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Carbohydrates as raw material from a Green BioRefinery

Carbohydrates analysis of green plant juices after wet-fractionation of grass

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Zusammenfassung

Grüner Presssaft von BioRaffinerie-Originalrohstoffen (Wiesenmischgras und Luzerne nach einer Nassfraktionierung und der Protein-Abtrennung) wurde mittels Gaschromatographie/Massenspektrometrie untersucht. Die im Presssaft enthaltenen Kohlenhydrate wurden derivatisiert und identifiziert mittels ihrer Retentionszeiten im Gaschromatogramm bzw. ihrer EI Massenspektren im Vergleich mit Daten der reinen Vergleichssubstanzen. Zusätzlich wurden CI Massenspektren zur besseren Charakterisierung der anwesenden Kohlenhydrate herangezogen. Die Kohlenhydrate, die auf diesem Wege identifiziert werden konnten, wurden mittels ‚response signals' bezüglich des inneren Standards ß-Phenyl-D-glucopyranosid quantifiziert.

Summary

Green juices from Green Biorefinery original raw-material (*wild mixed grass* and *alfalfa* after wet fractionation and protein separation) have been investigated by gas chromatography/mass spectrometry. The Carbohydrates, involved in the green juices, were derivatized and identified by both their retention times in the gas chromatogram and EI mass spectra compared to those of pure reference compounds. Additionally, chemical ionisation mass spectra were recorded for better characterizing of the carbohydrates present. The carbohydrates which could be identified in this way, were quantified by response signals with respect to that of the internal standard ß-phenyl-D-glucopyranoside.

Introduction

The Green BioRefinery (figure 1) is a system for whole substantial and energetic utilisation of green plant and waste biomass. The renewable resources, especially grass from an extensive land cultivation, or natural resources and alfalfa from an intensive land cultivation, are an excellent BioRefinery-raw material. Carbohydrates inherented in plant materials are fundamental secondary raw materials beside proteins, amino acids, fibres, hormones, enzymes and dyes etc.

The first step of the technology is the so-called wet-fractionation. This process separat the plant material into its water-insoluble contents (Press Cake) and a carbohydrates- and protein-rich water-soluble fraction (Green Juice or Brown Juice) by using pressure. A carbohydrate-rich juice (carbohydrate fraction) is available after precipitation of the plant protein by acidifying or heating. These juices are an excellent fermentation medium e.g. for the production of lactic acid. Additionally, the carbohydrates are suitable for application in pharmaceuticals, surfactants, medicine and building blocks for synthesis (chirality pool) [1].

Problems

In the first step we were looking for an appropriate analytical method that would able to detect the different single carbohydrates in the juice.

Juice from BioRefinery original raw-material *(wild-mix grass)* after fractionation and protein separation has been investigated by this method. GC-MS (gas chromatography-mass spectrometry) is the method of choice for the identification of small oligosaccharides, especially capillary GC-MS is made a routine technique.

The main problem for us was to optimise the method for the identification and reproducible quantification of derivatized carbohydrates from green juice in a short time.

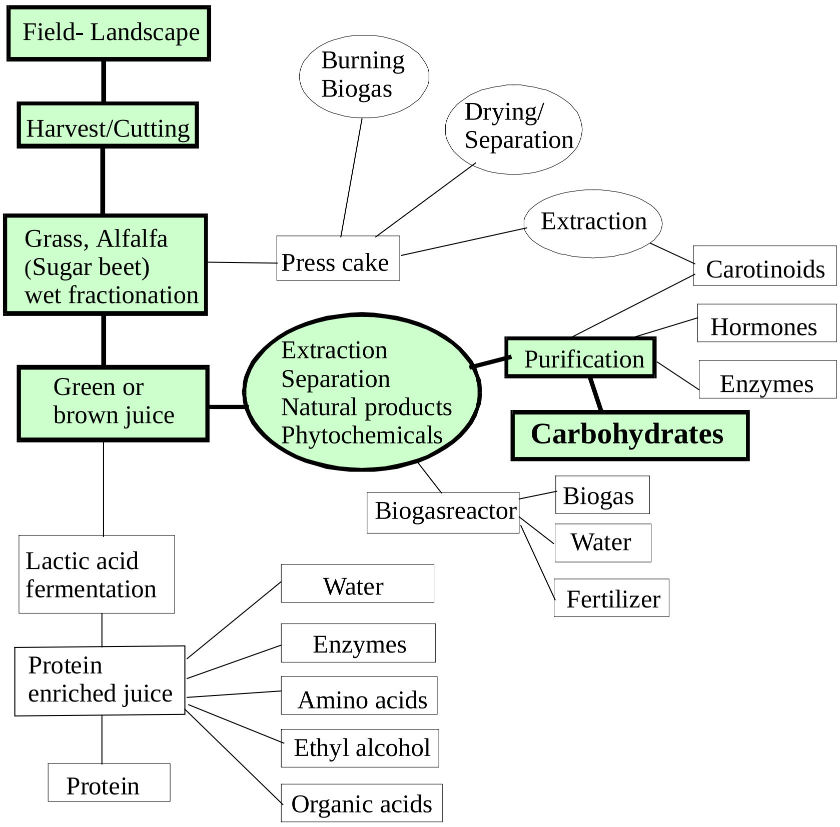

Figure 1: Carbohydrates from a system of a Green BioRefinery (see also Kiel, P. & Kamm in [1])

Raw materials and methods

Raw materials from a Green BioRefinery:

A) wild mixed grass

The green juices were obtained from the wild mixed grass of the area Vietnitz/Havelland. The grass was pressed and then the juice heated 10 hr at 80°C and later stored at 5° degree in the refrigerator.

B) *alfalfa*

The alfalfa (*Medicago sativa*) cutted from a field in the region of Selbelang/Havelland was used; the whole plant not only the leafs was used for the analysis. The proteins was separated according to the method *Carrez I* and *Carrez II*. The samples thus obtained were sterilly filtrated and then fast frozen at –18°C.

The sample preparation involves the hydrolysis of the saccharides in TFA; the sugars are dissolved in anhydrous pyridine and treated with HMDS or BSTFA to form the TMS- ether [2]. The saccharides-trimethylsilylethers were identified by their retention time on the GC compared to external standards (as well as their mass spectra). Additionally chemical ionisation spectra were taken for the characterisation. All identified saccharides were quantified by the response relating to an internal standard such as beta-Phenylglucopyranoside [3].

Results and Discussion

In the GC-MS study of the plant samples all components of more than 5% intensity of the peak area of the inner standard were identified.

The chromatogram of the detectable components in a grass mixture is shown in Fig. 2.

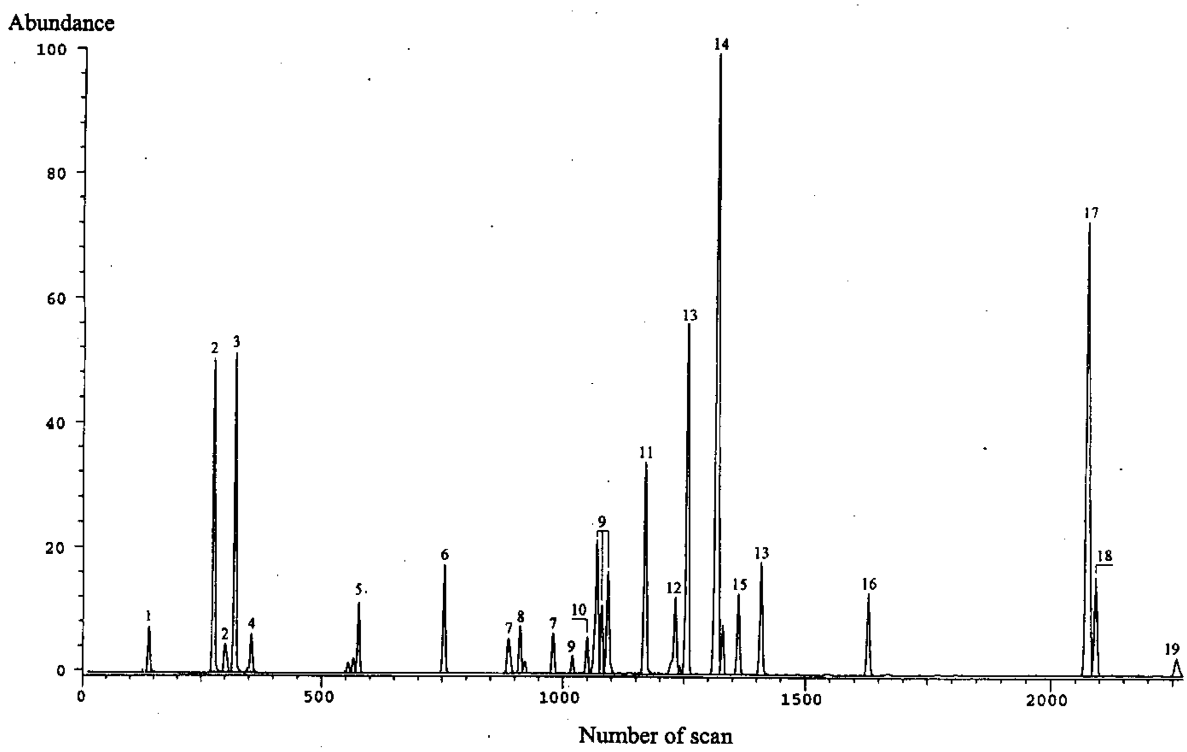

Figure 2: Gas Chromatogram of the TMS derivates of sample A) *wild mixed grass* juice (see also table 1.)

Linear relationships between the integrated peak areas Acarb/Ast on the total ion current (TIC) and the analyte/standard concentration Ccarb/Cst were found for all carbohydrates (see Table 1). The reproducibility proved to be of very good quality since the repeated estimation of the areas [%] of the different sugars change only < 10%.

Table 1: The results of the quantification of the carbohydrates of nonhydrolyzed and hydrolyzed samples of *wild mixed grass* juice (see also figure 2)

		Concentration of carbohydrates in grass juices [mg/l]	
Peak number	carbohydrates	nonhydrolyzed juice	hydrolyzed juice
2	Glycerol	80	77
5	Erythrose	12	8
6	Rhamnose	17	11
7	Xylose	8	7
9	Fructose	71	102
11	Galctose	52	64
12	Mannose	15	21
13	Glucose	85	91
14	Mannit	173	182
15, 16	Myo-Inositol	44	25
19	Maltose	56	8

All obtained results from *wild mixed grass* juice and *alfalfa* juice are described in [4].

Conclusions

The application of the method GC/MS described above proved to be effective for monosaccharides and carboxylic acids of green juices of wild mix grass and freshly pressed alfalfa juices. These investigations are the first steps for possible industrial application of natural sugars. If sufficient amounts of carbohydrates could be extractet, there is the possibility tot their further use in pharmacy, medicine and cosmetic.

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