approach. Small, acceptable changes in some variables can have a major impact on the results. The four key variables with the highest impact were determined.

5.2.1 Key variables

Four key variables are distinguished that have the highest impact on the scenarios outcomes (Table 8). These variables can change the profitability of the scenarios. Products that are used and produced in high quantity are sensitive to price changes. In the table below the four key variables are found with their corresponding numbers or prices. Fluctuating these variables gives inside in what threshold value is required for combinations of the variables to turn around a scenario's profitability. The estimations in this project are quite conservative, meaning that there is room for improvement.

Table 8 The key variables of the cost-benefit analysis

Variables	Scenario 1	Scenario 2	Scenario 3
Grass price (€/ton DM)			
Fresh grass	€ 150	-	-
Silage grass	-	€ 250	€ 250
Protein price (€/ton)			
Feed (protein)	€ 86	-	-
Human non-functional	-	€ 156	-
Human functional	-	-	€ 4.500
Labour (FTE)			
Mobile refinery	2	-	-
Central Refinery	-	8	8
Dry fibre fraction (€/ton)			
Feed (fibre)	€ 50	-	-
Insulation	-	€ 50	€ 50

5.3 Economical background

The global demand for animal proteins (such as meat and milk proteins) is increasing rapidly[96]. This chapter covers some aspects that are of importance when investigating a potential investment in a new type of business. Since grass refinement is quite a new business concept, it is essential to know which other companies operate on the same market. Also for the products, it is good to know with which established products they compete and whether this is likely to influence prices. In this section, the competition is first explored. After that, the expected price development of several products is discussed. Since prices fluctuate it is wise to predict how these price might change in the future.

5.3.1 Competition

For the competition, a distinction can be made between direct versus indirect competition and national versus international competition. Direct competition are businesses which sell essentially the same product, whereas indirect competitors sell substitute products. In the Netherlands the direct competition is very limited. Besides Grassa! no other noteworthy business is trying to refine grass into proteins. In the rest of Europe there are some projects involved in the bio refinement of grass, but as long as the products are sold on the national markets, these projects do not form a threat. For this report, international projects were out of scope. Therefore, we refer to a publication of O'Keeffe which includes an extensive list of projects in Europe[26].

While direct competition for protein production from grass is limited, indirect competition is intense, both national and international. There are many projects that are involved in the production of edible plant proteins, which are also named *green proteins*. The demand for these proteins is growing, because the demand for protein in general is growing. These green proteins are produced for direct consumption (e.g. soy or pea protein) or as ingredients for concentrated feed. This large indirect competition is two sided. On the one hand, the increasing demand for green proteins indicates that there is a possible (growing) market for grass protein. On the other hand, there are already established producers of green protein with which grass protein would have to compete.

When looking at the fibre product, not many options for valorisation of this product are known, besides feed purposes. Even for feed, it is debatable whether there is still enough nutritional value in the fibre product to make it a valuable product for feed (personal communication, A. Hamminga). Other potential buyers for this product can be found in the packaging industry, but only if the grass fibres are of sufficient quality. The grass fibres from the scenarios in this report are not suited for this and might be better used for the production of insulation material. However, there is currently only one company that produces grass-based insulation material. This limits valorisation options of the fibre fraction. Hence, the market for fibre products should be investigated more.

The competition for raw materials such as grass is intense, as grass refinement has to compete with dairy farming. The numbers of cows is expected to increase and thereby also the demand for grass. Besides, there are project running that also refine grass, but for other purposes than in this project. HarvestaGG (see appendix IV) for example uses grass to produce, amongst others, biogas. So the market for grass is expected to become even more competitive in case a large biorefinery for grass is initiated.

To conclude, there is a lot of competition that also extract green proteins from biomass. Expected is however that this number of businesses will not be able to foresee in the increasing protein demand. Therefore no problems are expected regarding the sales of the protein product. The purchase or raw material is more problematic as a refinery would have to compete with established digesters and dairy farms. Furthermore, the valorisation of the fibre fraction requires more investigation.

5.3.2 Price development

The aim of this section is to give an overview on the expected price development of grass and its refined products. The findings of this chapter were not taken into account for the cost-benefit analysis. However, due to price changes, the financial viability of bio-refinement might change in the future. This section is based on conversations with experts and data from the last 10-20 years. It must be noted that that no statistical analysis was performed and that the estimations made in this section do not offer any guarantee for the future.

- **Grass** Estimate: likely to increase
  Currently, grass is often sold for less than its nutritional value is worth. Wageningen UR Livestock Research publishes monthly prices for fodder based on the nutritional value and protein content. If this price is used for grass, estimates are that grass would cost as much as € 280/ton[18,29], while farmers assume a price of € 150 (personal communication, N. den Besten and J. van de Ven). This indicates that there is a chance that grass prices increase strongly in the coming years, especially if the dairy sector continues to grow, grass refinement further develops and grass becomes scarcer.

- **Protein** Estimate: stable/increase
  It is expected that protein prices for both feed and food purposes will remain stable or increase in the next few years. This is due to a growing demand which originates mostly from upcoming third world countries. For example, one research group calculated that the protein demand might increase by 110% from 2005 till 2050[97] and the FAO expects that the global demand for food, feed and fibre will increase 70% by 2050[96]. Currently, approximately 40% of the protein production derives from animal husbandry[98,99], for which a lot of plant-derived protein is needed.

  If the grass protein concentrate is used for feed purposes, it can best be compared to soybean meal. The price for Brazilian soybean meal 48% has been increasing since 2006[93] (see Figure 7A). This shows that there are certainly possibilities for grass protein as the protein market is still growing. The price of functional grass protein is based on chickpeas isolate for the CBA. However, for the price development of the functional protein fraction, prices for WPC34 (whey protein concentrate, 34% protein) were used. WPC34 contains significantly less protein than the protein product from scenario 3, but detailed historic prices of chickpeas isolate are not available. This is not problematic as the price development is approximately the same for each type of protein isolate. Prices for WPC34 are approximately 6 times higher than for soy protein. This is mainly to a difference in quality, origin and nutritional value. The price for WPC34 has been increasing since 2006, although it shows a lot of fluctuation (Figure 7B). With the increasing demand for protein from Asia it is likely that the price for whey protein will remain at this level or increase. Therefore, it is also likely that the price for grass protein would remain stable or increase in the coming years.

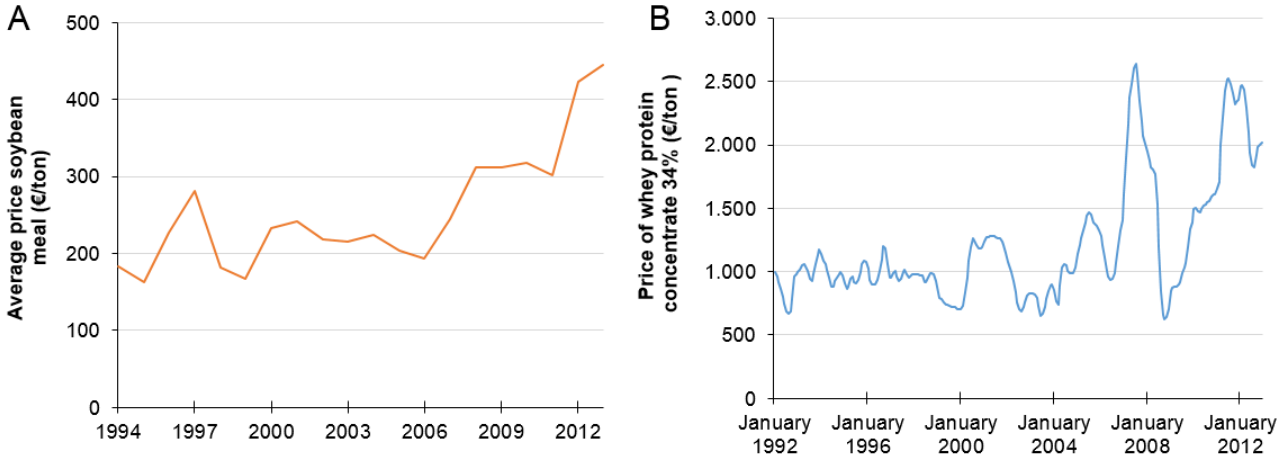

Figure 7 | Price development of different protein concentrates. A) Whey protein concentrate (34%). Average price for whey protein concentrate 34% from 2012-2014 is approximately € 2350,-/ton. Original data (in $/pound) from USDA Agricultural Marketing Service. Calculated €/ton with the $-€ exchange rate of 25-6-2014 (1 US $ = € 0,732845014). **B)** Price development of Brazilian soybean meal 48%. The average soy meal price from 2010-2013 was € 375,-/ton. Data from LEI.

- **Fibres for feed** Estimate: stable
  The grass fibre fraction for feed was compared to hay as this is the most comparable product. The price for hay in the Netherlands has been increasing since 2000, but there was a major price decline in 2012 (Figure 8A). After that the price has risen somewhat but no major changes are expected for this year. It is not expected that the price will increase rapidly due to the abolishment of the milk quota in 2015. Hay is not a direct product for high-producing cows due to its low nutritional value, so an increase in cows does not lead to increased consumption of hay.

- **Fibres for paper industry** Estimate: stable
  If the grass fibre fraction is to be used in the paper and packaging industry it can be best compared to waste paper. Since 2000, the prices of waste paper have been fluctuating between € 90 – 160 per ton (Figure 8B). Currently, the price lies between € 110 – 130 per ton and it is unlikely that there will be major fluctuations in the future.

- **Struvite** Estimate: stable/increase
  Phosphate prices have been relatively constant till 2007, but since then prices have been fluctuating[100,101] (Figure 9). There have been reports that deposits of phosphate rock will be near depletion in 2050. Although this will likely lead to a price increase, the timespan is too large to assume that this has a significant influence on the viability of grass refinement in the near future.

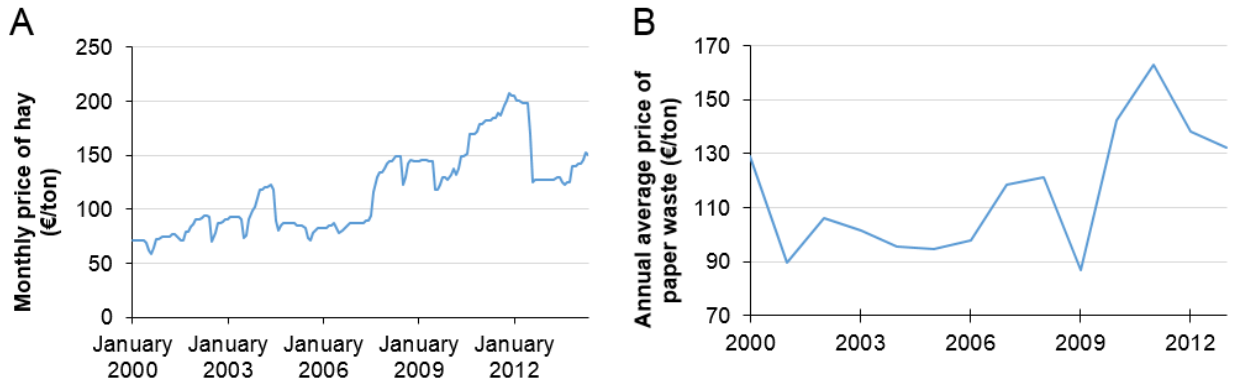

Figure 8 | Price development of different applications of the fibre fraction. A) The price of perennial ryegrass hay from January 2000 till May 2014. Prices are in €/ton and are for the whole product, including the water fraction. Data from LEI. **B)** Paper waste price development in the EU. Data from Eurostat.

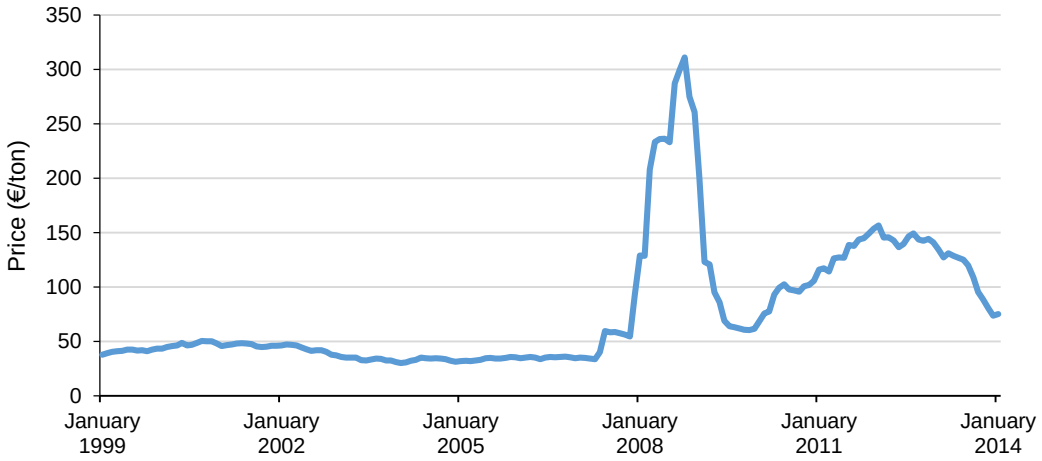

Figure 9 | Phosphate rock development. To estimate the price development of struvite, phosphate rock (Morocco, 70% BPL) was used as a reference as a world market for struvite is missing. Data from World Data Bank[101].

6 Conclusion

In our cost-benefit analysis all three scenarios were not financially viable. However, we believe that scenario 3 (functional protein) has the most potential to become viable with several adjustments to the process and fine-tuning of the assumptions. In this chapter we will first elaborate on the results of the CBA in general. After that we go into more detail about the different scenarios.

For scenarios 1 and 2 grass is the biggest cost, accounting for 30% and 61% of all costs respectively. In scenario 3 energy usage is the largest cost (46% of total costs) with grass being the second largest cost (43%). In each scenario, all benefits were due to the protein and fibre fractions. Scenario 2 mostly depended on the fibre fraction (53% of all benefits) while the protein fraction was more important in the other two scenarios (52% of total benefit for scenario 1, 88% for scenario 3). Because of the relatively large quantity of dry fibre product produced, a significant increase in the sales price of this product could turn the dry fibre fraction into the most valuable benefit. For scenario 2 and 3, it has to be noted that struvite precipitation was excluded from the cost-benefit analysis as the financial details of this process in the Netherlands are not known. Besides this, the feasibility of struvite extraction also greatly depends on the magnesium source that is used in the process, as magnesium is the main cost of struvite precipitation.

In addition to struvite extraction, the valorisation of the fibre fraction is not very well supported. In our cost-benefit analysis for scenarios 2 and 3 we assumed the price to be € 50 per ton fibre product. This might be an underestimation of the actual benefit as we do not know any details about the insulation material production process. However, the producer of grass insulation Gramitherm® states that its business case depends solely on the valorisation of the fibre fraction. In that case, one might argue that the revenue generated by the fibre fraction should at least cover the expenses for the raw material. In our CBA this is not the case as the purchase costs of grass are significantly higher than the benefit of the fibre fraction. This means that we might have underestimated the actual value of the fibre fraction.

Scenario 1
With the assumptions described in this report, the outcome of the scenario with the mobile refinery is negative. In our opinion it is unlikely that a mobile refinery such as we envisioned will become profitable. The Excel sheet that accompanies this report shows that significant changes in both costs and benefits do not lead to a profitable business case. Even when we set the four key variables at more optimistic values - 200% of fibre and protein price, 50% of grass price and labour- the scenario is still not profitable. This is mainly due to the energy and labour costs. In contrast to the other scenarios, the grass price accounts for only 30% of the total running costs. Thus, changing the grass price has a smaller influence on the overall financial situation. For this scenario, the most important factors for cost reduction are labour, energy and grass. Furthermore, the protein extraction efficiency for scenario 1 is assumed to be 45%. A higher efficiency will increase the profit of the protein fraction, but it is highly unlikely that this alone will be sufficient to make a profitable case.

Scenario 2
The central refinery for non-functional protein is without doubt a non-profitable scenario and we do not expect it to become profitable. This is due to two reasons. First, the protein product in this scenario generates significantly less revenue than the protein product in scenario 3, even though both are aimed at human consumption. This is due to the lesser quality and lack of functional properties of the obtained protein product in the second scenario. Secondly, the process of scenario 2 is more elaborate and expensive than of scenario 1 while the quality and price for the protein product do not differ that much. The result is an expensive process with a relatively low-grade protein product. Furthermore, we were not able to find a suitable application for this wet protein product. This further complicates this scenario.

Scenario 3

We have high expectations for this scenario even though the outcome of the CBA was negative. This is because slight, justifiable changes in the assumptions already lead to a profitable scenario. For example, the protein fraction accounts for 88% of the total benefits. This means that small changes in the protein price have a major effect on the total financial overview. In the CBA we assumed the protein price to be € 4500/ton protein, which is a price similar to that of chickpeas concentrate. However, TNO expects to earn around € 6000/ton for their Rubisco isolate. When we use this price for the CBA the scenario has a net profit of 9.8 million euro in the first year. Thus, in our opinion this scenario has highest financial viability. Next to the protein price assumption, the price for insulation material is also a very conservative estimate. In combination with the high quantity of dry fibre fraction, this suggests a higher profit for insulation material than currently assumed and thus a higher profitability of the scenario. On the grounds of these consideration, we expect that optimizations of the process will turn this scenario into a positive one.

7 Challenges

When deciding whether or not to invest in a project, the financial viability is highly important. There are however also considerations that cannot be quantified by money, but that do contribute to either the failure or success of a project. In order to provide the best possible recommendation for FrieslandCampina, these non-financial considerations (e.g. social or ecological) have been assessed as well. This chapter discusses several factors that could negatively influence the viability of grass refinement in the Netherlands.

Farmers might not support grass refinement

The first challenge is that there is a high possibility that farmers in the Netherlands do not want to get involved in the refinement of grass. In the Netherlands, a large part of the grassland is being managed by FrieslandCampina farmers (online interview with T. Kingma[3] and personal communication, Y. de Vries). Thus, the success of grass refinement for a large part depends on the cooperation of dairy farmers. Farmers have to choose between using their grass as dairy feed or for biorefinement. Currently, investing in dairy farming is relatively safe while financing the more experimental biorefinement carries a higher risk. This is partly due to the current status of dairy farming in the Netherlands. In 2015, the milk quota in the European Union (EU) will be abolished[102], which promotes growth of the dairy sector[31,103]. Combined with a growing export to other continents[103,104] this offers enough prospects for a financially healthy dairy sector. Thus, farmers are more likely to invest in their dairy farm and less likely in grass refinement. Besides this, farmers view dairy farming as their core-business and often as a family business as well. Therefore, even if grass refinement is financially attractive, farmers might still decide to continue farming as they have been doing for years.

However, farmers could be convinced to participate in biorefinement by two financial reasons. The first condition is that the profit per hectare of grassland is higher when the grass is used for biorefinement when compared to the current situation. In practice, this would mean that the grass price should increase, although it is outside of the scope of this project to calculate how much this increase would need to be. The second reason could be a guarantee of stable prices. Due to the fluctuation of the milk price, there are periods during which the milk price is extremely low and farmers have higher costs than benefits. If refinement of grass could guarantee a less fluctuating income, farmers might be persuaded to use their grass for refinement. An alternative option would be to move the focus from solely grass to include other biomass as well that is more readily available. Lastly, crop farmers might be persuaded to replace their usual crops (such as potatoes) with grass if this earns more money.

EU novel food-law hinders the application of grass protein

In 1997, EU regulation 258/97 was adopted by the European parliament and council[105]. This regulation, also called the *EU food law* or *novel food regulation* (NFR), was introduced to protect public health by ensuring food safety[106]. In general, all foods that were not consumed in the EU before the 15th of May 1997 have to be applied under the NFR[106]. As a results of this law it is much more difficult to introduce a new food product on the market that was not consumed before the establishment of this law. For a more elaborate explanation, see the article of Hermann (2009) or the latest version of the EU novel food law, regulation 178/2002[107].

As grass was not consumed in the EU before 1997, it is subject to the NFR, meaning that processed grass (components) may not be sold in the EU for human consumption. According to several experts this is an obstacle for using grass refinement to extract food-grade protein (personal communication, B. Smit and B. Koopmans). Indeed, one review reported that the average time spent from application to market authorisation is 39 months[106] and entails studies on nutritional information, production process, chemical composition, toxicology, allergy development and more[106,108]. It is not clear how much the application

procedure and the associated R&D costs, but the company PhytoTrade Africa reported that their 2-year during application for "baobab dried fruit pulp" cost more than £ 150.000[109] (or € 187.000). As this was a relatively easy and fast application, it is expected that the average expenses for an NFR application are higher[106].

In conclusion, the NFR is certainly an obstacle for FrieslandCampina, but not an insurmountable one. With an annual revenue of 11.4 billion euro and 157 million euro profit in 2013[2], the financial aspect of an NFR application is no obstacle for FrieslandCampina. In addition, it might be possible to process the grass in such a way that the end product is similar to an already established product and introduce it on the market within this product category (personal communication, B. Smit).

Grass is becoming scarce

Current initiatives for grass refinement started several years ago with the assumption that there was an excess of grass[110,111]. This was partly due to the nitrogen derogation for dairy farmers that was granted by the EU if at least 70% (since 2014 80%[112]) of the land on a farm consisted of grassland. However, the high feed prices of the recent years have led to a greater share of grass in the cows' diet. Also, the dairy chain is growing. Due to these two reasons, high-quality grass is becoming scarcer, although grass prices have not really increased yet (personal communication, N. den Besten and C. de Vries). To conclude, refinement of grass is no longer a solution for excess grass, but has to compete with the established dairy chain.

There are three options to manage this challenge. The first one is to guarantee a high grass price, as discussed in section 7.1. The second one is to set up a biorefinement in areas with a surplus of grass or in areas where grass is inexpensive. Such a region could be in Ireland or New Zealand for example. There, grass is abundant and biorefinement does not have to compete with other applications of grass, especially in regions with little or no dairy farms. The third possibility would be to supplement the grass with other green crops suitable for biorefinement.

Varying grass quality influences the refinement process

As discussed in Chapter 3, grass composition and quality may differ throughout the year. Especially the protein concentration requires attention. If this fluctuates too much, the process will need to be adjusted for every batch of grass. Besides this, the process might not be cost-efficient if the protein content of grass becomes too low. Regardless of protein content, the purity of the grass is also a point of attention, as not all grassland is a monoculture of *L. perenne*. Often, species of clover are present to increase the nitrogen fixation. Also, harmful or poisonous plants can infiltrate the grassland, which might be difficult to spot when the grass is already harvested.

For the application of grass protein, a strict quality-control for processed grass has to be established. To ensure a more constant quality of grass, the process can be adjusted to use only ensilaged grass. This makes the process less season bound and offers more freedom in the selection of raw material. For example, one can decide to use a specific silage for cattle feed if it does not meet the criteria for refinement of grass. An alternative solution would be to develop a grass species that is more stable, better adapted to the Dutch climate and grows better throughout the year.

8 Recommendation

The outcome of the cost-benefit analysis has shown that with our assumptions none of the scenarios are financially viable. However, as mentioned in the conclusion of this report, the scenario with functional protein shows enough potential for further investigation. We expect that this scenario will be financially profitable when our assumptions have been fine-tuned and the process has been further specified. This is not the case for scenarios 1 and 2, for which adjusting to more optimistic assumptions was not sufficient to demonstrate any potential viability. For scenario 3, we suggest three approaches for further research:

1) **Elaborate on the original scenario**
   The original scenario has the potential to be financially viable. In order to continue with this scenario, research should focus on improving the process. Aside from this, more accurate assessments of prices for grass, functional protein isolate and dry fibre are needed for this scenario.

2) **Use original scenario but with other biomass**
   Another approach for future research is to investigate whether, aside from grass, other biomass can be processed in the same biorefinery. As mentioned in chapter 6, grass is one of the main costs for a biorefinery. Cutting down on the costs of raw material can be sufficient for a solid business case. Besides this, using other green biomass makes a biorefinery less dependent on seasonal changes.

3) **Investigate grass refinement in other countries**
   The third approach to further develop this scenario is by investigating the refinement of grass in other countries where grass is cheaper and more abundant. Furthermore, other countries might have a climate that is better suited for the production of grass (longer seasons) which would enable a biorefinery to use fresh grass instead of ensilaged grass. This would both lower costs, as grass does not need to be ensilaged, and increase profit as fresh grass contains more protein.

In summary, we recommend that FrieslandCampina does not immediately invest in setting up a grass refinement facility at the present time. However, we think that grass refinement has enough potential and deserves further research. An interesting partner for this would be TNO as they have developed the process on which our functional protein scenario is based.

8.1 Future research

As this study was limited in scope as well as in time and resources, we were not able to answer all questions and also some new questions were raised. These questions are important to answer in future research, in order to get a more complete overview of all the possibilities regarding refinement of biomass.

- **Elaboration of the technical details**
  Limitations in time and knowledge impeded the construction of a detailed process overview. Due to this, assumptions about investment costs, energy usage, hourly throughput and more had to be made. In order to determine several of these factors, trials have to be performed as some details cannot be accurately predicted. Besides this, more access to the technical details of machinery will provide a more accurate estimation of the financial viability of grass refinement. Due to confidentiality issues we were unable to gather some technical details.

- **Market investigation**
  In this report, the opinion of the food manufacturer and consumer was not taken into account. We recommend that a market investigation is performed before the protein is applied in different feed or food products. Otherwise, the average consumer might disregard new products that contain grass proteins.

- **Valorisation of the waste stream**
  Part of the process that needs to be elaborated is the valorisation of the waste stream. This stream contains sugars, minerals and amino acids but the isolation of these components is either too expensive or still in the testing phase. Other possible applications for the waste stream are cattle feed or fermentation. Likewise, more research is needed before the cost-efficiency of these applications is known. O'Keeffe (2010) investigated the financial viability of a grass digester in Ireland[26]. She found that a large scale production plant would be profitable without government subsidies, while a medium scale plant is the easiest to implement due to the low investment costs and can perform with few subsidies.

- **Other crops suitable for biorefinement**
  For this report, only the refinement of grass was investigated. However, perennial ryegrass is not the only crop suited for biorefinement. Another grass species, tall fescue, has a higher protein yield and might be more suited for biorefinement than perennial ryegrass. Besides this, there are currently several biorefinement initiatives that use crops other than grass. For example, a consortium with among others TNO is investigating a scenario in which protein is isolated from sugar beet leaves[113,114] and WUR is developing a package from tomato plant material[115]. Furthermore, alfalfa has also been identified as a potential source for refinement[10,63]. This shows that biorefinement is not limited to grasses but can be applied to any kind of green plant material. Future research could focus at processing different green waste streams in one biorefinery. This can enhance the financial viability of biorefinement as the production plant is less dependent on the seasonal growth of crops. For example, sugar beet leaves and waste material of greenhouse crops can be processed when grass is not available.

- **Biorefinement abroad**
  There are also opportunities for biorefinement in other countries than the Netherlands. Especially countries with an optimal climate for growing grass and regions with no or limited dairy farming are interesting locations. In these regions there is still an abundance of grass and costs for raw material and labour are usually lower. Options for biorefineries outside the Netherlands have already been explored, for instance Ireland[26], Austria[83] or Africa (personal communication, B. Koopmans).

- **Practical applications of the protein product**
  The different applications of the protein products from grass refinement still needs elaboration. In this report, we gave a rough overview for the different protein products. However, we have not yet determined into which specific foods the protein can be processed. In particular the price of functional grass protein heavily depends on its possible applications in different food products. Furthermore, it is important to know who potential consumers are.

- **Subsidy for protein production (EU)**
  For the initial investment in a grass refinery a subsidy might be requested. This can be either a subsidy from a local government or from the EU. As part of the Horizon 2020, the EU has opened a call of € 128,000,000 for 'Protein of the future'. Unfortunately, this call has closed recently and no new applications can be submitted. However, chances are that in the near future a new call that applies to protein production or biorefinement might be opened.

- **Comparison of biorefinement with the dairy chain**
  In the Netherlands, grass refinement would compete with the dairy chain for raw material (grass). The question that needs to be answered in that case is which application is more cost-efficient but also more sustainable. Grass refinement could lower the import of soybeans and less cows would

decrease the emission of methane. However, the biorefinement process requires a lot of energy and water. If we want to claim that grass refinement is sustainable, then more details about the effect of biorefinement on the environment need to be determined. Besides this, sustainability is not well defined. One could also interpreted sustainable as outdoors grazing of cows ('weidegang') in contrast to being penned-up in a stable.

- **Interesting partnerships for grass refinement**
  As the industrial scale of grass refinement is still new, a possible investment also carries a high risk. This risk can be lowered by finding partners for the initial investment and/or future purchase of goods produced. Establishing a direct relationship with possible buyers of the fibre and protein product might increase security of the business. Examples are Unilever (protein), Agrifirm (feed) and Gramitherm (fibre). Furthermore, partnerships can increase knowledge sharing which benefits all involved parties.

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About the authors

Babette Paping (1988) was born and raised in Schiedam, but decided after a year of traveling and studying abroad that she was ready for a new city and moved to Amsterdam. Here she completed the interdisciplinary bachelor 'Bèta-gamma' with a major in mathematics. Currently she is a master student in Logic at the University of Amsterdam, which meets her needs in working on various exciting subjects. In this master she can apply the abstract way of thinking she has developed during her mathematics to different fields within logic. Her education and extracurricular activities, like playing hockey at high level, participating in a board, teaching at school, showed her how useful and important it is to interact and collaborate in a team or group. Also during her long travels she has noticed that she was inspired by new challenges and experiences. These reasons made her highly motivated to participate in this innovative project.

Koen van de Ven (1990) originates from a family of farmers. His parents started a goat farm in 1995 and two uncles manage a pig farm and a dairy farm. After middle school, Koen left to study for a bachelor in Biomedical Sciences at the University of Amsterdam. In 2012, he graduated *cum laude* and with honours. After this he continued his education with a master in Biomedical Sciences. During his bachelor, Koen was a student-member of the visitation committee of the bachelor and master programme Biomedical Sciences. This committee was set up by QANU and visited all the universities in the Netherlands that offers a programme in Biomedical Sciences. In 2012 and 2013, Koen also worked as a practical assistant for a course in Immunology. During this course, he assisted second-year students with their lab work and translated knowledge into practice.

Rami Wohl (1987) was born in Enschede, from a family of mostly musicians. In order to be able to put some emphasis on the arts and music, he attended a Waldorf school in Zutphen before starting his studies. In 2007, he moved to Utrecht to study for a bachelor in Chemistry at the Utrecht University, during which he was a student member of the program committee of the bachelor Chemistry, as well as an active member of Utrecht's Chemistry study association. In 2009 he switched from Chemistry to a bachelor in Psychobiology at the University of Amsterdam, from which he graduated in 2012. That same year, Rami started a master's program Cognitive Neurobiology & Clinical Neurophysiology at the University of Amsterdam, during which he also joined the Psychobiology program committee as a representative of master students.

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Appendices

I Experts contacted

Expert	Contacted	Institution	Background
Bertus Tulleners MSc	Weekly contact	University of Amsterdam	Tesla minor supervisor
Joris Buis MSc	Weekly contact	University of Amsterdam	Tesla minor supervisor
Prof. Chris Kruse	Multiple times	University of Amsterdam	Internal supervisor
Dr. Ynte de Vries	Multiple times	FrieslandCampina	Client from FrieslandCampina
Dr. Ir. Jeroen Heck	07 April 2014	FrieslandCampina	Expert on dairy-chain
Albert van der Padt	28 May 2014	FrieslandCampina	Expert on process technology.
Cynthia Klostermann and others	10 March 2014	Wageningen University & Research centre (WUR)	Third year student 'Levensmiddelentechnologie' at the WUR. Wrote a report on grass refinement together with her colleagues.
Dr. Bart Smit	18 March 2014	NIZO	Expert on translation and application of science into products. Is investigating protein extraction from grass, mainly focusses on Rubisco.
Dr. Maurits Burgering	1-4-2014 24-6-2014	TNO	Expert on bio-based business. Involved in the TNO project about protein extraction from sugar beet leaves.
Ir. Peter Geerdink	01 April 2014	TNO	Scientist food & bio processing. Involved as a process technologist in the TNO project concerning protein extraction from sugar beet leaves.
Prof. Dr. Johan Sanders	11-03-2014	Grassa!, WUR	Professor 'agrotechnologie en voedingswetenschappen'. Co-owner Grassa!
Jan Cees Vogelaar		HarvestaGG	Founder of HarvestaGG, which aims to produce bio-gas from grass.
Bram Koopmans	23 May 2014	Grassa!	Process technician and co-owner of Grassa!
Ing. Carel de Vries	14 April 2014	De Vries Projectregie	Innovationmanager. Guided and supported the Grassa! initiative in their start-up phase.
Edward Ensing	19 March 2014	Barenbrug	Project manager and expert on culturing grass.
Jan van de Ven		Farmer	
Nils den Besten	06 June 2014	Farmer	Involved in several advisory and management committees.
Anke Hamminga MSc	13 May 2014	Provimi	Expert on cattle feed and diet. Previously worked for FrieslandCampina as dairy farm advisor.
Ir. Michiel Adriaanse	28 April 2014	KCPK	Programme leader alternative fibre sources.
Ruud Nijs	19 June 2014	TheRockgroup	Financial expert. Focusses on arranging financing for innovative businesses.
Christian Roggeman	01 July 2014	Gramitherm®	Owner of Gramitherm®, a producer of insulation material made from grass fibre.

II Methodology Excel file

This section will outline how the Excel file used for the CBA was constructed and shortly explain the framework of the Excel file that was used to carry out the CBA. Besides this, a thorough readme is present in the Excel file itself.

The analysis

The first step in the analysis is to compose the process for each scenario and determine the required machinery and accompanying equipment. Next, the amount of grass to be processed has to be defined. This amount of grass is based on the processing volume of the machines combined with the number of hours that they run per year. For each step in the process, the purchase price of machinery and the operating cost (energy and water consumption) is estimated. On basis of this information the in- and outflow of the products are calculated. This provides an overview of all the input and output quantities for the process. When these quantities are linked to their corresponding prices, the investment, fixed and variable costs and benefits can be calculated.

In summary the following steps are undertaken:

1. Define the process with the associated machinery and equipment
2. Estimate the quantity of grass to be processed
3. Determine the machine purchase prices and use of energy and water
4. Compute the product in- and outflow for each step of the process
5. Estimate prices for products and side products, variables and quantities
6. Link the quantities to the set prices
7. Calculate the investment, fixed and variable costs
8. Calculate the benefits

To determine the EBIT or Net Profit of the project the costs and benefits have to be compared over a certain time. In this report a comparison is made between het amount of euros that are paid and earned per year. To do this the fixed costs (€/year) and the variable costs (€/quantity) have to be taken into account. These two cost categories together are referred to as running costs. In order to sum up these two cost categories, the variable costs have to be expressed in €/year as well. In this CBA, a substantiated assumption is made on the amount of grass that is processed per year. Based on this, the variable costs per year are calculated. This cost category together with the fixed costs account for the running costs. The benefits are also quantified in euros per year. In this way, the running costs can be compared to the benefits.

The investment is not taken into account to calculate the EBIT. To evaluate the investment in relation to the earnings each year, the profitability indicators described in section 5.1.6 are used.

Framework of the Excel file

The analysis of the costs and benefits is performed by means of an Excel file. The Excel file is built-up in such a way that all scenarios are evaluated in one document. Within this document, a distinction can be made between general sheets and scenario specific sheets. The general sheets contain all input and output data that are relevant for the three scenarios.

- On the main output sheet, the three different scenarios are shown with their financial results (EBIT, net profit, investment, NPV, IRR and ROI). For all scenarios the most important and influential variables are determined and also displayed on this sheet. These key variables are grass prices, protein prices, labour in FTE (full time equivalents) and fibre prices. The magnitude of these values has a major impact on the financial results of the various scenarios. For each of the values, an estimate, minimum and maximum is given. The value of the variable can be adjusted by means of

a scroll bar. This enables the user to compare the financial results in a low (pessimistic), medium (realistic) and high (optimistic) case and everything in between. This also provides the possibility to determine what should be the minimum value of a particular variable or a combination of variables in order to make a scenario cost-effective.

- On the second general sheet an extensive list of assumptions and variables is displayed. Most of the used prices can be found in this list. Some of the variables or assumptions are supported by a comment, when required. Furthermore, sources are given here such that it is easy to trace back and update the original number.

- The last general sheet provides an overview of the composition of the silage and fresh grass in terms of water and dry matter. The composition of grass is linked to other sheets so that adjustments to this automatically changes the output of products for each scenario. Also, the calculation for the amount of grass processed per year is described on this sheet.

Each of the scenarios has four scenario specific sheets.

- The first sheet provides a general overview of all costs and benefits.

- The second sheet contains the profit and loss statement for the project estimated for the next seven years. The profit and loss statement gives an overview of all expenses (fixed and variable costs) and revenues over a certain period. It can be seen as a receipt, where at the top the gross revenue is stated and at the bottom the net profit, after subtracting all costs:

  - ***Gross Revenue***
    The gross revenue represents all benefits earned by the sales of products.
  - ***Costs of Goods Sold (COGS)***
    The COGS represent the costs that are incurred by the production of the goods. In this case these are the costs for grass, chemicals, transport and storage.
  - ***Operating Expenses (OPEX)***
    The OPEX are the costs that are made to keep a business running. For this project maintenance, research and development, labour, water and energy are considered as OPEX.
  - ***Depreciation***
  - ***Interest***
    There is no loan, so in this case no money has to be paid off to the bank.
  - ***Corporate Taxes***
    The corporate taxes in the Netherlands are 20% for all amounts up to € 200,000 and after that the tax rate is 25%.
  - ***Net Profit***
    The net profit is the result after subtracting all the above costs. As one might have noticed, are the fixed and variable costs divided over the COGS and OPEX, except for depreciation.

  This sheet also contains the cash flow statement and the profitability indicators.

- The third sheet displays the in- and outflow quantities of the different steps of the process, the produced products and the required energy and water per step.

- Finally, the fourth sheet shows the costs and benefits for each step separately. This sheet includes a function to include or exclude different steps in the process for the price calculation. For example, this function was used to exclude the struvite precipitation step as too little is known about the costs and benefits of this process.

III Recommended reading

O'Keeffe, SM. Alternative Use of Grassland Biomass for Biorefinery in Ireland : A Scoping Study. (2010).

- Thesis in which the possible introduction of grass refinement in Ireland is extensively discussed.

O'Keeffe, S, Schulte, RPO, Sanders, JPM & Struik, PC. I. Technical assessment for first generation green biorefinery (GBR) using mass and energy balances: Scenarios for an Irish GBR blueprint. *Biomass and Bioenergy* 35, 4712–4723 (2011).

- First of two papers in which the introductino of a green biorefinery is discussed. Focusses on the technicall aspect.

O'Keeffe, S, Schulte, RPO, Sanders, JPM & Struik, PC. II. Economic assessment for first generation green biorefinery (GBR): Scenarios for an Irish GBR blueprint. *Biomass and Bioenergy* 41, 1–13 (2012).

- Second of two papers in which the introductino of a green biorefinery is discussed. Focusses on the economicall aspect.

Petrides, D. *Bioprocess Design and Economics*. (2003). Available from http://people.ufpr.br/~meleiro/graduacao/tq066/Bioproc_Design.pdf.

- Bookchapter that explains the fundamentals of bioprocess designs. Contains price estimations for severall processes and a guideline on how to design a biorefinement process. The information is s0omewhat outdated but is a good starting point.

IV Overview current initiatives

Initiative	Resource	End products	Stadium
Grassa!	Culture grass	▪ Protein (feed) ▪ Fibre (feed) ▪ Struvite	Commercially active, started demonstrating/promoting their mobile refinery in 2014.
HarvestaGG	Specifically produced Culture grass	▪ Liquefied Biogas (LBG) ▪ Protein (feed) ▪ Compost	First production plant end 2014 – begin 2015
Solidpack	Natural grass	▪ Fibres for cardboard	Pilot plant is ready for production
Newfoss**	Biomass	▪ Fibres for paper industry ▪ Biogas ▪ Fertiliser	Quality of fibre still insufficient for use in paper industry. Started building production plant in Heerenveen.
Indugras	Ensilaged nature grass	▪ Energy rich natural grass for cattle ▪ Sugar crude ▪ Biogas	Started second pilot project in December 2013. Commercial exploitation planned for 2015.
BELW Advies**	Culture grass	▪ Protein juice (feed) ▪ Waste fraction used for energy production, building industry or chemical industry.	First tests have been conducted. Contractor is going to produce 1.000 tons.
TNO/ ProLeaf	Sugar beet leaves	▪ Protein, Rubisco (food) ▪ Organic acids for biopolymers ▪ Biogas	TNO has a patent on the extraction process of Rubisco. Proof of concept on lab scale, pilot studies have been conducted.
ABC Kroos	Duckweed	▪ Protein (feed and food) ▪ Fibre fraction (feed) ▪ Cellulose ▪ Biogas	Several pilot locations in the Netherlands.

*) Table adapted from Visiedocument Valorisatie van Eiwithoudende Reststromen (2013).

**) Information not verified, might be outdated.