Waxes and Lipids Extraction from Miscanthus Giganteus Stems.

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WAXES AND LIPIDS EXTRACTION FROM MISCANTHUS × GIGANTEUS STEMS

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Introduction

In the framework of the project BIORAF, supported by Technology Agency CR (project no. TE01020080), plantations for experimental production of *Miscanthus* × *giganteus* and *Miscanthus sinensis* have been founded by the project partner AGRA Co. Tall stems of this plant contain a broad spectrum of various substances (waxes, lipids, carotenoids, etc.) being potentially exploitable in cosmetics. Extraction processing of crushed dry stems using non-polar solvent is a promising way for separation of these substances into an extract, whereas the waste raffinate containing waste biomass has, after being pressed into the form of pellets, the potential for energetic utilization as "green fuel".

Method

Large production plantations of bio-energetic plants *Miscanthus sinensis* and *Miscanthus* × *giganteus* were established and harvested (see Figure 1). Subsequently, the dried stems were crushed (see Figure 2). One part of it was used for extraction experiments described in this text and the other for pelleting and incineration tests.

The aim of extraction experiments was to isolate non-polar components from the stems; therefore, hexane as a solvent was used. Both the Soxhlet extractor with a stationary layer of crushed raw material operating at solvent boiling point temperature (69 °C) and the batch extractor with dispersed solid phase operated at room temperature were used for extraction tests.

Application of carbon dioxide under supercritical conditions (temperature 70 °C, pressure 16 MPa) as a solvent for extraction was also tested. Nevertheless, efficiency of this method was very low and moreover, this method required very expensive high pressure equipment. Regarding this, it was stated that this procedure has no technological perspective and is not recommendable for practical usage.

Soxhlet extraction

When using a Soxhlet extractor for the extraction, 20 g of the sample was placed into a paper thimble and approximately 600 ml (i.e. 400 g) of hexane into a boiling flask. The weight ratio of the raw material/solvent 1/20 was always applied. The extraction took place at the boiling point of the solvent (69 °C). The condensation solvent ran down through the layer of the extracted material back into the boiling flask afterwards. The sample was extracted for 2 h. Subsequently, a further experiment was conducted. In this case one batch of solvent per three freshly filled paper thimble was used. The weight ration applied in this case was the sample/solvent 1/6.7 (3 x 20 g to 400 g of hexane). The extraction of each of these thimbles lasted for 1 h. By using the Soxhlet extractor, the obtained extracts possessed a clear yellowish colour.

Single batch extraction

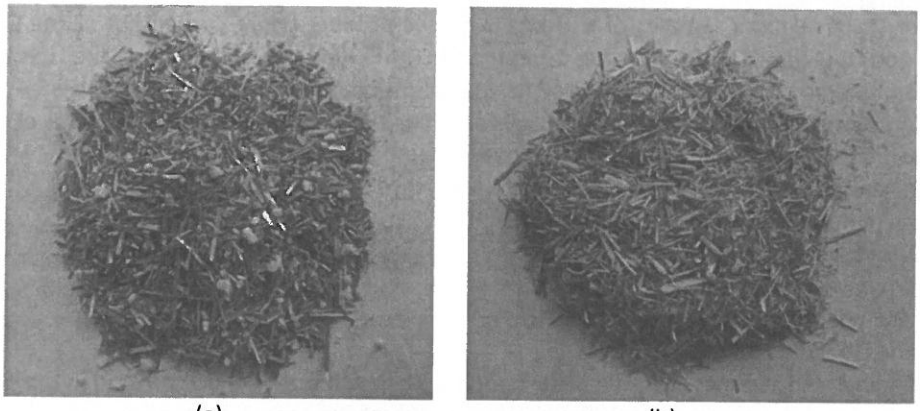
Batch extraction in stirred extractor was made under inert atmosphere, absence of light and at the ambient temperature in a sealed glass flask. The mixture was stirred for 6 h maintaining the weight ratio of the raw material/solvent 1/20 (corresponding to the preceding case). The solid part was filtered by Büchner funnel afterwards. This process resulted in a slightly turbid, yellowish extract.

Supercritical extraction

Applying liquid carbon dioxide for supercritical extraction utilizes its non-polar characteristics and considerate experimental conditions. On the other hand, this method of separation is a demanding process requiring high pressure and expensive equipment. Nevertheless, this method was experimentally tested twice. In this case, the raw material was crushed and sieved for fraction of 0.125 mm. The sample of 3.6 g (25 ml) was placed into a press cartridge. Liquid CO_2 was flowing through the cartridge for 5 h at the speed of 0.3 ml/min. The extracts

from both experiments were removed into a vial of 5 ml in which the lipophilic product deposited after the pressure release. Figure 3 shows the results of both tests in which 7.18 g of *Miscanthus* × *giganteus* was extracted but only a very low amount of extract of 0.0214 g was obtained. The product was dissolved in 4 ml of cyclohexane and subsequently analysed. It was concluded that from the technological point of view this method of processing stems of *Miscanthus* × *giganteus* is by no means applicable.

Figure 1. Laboratory tests of cultivars and flowering *Miscanthus* × *giganteus*.



(a) (b)

Figure 2. Crushed stems of *Miscanthus sinensis* (a) and *Miscanthus* × *giganteus* (b).

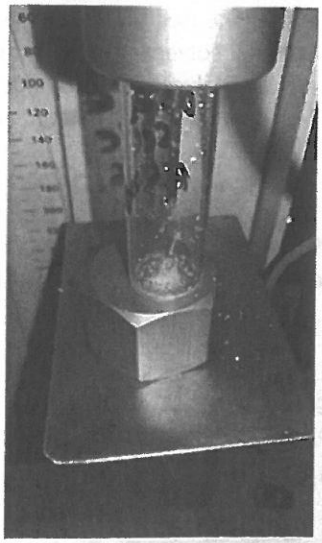
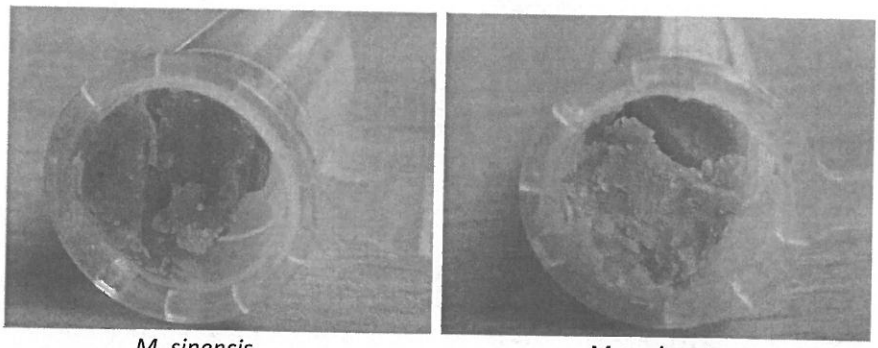


Figure 3. Particles of lipophilic product after supercritical extraction of *Miscanthus* dust proportion.

Characterization of the products

The extraction solvent was distilled from obtained extracts on a rotary evaporator under reduced pressure. The conditions were carefully prepared in order to prevent potential oxidation of obtained components and the working temperature was 40 °C. The obtained yellowish orange waxy solid product (see Figure 4) was analysed. In general it might be claimed that *Miscanthus sinensis* provided higher yields and its solid product was of deeper colour shade within the range of yellow tending to orange in colour.



M. sinensis *M. × giganteus*

Figure 4. Samples of solid products after the extraction from the stems of *Miscanthus*.

Analysis of extracts and solid products

A detailed analysis of selected samples of extracts and solid products were carried out at the Department of Food Analysis and Nutrition FFBT UCT Prague using the HPLC/MS method. Screening via the LipidView software and information based on literature[1-3] were used for identification of the searched substances.

The chromatograms confirmed the following categories of components contained in the stems of *Miscanthus*:

- Sterols and their derivatives (3 and 18 derivatives)
- Waxes (esters of fatty acids and alcohols, 15 derivatives)
- Ceramides (4 derivatives)
- 1-Alkyl-2-acylglycerols (2 derivatives)
- Oleamide
- Alkanes and polykosanoles (3 and 7 derivatives)
- Triglycerides (45 derivatives)

It is evident that analysed samples are extremely complex. Their composition is illustrated in the Tables I and II. The data in Tables I and II indicate the relative signal strength of the chromatograph detector (a.u.) and define particular components or groups of substances.

The first four columns from the left correspond to the analyses of solid products, whereas the right four columns represent the analyses of liquid extracts. Based on the analyses, it is evident that the heat treatment of the extract during the extraction solvent evaporation (hexane solvent) causes the degradation/transformation of mono- and diacylglycerols, ceramides, stigmasterols, or campesterols. The amount of extracted components and their groups in the extract and solid products varies in the case of triacylglycerols.

Acknowledgement

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References

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Table I
Relative representation of key components in the extract and solid products (a.u.)

	Solid products				Extracts			
Compound / sample	M7E6h	M4E6h	M7S2h	M4S2h	M7E6h	M4E6h	M7S2h	M4S2h
Sterols and their derivatives								
stigmasterol/stigmast-4-en-3-on	10173125	4335919	1894307	3097894	5892364	9727372	1323061	1152025
campesterol	61265	38500	33887	34375	36464	41703	18266	17731
sitosterol	636976	159592	130588	120286	343374	195562	51752	54073
sitosteryl ester 18:2	4496027	422471	773265	1075848	2988482	2167868	460633	406896
sitosteryl ester 18:3	1276752	169376	267596	435202	1030963	848794	160413	161704
campesteryl 18:1	78445	15416	28428	68408	74058	94501	14354	21333
campesteryl 18:2	754593	126746	162177	260730	687749	564974	99252	95946
campesteryl 18:3	281758	35738	64705	100509	275602	238900	38072	36906
stigmasteryl 18:3	323025	38141	64324	122810	347547	250169	45174	39165
Waxes (fatty acids and alcohol esters)								
Palmityl palmitate	7586	2458	3073	5751	10052	57970	3589	3510
Palmityl palmitoleate/Myristoleyl stearate/Myristyl oleate	11911	ND	8859	11621	47347	134753	6766	8652
Arachidyl palmitoleate/Oleyl stearate	18431	ND	14664	24579	80204	294795	13691	26823
Stearyl palmitoleate/Arachidyl myristoleate/Oleyl palmitate	15232	ND	12162	23872	108568	234608	13847	23270
Ceramides								
Cer 36:0;2	ND	ND	ND	ND	4153816	8132289	525812	1233483
Cer 34:0;2	ND	ND	ND	ND	11222166	13423396	3339530	6270314
Cer 32:0;2	ND	ND	ND	ND	11011661	13702342	2966311	4829812
Cer 38:0;2	ND	ND	ND	ND	563841	835736	89256	295382
1-Alkyl-2-acylglycerols								
MADAG 32:0	228157	34196	33631	45993	2066328	4019396	330147	539662
MADAG 34:0	64422	11067	12267	18040	7224063	10388213	1248029	1408694
Oleamide	65199	20005	16964	31391	7342419	8956457	3198631	3517955

The codes in Tables I and II for each of the samples indicate the raw material used, the method of extraction, and extraction time, e.g.:
M7E6h: *Miscanthus* × *giganteus*, one-stage batch extraction for 6 h; M4S2h: *Miscanthus sinensis*, Soxhlet extraction for 2 h.

Table II

Relative representation of triacylglycerols in the extract and solid products (a.u.)

Sample	Solid products				Extracts			
Triacylglycerols	M7E6h	M4E6h	M7S2h	M4S2h	M7E6h	M4E6h	M7S2h	M4S2h
TAG 42:0	17607	8697	14655	22709	48643	64125	11128	13433
TAG 50:1	633572	184827	177389	187801	545976	1027880	116535	104109
TAG 50:2	1239295	255728	272208	360231	1290335	1813069	180474	170386
TAG 50:3	733332	116471	130742	187928	570552	746692	93404	72692
TAG 50:4	209608	29209	43678	52468	208100	244602	21776	16405
TAG 52:0	167684	19238	16458	23872	3585154	5377101	501170	643674
TAG 52:2	4144036	590394	609502	1081790	2895957	4966669	356692	402910
TAG 52:3	4979895	815622	467312	899683	2925571	5195636	333463	348287
TAG 52:4	6196901	714947	713096	1090636	4808550	5060046	447953	435991
TAG 52:5	5332772	481411	646717	888084	4147440	3719004	341118	302234
TAG 52:6	1819092	146911	203354	305750	1334433	983887	131353	103646
TAG 52:7	131747	15341	13991	20914	116662	113422	10197	9526
TAG 54:2	594252	182971	112002	196124	456735	849459	71096	88559
TAG 54:3	3341674	692577	470324	1225483	2651418	5243622	300606	442941
TAG 54:4	4064950	641874	407124	854099	2282837	4614298	236262	328575
TAG 54:5	4652003	613829	440016	804942	2936602	4143981	277312	285587
TAG 54:6	5372514	579075	518222	900466	3697231	4305912	291377	300491
TAG 54:7	5066362	400194	426353	772586	4161117	3474401	282800	275962
TAG 54:8	2626586	196790	235880	410728	2005474	1347650	138884	139267
TAG 54:9	744316	52872	57544	100765	626035	390981	45141	36031
TAG 56:2	66376	19693	14631	27898	50463	97152	9924	11658
TAG 60:1	272265	67521	31270	89338	208094	312204	16566	31406
TAG 60:2	348518	69508	38813	100104	243992	353941	25035	41232
TAG 60:3	183956	37115	22200	46283	120778	166595	11557	18832