PROTEIN EXTRACTION FROM RED CLOVER

The supply of organic protein feed for monogastric animals (i.e. poultry and pigs) with the right amino acid profile and a competitive price is one of the major challenges for organic agriculture nowadays. In this project, the development of an organic protein concentrate able to substitute the soybean protein while competing with the current market prices is assessed. The product is developed from red clover biomass, using a sustainable technology without the use of inorganic acids or organic solvents. It also contains some lactic acid, which is supposed to be beneficial for the monogastric livestock. The quality of this protein concentrate is evaluated in terms of amino acid composition. The suitability of the obtained product as feedstock for poultry will be also studied.

Description of the biorefinery process (harvest-separation and protein precipitation)

Red clover was harvested on the 14th of May 2014; at Vamdrup, Denmark. Right after the harvest, the biomass was screw pressed obtaining a solid and a liquid fraction, namely press cake and green juice; respectively. The pressing was done using a Vincent CP4 screw press. 62.2 kg freshly harvested red clover were pressed, obtaining 37.3 kg green juice and 24.9 kg press cake. 3.5 kg biomass was lost during the mechanical process. The temperature and pH of the green juice after the mechanical separation were around 14.8^{0}C and 6.2, respectively. After the solid-liquid separation, the green juice was inoculated with an overnight culture of *Lactobaciluus salivarius* BS 1001 (20 ml per liter of green juice) and fermented at 38^{0}C overnight. At that time, the pH was 4.7 and the proteins were precipitated obtaining two fractions, namely brown juice and protein concentrate. In order to separate those two fractions, a centrifugation step (Centrifuge Beckman GS-6 cooling centrifuge equipped with a GH-3.8 horizontal rotor) was performed during 10 minutes at 3800 rpm and 5^{0}C.

Chemical composition of the different fractions and mass balances

The chemical composition of the different fractions obtained during the biorefinery process is presented in Table 1. The dry matter content of the fresh material was 164 g/kg, where organic matter represented 88%. Dry matter content in the press cake after the mechanical fractionation was increased 1.5-fold, when compared with the fresh biomass. The proteins in the press cake represented around 18% (dry matter basis), which was similar to the approx. 20% protein content in the fresh material on a dry matter basis. Most of the free sugars in the fresh biomass were recovered in the green juice after the mechanical fractionation. Those free sugars were fermented into lactic acid, decreasing the pH to final values of 4.7 and 4.3, in the brown

juice and protein concentrate, respectively. The protein concentrate presented a dry matter of 191 g/kg, of which 93% was organic. The crude protein content of the protein concentrate was 39.3% (dry matter basis).

Table 1. Chemical composition of the different fractions obtained during the biorefining process.

Parameters		Fresh material		Press cake		Green juice		Brown juice		Protein concentrate	
		Average	*STD*	*Average*	*STD*	*Average*	*STD*	*Average*	*STD*	*Average*	*STD*
pH		5.7	*n.d.*	5.5	*n.d.*	5.0	*n.d.*	4.7	*n.d.*	4.3	*n.d.*
Total solids	*g/kg*	163.6	*9.6*	236.6	*3.4*	62.2	*0.8*	24.6	*0.1*	191.3	*1.4*
Volatile solids	*g/kg*	144.5	*9.2*	217.7	*2.8*	51.0	*0.9*	18.8	*0.1*	178.2	*1.3*
Ash	*g/kg*	19.1	*n.d.*	18.9	*n.d.*	11.2	*n.d.*	5.8	*n.d.*	13.1	*n.d.*
Total Kjeldahl Nitrogen	*g/kg*	5.3	*0.2*	7.0	*0.2*	2.5	*0.1*	0.8	*0.1*	12.0	*1.5*
Crude protein	*g/kg*	33.1	*n.d.*	43.6	*n.d.*	15.7	*n.d.*	4.8	*n.d.*	75.1	*n.d.*
Free sugars	*g/kg*	23.0	*2.7*	n.d.	*n.d.*	23.6	*0.03*	7.8	*0.22*	15.3	*0.2*
Lactic acid	*g/kg*	0.0	*0.0*	0.0	*0.00*	0.0	*0.00*	6.8	*0.24*	14.8	*0.1*

n.d. stands for not determined

Mass balances are presented in Table 2. Although some loss of material was observed during the process, it was decided not to take them into account since they will be minimized when scaling up the biorefinery process. According to the mass balance based on fresh biomass, 60% of the fresh weight mass was recovered as green juice, obtaining 67 kg of protein concentrate per tonne of fresh red clover biomass.72% and 14% of the dry matter was recovered in the press cake and brown juice, respectively, which is convenient, as these two fractions will be used to produce biogas. 65% of the crude protein in the fresh biomass was recovered as fiber-bound protein in the press cake, whereas 23% of the crude protein in the fresh biomass was recovered in the protein concentrate.

Table 2. Mass balances for red clover separation-protein precipitation.

	Fresh biomass	Dry matter	Crude protein
	kg/t	*kg/t*	*kg/t*
Fresh material	1000	1000	1000
Press cake	401	718	649
Green juice	599	282	351
Brown juice	532	142	117
Protein concentrate	67	140	234

Amino acid profiles

The amino acid profile in the fresh material, green juice and protein concentrate was analyzed and it is presented in Tables 3 and 4. The data corresponding to the amino acids tryptophan and tyrosine are not included since the method that was used does not measure these two amino acids. The amino acid profile in terms of grams of amino acid per kilogram of true protein (i.e. sum of each amino acid concentration) was in the same range for the fresh material and the protein concentrate. However, it was slightly lower in the green juice, with exception of asparagine which up-concentrated compared with the fresh crop and the protein concentrate (Table 3). This fact explains that the amino acids contained in the fresh material were recovered during the biorefinery process ending up in the protein concentrate. The three fractions presented a balanced content of amino acids.

Table 4 presents the amino acid concentration on dry matter basis. As expected, the amino acid concentration in the protein concentrate increased up to 2.7 times when compared to the fresh material. The amino acid profile in the protein concentrate is comparable with an organic basal diet for poultry and with the soybean meal, which is the main source of protein for organic monogastric livestock. The concentration of essential amino acids such as methionine is higher than in the commercial organic basal diet and in the same range as the soybean meal.

Table 3. Amino acid profile in the different fractions through the biorefinery process.

	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser
	g/kg TP	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*	*g/kg TP*
Fresh material	58.8	23.8	52.3	89.8	65.7	17.7	58.5	50.2	65.9	63.5	171.7	8.0	115.8	55.8	49.6	53.1
Green juice	42.1	18.9	47.5	78.2	57.1	14.7	50.3	46.5	61.2	64.8	255.7	6.6	110.1	51.4	45.5	49.4
Protein concentrate	67.7	27.3	59.2	99.4	67.7	21.3	66.9	50.8	71.2	66.2	121.7	6.4	118.9	59.0	48.3	48.1

TP stands for true protein

Table 4. Amino acid composition of fresh crop, green juice and protein concentrate of red clover. Amino acid composition for organic basal diet for poultry (Hammershøj and Steenfeldt, 2012) and soybean meal (Sriperm et al., 2011).

	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser
	g/kg DM	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*	*g/kg DM*
Fresh material	10.2	4.1	9.1	15.6	11.4	3.1	10.2	8.7	11.5	11.0	29.8	1.4	20.1	9.7	8.6	9.2
Green juice	7.5	3.4	8.5	14.0	10.2	2.6	9.0	8.3	11.0	11.6	45.8	1.2	19.7	9.2	8.2	8.9
Protein concentrate	26.8	10.8	23.5	39.4	26.8	8.5	26.5	20.1	28.2	26.3	48.2	2.5	47.1	23.4	19.2	19.1
Organic basal diet	11.5	4.9	8.7	16.7	9.3	3.6	9.8	7.5	9.4	9.8	17.0	3.6	41.3	8.9	13.9	10.3
Soy bean	37.4	13.5	23.1	39.0	32.3	7.7	26.5	20.2	24.1	22.4	59.5	6.9	92.5	21.6	24.1	25.8

Final remarks and future perspectives

Up to 67 kg of protein concentrate per tonne of fresh red clover is obtained after the biorefining process. With a dry matter content around 200 g/kg this would lead to 13-14 kg of dry protein concentrate. This dry organic product contains 40% of crude protein and a balanced amino acid profile, comparable with the soybean meal. Further investigation is needed to evaluate the economy of the biorefinery process in order to obtain a product able to compete with the current market prices.

Moreover, two different sub-products are obtained during the biorefinery process, namely press cake and brown juice. The high content in lignocellulosic material and fiber-bounded protein in the former one make it very likely a suitable product to be used as feed for ruminants. Both sub-products are also adequate to be used as substrate for biogas production since the brown juice presents high content of minerals and easily degradable organic matter while the press cake presents high organic matter content and proteins. Finally, the organic digestate obtained after the anaerobic digestion of the sub-products could be utilized as organic fertilizer.