Grassland at the heart of circular and sustainable food systems

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Mineral concentration in fractions of green forages after screw-pressing

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Abstract

The concentrations of minerals (K, Ca, Mg, P, Zn and Cu) were analysed in the fractions whole plant, pulp, green juice, green protein, and brown juice from biorefining of white clover, red clover, lucerne, and perennial ryegrass. Forages collected in July, September, and November were separated by screw-pressing and green protein was extracted from the green juice by heat precipitation. Following centrifugation, all samples (n=52) were analysed for minerals. The concentrations of K, Mg, and Cu were lower in pulp (10.0 g kg^{-1} of DM, 1.16 g kg^{-1} DM, and 4.2 mg kg^{-1} of DM, respectively) compared to the whole plant (22.6 g kg^{-1} of DM, 1.95 g kg^{-1} of DM, and 6.61 mg kg^{-1} of DM, respectively), whereas Zn and Cu in green protein (54.5 and 21.0 mg kg^{-1} of DM, respectively) were more than double the whole plant concentrations (25.9 and 6.61 mg kg^{-1} of DM, respectively). Especially green protein of perennial ryegrass had five times higher concentration of Cu than that of whole plant. Concentrations of K, Mg and P were higher in brown juice (48.9, 4.14, and 6.02 g kg^{-1} of DM, respectively) compared to the whole plant (22.6, 1.95, and 3.28 g kg^{-1} of DM, respectively), whereas the concentrations in green protein were similar to the whole plant.

Keywords: biorefining, mineral, pulp, green juice, green protein, brown juice

Introduction

From an environmental point of view, locally grown protein sources as alternatives to soybean meal are highly desired. In northern Europe, green forages such as grass, lucerne and clover give high yields of crude protein, but in order to utilize the protein efficiently in monogastric animals, soluble protein has to be up-concentrated and separated from the fibre fraction. This is done during a biorefining process using, e.g. a screw-press followed by protein precipitation and centrifugation of green juice to obtain a green protein concentrate. The process leaves a side-stream of pulp and brown-juice. Other compounds such as minerals might be recovered in each of the fractions depending on the solubility and binding properties of the individual mineral to, e.g. protein and fibre. Surplus amounts of minerals (e.g. P) might have negative environmental effects, whereas naturally occurring Zn in the green protein could be beneficial for animal health. Knowledge, however, on mineral content in the biorefining fractions is very limited and is needed in order to optimally utilize the different fractions. The aim of this study was to determine the concentration of minerals in whole plant, pulp, green juice, green protein, and brown juice after screw-pressing and protein precipitation of white clover, red clover, lucerne and perennial ryegrass.

Materials and methods

Forage samples, weighing 2-5 kg, of white clover (*Trifolium repens* L., var. Klondike and Silvester; WC), red clover (*Trifolium pratense* L., var. Rajah and 'Suez'; RC), lucerne (*Medicago sativa* L., var. Creno; LU), and perennial ryegrass (*Lolium perenne* L., var. Trocadero and Calvano 1; PR) were sampled (July, September, and November) from the experimental farm at Aarhus University, Foulum, Denmark, and immediately frozen at -20 °C until processing. Sampling and processing are described in detail in Damborg *et al.* (2020). The plant material was thawed overnight at 5 °C before being processed at room temperature in a lab-scale twin-screw press (82 rpm) without prior chopping. Pulp and green juice obtained from processing were stored at -20 °C until further processing or analysis. Thawed green juice was heated in a two-step procedure to precipitate protein. First the green juice was heated to a targeted 60 °C in a water bath and kept at that temperature for 20-30 seconds, after which it was cooled to 5 °C. The green juice was centrifuged (1,950×*g* for 10 min) and the pellet was defined as the green protein. The supernatant was heated again to 80 °C and kept at that temperature for 20-30 s before it was cooled to 5 °C and centrifuged (1,950×*g* for 10 min). The supernatant was defined as the brown juice. The pellet was defined as white protein, but the sample amount was too small for mineral analysis. The concentrations of K, Ca, Mg, P, Zn, and Cu were analysed by ICP in the fractions whole plant, pulp, green juice, green protein, and brown juice. The statistical analyses were conducted in R using a linear model, which included sampling time (n=3), forage type (n=4), fraction (n=5), and the interaction between forage type and fraction as fixed effects. Results are given as least square means and largest standard error of mean (SEM) is provided. Pairwise comparison of means was made using Tukey's test. Fractions of green protein and brown juice from November were not available due to limited sample material.

Results and discussion

Sampling time had no effect on mineral concentrations. The mineral composition of the four different forages (Table 1) reflected expected concentrations (NorFor, 2021). Across forages, K was higher ($P<0.01$ for both) in green and brown juice (48.5 and 48.9 g kg^{-1} DM, respectively) compared to the whole plant (22.6 g kg^{-1} DM), whereas no difference was found between green protein and whole plant, reflecting the high water solubility of this element. The concentration of Ca was more than twice as high ($P<0.01$) in LU compared to PR (16.1 vs 6.76 g kg^{-1} DM, respectively). The Ca is both fibre-bound and located in enzymes, which could explain why the concentration of Ca in individual fractions differed less from the whole plant compared to that of the other minerals. Mg is more water-soluble than Ca, which explains the higher ($P<0.01$ for both) concentrations found in green and brown juice (3.46 and 4.14 g kg^{-1} DM) relative to the whole plant across forages (1.95 g kg^{-1} DM). Moreover, Mg concentration in brown juice, especially for RC and PR, was 2.5 times higher ($P<0.01$ for both) than in the whole plant. In contrast, Mg concentration was 40% lower ($P<0.01$) in pulp (1.16 g kg^{-1} DM) compared to the whole plant across forages. Across forages, P is mainly present as either inorganic PO_4^{3-} or in phospholipids. The inorganic PO_4^{3-} follows the liquid phase, which could explain why the P concentration in green and brown juice (5.71 and 6.02 g kg^{-1} DM, respectively) was almost twice as high ($P<0.01$ for both) as in the whole plant (3.28 g kg^{-1} DM). The P concentration in green protein, which has a reactive high lipid content, was similar to that of the whole plant, probably because of the phospholipids found in the green protein. Zn is often associated to proteins as part of many enzymes, and can explain the more than twice as high Zn concentration ($P < 0.01$) in the green protein compared to the whole plant (54.5 vs 25.9 mg kg^{-1} DM). Cu is also a part of many enzymes and can explain the three times higher ($P<0.01$) concentration of Cu in green protein compared to the whole plant across species (21.0 vs 6.61 mg kg^{-1} DM, respectively). The exception was WC, where the Cu concentration in green protein was less different from that of the whole plant. In green protein of PR, the Cu concentration was five times higher ($P<0.01$) than that of the whole plant of PR (Table 1).

Conclusions

The investigation showed that biorefining of grass and legume forages resulted in differentiation in mineral composition between the different fractions. On DM basis, the concentration of all minerals analysed (K, Ca, Mg, P, Zn, and Cu) was either significantly or numerically lower in pulp compared to the whole plant, and for Zn and Cu, the concentration was higher in green protein compared to the whole plant. Brown juice had a lower concentration of Cu and a higher concentration of K, Mg, and P compared to the whole plant. Concentration of Ca was more equally distributed between fractions than the other minerals.

Table 1. Concentration of minerals in white clover, red clover, lucerne, and perennial ryegrass, and in the five fractions; whole plant, pulp, green juice, green protein, and brown juice, produced during the juice extraction and heat precipitation.[1]

Forage	Fraction	K	Ca	Mg	P	Zn	Cu
White clover	Whole plant	25.9	11.6	2.00^{b}	3.30	21.8	6.26^{b}
	Pulp	11.2	10.2	1.36^{b}	2.20	17.4	4.11^{b}
	Green juice	51.6	13.4	3.17^{a}	5.49	30.7	11.0^{a}
	Green protein	21.2	12.0	2.28^{ab}	2.87	36.1	13.1^{a}
	Brown juice	41.0	12.6	3.44^{a}	5.12	24.6	5.99^{b}
Red clover	Whole plant	18.7	12.3	2.24^{cd}	2.78	30.9	8.43^{c}
	Pulp	8.32	10.3	1.24^{d}	1.73	25.9	5.93^{cd}
	Green juice	44.7	15.6	3.67^{b}	4.62	41.4	13.0^{b}
	Green protein	17.2	12.5	2.44^{c}	2.33	65.0	22.0^{a}
	Brown juice	57.6	17.4	5.43^{a}	5.62	48.2	3.38^{d}
Lucerne	Whole plant	17.3	15.5	1.82^{cd}	3.07	24.3	6.37^{c}
	Pulp	8.77	12.7	1.06^{d}	2.13	18.7	3.89^{cd}
	Green juice	40.8	22.7	3.78^{a}	5.21	37.4	12.9^{b}
	Green protein	20.8	16.2	2.26^{bc}	4.17	64.6	22.2^{a}
	Brown juice	31.1	13.4	3.16^{ab}	3.55	21.6	0.298^{d}
Perennial ryegrass	Whole plant	28.5	5.00	1.75^{cd}	3.98	26.6	5.39^{c}
	Pulp	11.7	3.74	0.988^{d}	2.35	16.2	2.73^{c}
	Green juice	56.8	6.96	3.23^{b}	7.52	34.4	11.0^{b}
	Green protein	31.2	9.05	2.78^{bc}	5.69	52.2	26.6^{a}
	Brown juice	65.8	9.06	4.56^{a}	9.78	53.3	2.46^{c}
SEM[2]		7.42	1.985	0.312	0.925	7.46	1.261
P-value	Forage	0.02	<0.01	0.03	<0.01	0.01	0.02
	Fraction	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	Forage × Fraction	0.57	0.18	0.01	0.14	0.24	<0.01

[1] The values for K, Ca, Mg, and P are given as g kg^{-1} DM, and the values for Zn and Cu are given as mg kg^{-1} DM. Values within each column and forage with different subscript letters differ ($P<0.05$).

[2] Largest standard error of mean.

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