Bio-value chains for high-value products and compounds as an example for innovation and management for sustainable agroindustry

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Abstract. The rapid rise in the use of natural resources and the emission of greenhouse gases and extensive use of fertilizers to satisfy rising demand for food have led to a dynamic but unsustainable development even in agricultural areas. The principle of sustainability aims to conserve land, water and resources for future generations and to preserve the natural fertility of the soil. The BIVAC project seeks to develop novel, economically interesting products from residues streams produced in the agricultural sector. This surplus biomass could be very diverse. Residues are presently mostly returned to the fields, used in biogas plants or treated as waste. The material often contains valuable components, which could still be of use for the production in other companies. The project aims to determine which components can be found in these residue streams. To this end, different analytical methods are used ranging from photometric determinations to HPLC but also including determination of proteins using the Kjeldahl method or determination of parameters as dry matter, carbon and nitrogen content, analysis of heavy metals or the vitamin C content. The analyses carried out so far show that different residue streams contain candidate components that could be valuable for companies that do not primarily deal with agricultural products.

1. Introduction
In the last years, the effects of climate change have become visible by an increased number of extensive droughts, heavy rainfall events and extreme weather situations. In 2015 Steffen et al. published a research on socio-economic and earth system trends (1). The data presented there show exponential post-industrial rise since 1950. The use of natural resources and the emission of greenhouse gases have exponentially increased since then. This intensive use of our natural resources contrasts with the principle of sustainability, which was first described in 1713 by Hans Carl von Carlowitz (2). An overuse of mineral resources, also of soil, water and nutrients for increasingly intensive agricultural production does not represent a sustainable solution. The rising demand for food and extensive use of our natural environment led to a production exceeding the world bio-capacity in the 1990s. In today's world, the demand for food production continues to grow. This is not only due to the constantly growing population, but also the greatly changing human habits in recent decades. However, the global agricultural area has not increased. Ideas and agricultural methods are needed to intensify agricultural production while protecting the environment and consuming fewer resources.

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The agricultural production of food and other raw materials for the industry is an extremely complex system of methods that also differs greatly from region to region. Thus, not only the climatic conditions vary globally, but also the differences in traditions and cultural peculiarities play an important role. In some regions, grain production dominates, in others it is oil fruits, in others horticulture dominates, and so on. Due to these different circumstances, the approaches for an ever more sustainable agriculture will also have to look very different from region to region. The ever more globalised agricultural market also represents a challenge for sustainable agriculture. The relocation of agricultural production to areas with particularly favourable conditions certainly has economic advantages and promises high efficiency. However, the intensification and change of land use forms is often also a cause of emissions and social difficulties for the regional population. Diversification of agricultural production can help to address these difficulties and other problems such as biodiversity reduction. Thus, the solution to the problem certainly does not consist solely in an ever-increasing intensification. The further technical development of agriculture will certainly make an important contribution. Methods such as smart farming should and will combine economic and ecological advantages. Fertilizers and pesticides can be saved and less energy consumed. But local low-tech solutions also make an important contribution to maintaining village structures and regional identity, as well as the diversity of agricultural structures and thus the diversity of species. In order to make the development of agriculture more sustainable, we need not only the economic profitability but also the environmental stewardship and the social responsibility. This form of development of agricultural production is already strongly demanded in many countries. Producers and processors of agricultural products will have to take greater account of this in future.

Another important approach to improving sustainability in the agricultural industry is to avoid losses. Losses due to pest infestation, but also during harvesting and storage, play an important role here. However, many harvested products are not fully used. Large parts of the field crops, which are cultivated with great effort, become a waste stream immediately after harvesting or during processing. This, too, is a great potential for the agricultural industry. All these substances contain carbohydrates, fats and proteins, which can be a potential raw material for the food and other industries.

These industries often do not exactly know, when and where possible residue streams are available and thus opportunities remain unused. The use of these hitherto unused residue streams provides a chance for sustainable use. Here, the project BIVAC tries to make a first step.

1.1. BIVAC-project

The project BIVAC (acronym for bio-value-chains) is funded by Interreg Deutschland Nederland and addresses the development of novel, economically interesting products and products that are based on residue streams originating mainly from the cabbage and wood production. Other residue streams from the agroindustry (like leek and onion residue streams) have proven to be promising as well and are thus included in the initial analysis of potentially interesting compounds. Project partners from industry and academia situated at very diverse points along the value chains are brought together to exchange knowledge with the aim to develop new methods or products from the hitherto unused residue streams to fulfil the growing needs for products and at the same time to use the agroindustrial products in a sustainable manner. The envisioned products range from health-promoting food additives to the use of fermentable sugars for the production of astaxanthin and enzymatically optimized feed-additives as shown in figure 1.
Figure 1. Main tasks addressed in the BIVAC project. Next to astaxanthin and proteins, the project aims at the identification and extraction of high value compound from vegetable and wood residues.

The project further aims to set up a regional residue streams database and to bring producers together to enable an exchange of the streams. This aims at a sustainable use of the produced feed and food streams to meet the growing need for sustainable production.

1.2. Residue stream diversity
The residue streams that were analysed throughout the project were very diverse, reaching from the outer leaves and stalks red and white cabbage to cut grass from roads and including outer peels of red and white onion, rhubarb leaves, as well as the cut-off green parts of carrots and cabbage turnip to be sold in supermarkets. We even received underwater plants from a nearby stream that are regularly removed to prevent eutrophication and reduction of oxygen levels due to degradation of organic matter.

All of these residue streams are currently either returned to the fields where the vegetables are harvested or are transported to recycling stations where they are transformed into compost. All the valuable ingredients are lost and additional organic matter and substances like phosphates are transferred onto the field. For the transport, additional fossil fuels are used and emissions are produced.

1.3. Analysis of residue streams
For all of the residue streams, some basic parameters like wet and dry matter, the amount of heavy metals, the protein content following Kjeldahl were determined. Additionally, some more parameters like the presence of different sugars, the amount of vitamin C and the glucose amount were determined.

Some of the samples, especially the white and red cabbage samples were also analysed for their polyphenol content.

2. Materials and methods for analysis

2.1. Determination of dry matter
Depending on type and size of the sample, the samples were split into even portions. The wet weight of each of these portions was determined. Liquid samples were dried using the Eppendorf Concentrator Plus set to 60 °C and water-based samples. After completion of the drying, the dry matter was determined gravimetrically.

Solid samples were dried using an incubator set to 65 °C with forced air ventilation for 24 hours. After drying, the dry matter was determined gravimetrically. For red and white cabbage, portions of the sample were dried at different temperatures. Part of the sample was freeze-dried.

2.2. Nitrogen determination after Kjeldahl
The method is adapted from the publication by Kjeldahl (3) and a standard reference for food analytics (4).
The dried sample is first milled to maximize the surface. An amount of 0.5 g is mixed with one ground Kjeldahl tablet (Carl Roth, Germany), a few glass beads and 20 mL sulphuric acid in a Kjeldahl flask. The mixture is carefully heated and kept slightly boiling until it is clear and only slightly bluish in colour. After 15 min post heating with low heat input, the mixture is allowed to slowly cool to room temperature.

After cooling, the content is gradually and carefully diluted with 40 mL distilled water, transferred to a 100 mL volumetric flask and filled up to the mark after cooling.

10 mL of the diluted acid digestion are transferred to a 250 mL round bottom flask and mixed with 45 mL 50% sodium hydroxide solution (the sample should become cloudy during this step). The flask is put into a distillation apparatus and the outlet of the apparatus should be dipped into 20 mL of a mixture of boric acid and an indicator (containing bromocresol green, p-nitrophenol, aqueous neucoccein solution and sodium hydroxide).

Distillate until the colour of the indicator solution changes from green to blue, then continue distilling for another 10 minutes, lower the original and finish the distillation after another minute.

The distillate is quickly titrated with hydrochloric acid solution until the first colour change (from blue to green), then dropwise (from green to yellow).

The amount of solution consumed is used to determine the percentage protein content (P) using the following formula:

\[
P = \frac{(a - b) \cdot 1.4008 \cdot F}{E}
\]

- \(P\) protein content of the sample (in %)
- \(a\) amount of hydrochloric acid solution (0.1 mol/L) in main test in mL
- \(b\) amount of hydrochloric acid solution (0.1 mol/L) in blank test in mL
- \(F\) conversion factor for calculating the protein content (depending on sample type, here 6.25 for food is used)
- \(E\) sample weight in g

The factor 1.4008 is used because the consumption of 1.4008 mL hydrochloric acid solution (0.1 mol/L) corresponds exactly to 1.4008 mg nitrogen.

2.3. Determination of heavy metals by inductively coupled plasma mass spectrometry (ICP-MS)

The milled sample is extracted to release all the metals. This extract is used for the analysis. Different dilutions of the sample are measured as well as standards. From the comparison of the peaks with each standard, the presence of heavy metals and also the concentration can be determined.

2.4. Detection of the vitamin C content

The vitamin C content was photometrically determined using the L-ascorbic acid determination kit by R-Biopharm (10409677035, Boehringer Mannheim, Germany) (5). The instructions for flour were followed for the milled samples.

The sample is mixed with meta-phosphoric acid to form a homogenous dispersion. A filtration step removes all particles that could interfere with the photometric analysis.

The amount of ascorbic acid is measured against the blank where all ascorbic acid is removed with ascorbate oxidase during the test. From the measurements, the concentration of ascorbic acid is calculated.

2.5. Detection of the glucose content during cellulose enzymatic breakdown

The samples are dried and milled. Of the milled sample, 0.5 g is mixed with a cellulose enzyme solution containing 100U/mL. The solutions are incubated at 37 °C under agitation (300 rpm) for 72 h. From the solutions, 1 mL samples are taken at the beginning, after 4 h, 24 h, 48 h and 72 h. The enzyme in the samples is inactivated by incubation at 90 °C for 5 min. The samples are then kept frozen until analysis.
For analysis, samples are thawed and centrifuged for 5 min at 17000 g to pellet the solid phase. The supernatant is transferred to new tubes. Of this, 10 µL is used for the protocol provided with the Megazyme D-Glucose-HK assay (6).

2.6. Detection of sugars via HPLC
For HPLC analysis, the milled samples were extracted under agitation in demineralized water for 24 h. The extracts were centrifuged for 5 min at 4600 g and the supernatant was then filtered through a 0.2 µm syringe filter.

Analysis of the pre-treated samples was performed using the Rezex™ RCM-Monasaccharide Ca⁺² (8%), LC Column 300 x 7.8 mm (part No:00H-0130-K0, Phenomenex, Aschaffenburg, Germany).

Water was used as mobile phase, with a flow rate of 0.5 mL ∙ min⁻¹. The column temperature was set to 70 °C and the temperature of the RI detector to 35 °C.

An injection volume of 10 µL was used. Peaks are identified by using standards with different concentrations which are measured under the same conditions.

3. Result

3.1. Determination of dry matter
The different samples were all collected at different times and from different locations. All were treated as described in the materials and methods section. What is remarkable here is that the red and white cabbage sample that was split into portions, does give different ratios of dry to wet weight depending on the treatment during drying. All results are given in table 1.

| Sample                        | Wet weight (g) | Dry matter (g) | Dry/Wet (%) |
|-------------------------------|----------------|----------------|-------------|
| carrot-green                  | 187            | 23.08          | 12.34       |
| carrots                       | 239            | 23.37          | 9.78        |
| grass                         | 100            | 18.75          | 18.75       |
| Kohlrabi                      | 244            | 14.87          | 6.09        |
| Kohlrabi-green                | 89             | 9.13           | 10.26       |
| pea (pods and stems)          | 50             | 6.9            | 13.80       |
| radish                        | 343            | 12.11          | 3.53        |
| radish-green                  | 93             | 6.21           | 6.68        |
| red cabbage dried at 60 °C    | 375            | 42             | 11.20       |
| red cabbage dried at 80 °C    | 490            | 33             | 6.73        |
| red cabbage freeze-dried      | 432            | 48             | 11.11       |
| rhubarb                       | 50             | 2.8            | 5.60        |
| white cabbage (old variety)   | 717            | 48.95          | 6.83        |
| white cabbage dried at 60 °C  | 336            | 18             | 5.36        |
| white cabbage dried at 80 °C  | 247            | 21             | 8.50        |
| white cabbage freeze-dried    | 288            | 21             | 7.29        |

3.2. Nitrogen determination after Kjeldahl
The nitrogen determination following Kjeldahl was performed in duplicates. The difference between the different treatments of the white cabbage samples is most likely due to nitrogen loss during drying at the higher temperature. It remains unclear why this is not the case in red cabbage. The detailed results are given in table 2.
Table 2. Overview the protein content of different samples.

| Sample                  | Protein (mg/dry matter) |
|-------------------------|-------------------------|
| alfalfa                 | 16.93                   |
| carrot                  | 22.76                   |
| carrot-green            | 12.91                   |
| grass                   | 14.00                   |
| kohlrabi                | 76.38                   |
| kohlrabi-green          | 23.85                   |
| onion                   | 7.44                    |
| pea (pods and stems)    | 50.25                   |
| radish                  | 51.21                   |
| radish-green            | 12.91                   |
| red cabbage dried at 60 °C | 17.32               |
| red cabbage dried at 80 °C | 102.45              |
| red cabbage freeze-dried| 8.01                    |

3.3. Determination of heavy metals by inductively coupled plasma mass spectrometry (ICP-MS)

Table 3 shows the heavy metals detected by ICP-MS in the different samples. In most of the cases, the concentration was below the detection limit. Onions have been reported to contain lead between 2 and 6 mg/kg previously (7), so it is not surprising that this value was detected.

Table 3. Overview of the amount of different heavy metals detected by ICP-MS.

| Sample                        | Cd (mg/mL) | Cr (mg/mL) | Cu (mg/mL) | Ni (mg/mL) | Pb (mg/mL) | Zn (mg/mL) |
|-------------------------------|------------|------------|------------|------------|------------|------------|
| alfalfa extract               | < 0.1      | < 0.1      | 0.133      | < 0.1      | < 0.1      | 3.599      |
| carrot extract                | < 0.1      | < 0.1      | < 0.1      | < 0.1      | < 0.1      | 0.184      |
| onion extract                 | < 0.1      | < 0.1      | < 0.1      | < 0.1      | < 0.1      | 0.104      |
| rhubarb                       | < 0.2      | < 0.2      | 0.230      | < 0.2      | < 0.2      | 0.228      |
| white cabbage extract         | < 0.1      | < 0.1      | < 0.1      | < 0.1      | < 0.1      | 0.311      |

3.4. Detection of the vitamin C content

The milled samples of cabbage were screened for their vitamin C content. White cabbage is known to contain 50 mg/100g vitamin C, red cabbage 55 mg/100g (8). Surbhi et al. did analyse the vitamin C content of white cabbage outer leaves and found the content to be 399.2 ± 1.42 mg/100g in uncooked leaves and 81 mg/100 g in white cabbage that had been dried at 80 °C (9). The results of the white cabbage dried at 80 °C were under the detection limit of the kit (0.3 mg/100g). All results are shown in table 4.

Table 4. Overview of the vitamin C amounts in different red and white cabbage samples.

| Sample                              | Vitamin C (mg/100 g) |
|-------------------------------------|----------------------|
| red cabbage dried at 60 °C          | 3494.59              |
| red cabbage dried at 80 °C          | 114.52               |
| red cabbage freeze-dried            | 8317.08              |
| white cabbage dried at 60 °C        | 2258.02              |
| white cabbage dried at 80 °C        | < detection limit     |
| white cabbage freeze-dried          | 3428.97              |
The detected vitamin C contents were generally higher than those found in literature. Only the amount in the white cabbage was lower than the one determined by Surbhi. This can be due to differences in the duration of the drying.

3.5. Detection of the glucose content
Figure 2 shows the amount of glucose detected in samples during the degradation of cellulose by cellulase.

For onion, the glucose content does rise from 0.79 mg/g dry matter to 3.34 mg/g dry matter after 72 h. A similar constant rise does show with wheat straw, although the total content is a bit lower. For the water-plants, the glucose content does rise in the first 4 h, but then continually decreases. For cellulose, there is a rise in the glucose content up to 24 h, then the glucose content does decrease.

![Figure 2. Glucose content in different samples during cellulase enzymatic breakdown.](image)

3.6. Detection of sugars via HPLC
The results of the HPLC sugar detection for the different samples are shown in the figures 3 to 7. Figure 3 shows the peaks for the rhubarb sample. The peaks show (from left to right): fructo-oligosaccharides and dextrin, sucrose (and possibly lactose if present), glucose, galactose and a combination of arabinose and fructose in the last peak.

![Figure 3. Sugars detected by HPLC in the rhubarb sample.](image)
Figure 4 shows the peaks for the pea sample. The peaks show (from left to right): fructooligosaccharides and dextrin, raffinose, sucrose, glucose, galactose, arabinose and fructose, an unidentified peak (16.902 min), mannitol and another unidentified peak (19.391 min).

![Figure 4](image_url)

**Figure 4.** Sugars detected by HPLC in the pea sample.

Figure 5 shows the peaks for the white cabbage sample. The peaks show (from left to right): fructooligosaccharides and dextrin, sucrose, glucose and arabinose and fructose combined in the last peak.

![Figure 5](image_url)

**Figure 5.** Sugars detected by HPLC in the white cabbage sample.

In this case, the amounts of some of the sugars could be calculated based on the standards. The results are shown in table 5.

| Time (min) | Sugar            | Concentration (mg/mL) |
|------------|------------------|-----------------------|
| 7.212      | Unidentified     |                       |
| 9.083      | Sucrose          | 0.36                  |
| 10.935     | Glucose          | 4.63                  |
| 14.054     | Arabinose/Fructose|                      |

**Table 5.** Overview of the sugar concentration white cabbage sample.
Figure 6 shows the HPLC analysis results of white cabbage (left) and red cabbage (right) that have been dried at 80 °C before analysis. When this spectrum is compared to the spectrum shown in figure 5, it becomes clear that there are more peaks. These are most likely degradation products of the sugars that are formed during the drying at the high temperature of 80 °C.

Figure 6. Degradation products detected by HPLC in white (left) and red (right) cabbage dried at 80°C.

Figure 7 shows the peaks for the onion sample. The peaks show (from left to right): fructo-oligosaccharides, an unidentified peak (8.12 min), sucrose, glucose, a peak that can represent a mixture of mannose, xylose and galactose, arabinose and fructose and two more unidentified peaks at the end.

Figure 7. Sugars detected by HPLC in the onion sample.

4. Conclusion and outlook
The analyses performed show that there are some candidate ingredients in the analysed residue streams. Up to now, the sugar contents found are too low for fermentation and thus one of the current steps is to determine whether a concentration of the streams is worthwhile.

The cellulose breakdown is currently further analysed to optimize the amount of glucose released from it. If this is successful, this could provide a method to bring up the concentration of glucose by combining several residue streams.

The main task now is to bring producers of the residue streams and possible users together and set up an availability database that also covers the availabilities and variations of the stream components. The variations in the streams can have different causes ranging from differences in fertilizers used, but also factors like the distance to roads, concentrations of nitrate and nitrite in the water, possible water stress conditions or even longer transport after harvesting.

To get an accurate picture, analysis of many more samples from different fields, different harvesting times and also with different pre-treatments is necessary. Thus far, there are first ideas for cooperation, but they are not yet fully developed.
Now that the project is progressing, the idea is spreading to more and more producers and there are plans for meetings between different producers and companies who are interested in the use of the residue streams.

Most of the streams can not be used as they are directly, so refinery steps of one kind or the other will be necessary. Several companies are looking into the chances but also at the costs for the necessary steps.

This one project will surely not be able to save the world on its own, but if several of these activities are carried out, they can together cause a positive change towards a more sustainable use of the available resources.

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