Wilting of sainfoin affects tannin composition as determined by thiolytic degradation

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Introduction

Condensed tannins (CT)-rich legumes such as sainfoin (*Onobrychis viciifolia*) or birdsfoot trefoil (*Lotus corniculatus*) have been investigated for their effects on the quality of ruminant-source foods (Schreurs et al., 2008; Vasta et al., 2009). It has been shown that CT have the ability to bind protein in the rumen thereby affect bacterial activities like biohydrogenation of dietary polyunsaturated fatty acids (PUFA). In some studies, a higher PUFA and lower saturated fatty acids (SFA) content have been reported in meat and milk of ruminants fed CT-rich legumes (Cabiddu et al., 2009; Vasta et al., 2009). However, depending on whether these legumes are offered as fresh plant, hay or silage might impact their effects in the rumen. Thus, the aim of this experiment was to study the effect of wilting on content, composition and mean degree of polymerisation (mDP) of sainfoin CT.

Material and methods

Sainfoin (cultivar: Perly) was sown in March and harvested first in July 2012 in Posieux (Switzerland). Fresh and wilted samples (1 day after cutting) were collected in the field from three different locations. Subsequently, the three batches per harvest time were lyophilised, ground through a 1-mm sieve and then ball-milled before analysis. Using the thiolysis method as previously described by Gea et al. (2011), samples were depolymerised with benzyl mercaptan as nucleophile, and analysed by high performance liquid chromatography (UV detector UVD340U Dionex, Gilson Pumps 306, Dynamic mixer Gilson 811C, Manometer module 805 Gilson, Autoinjector 234 Gilson). Thiolysis was performed either directly on lyophilised plant material or after extraction of the CT with acetone:water (7:3; v/v). This method allowed to determine some qualitative CT attributes such as the CT content, the mDP, which is a measure of the average polymer size, and the CT profile as given by the proportions of the four different flavan-3-ols (catechin, C; epicatechin, EC;

gallocatechin, GC and epigallocatechin, EGC) in the terminal units and in the extension units. Catechin and epicatechin are procyanidins (PC) whereas gallocatechin and epigallocatechin are prodelphinidins (PD). A t-test was performed to compare mean values of CT levels and composition of fresh and wilted samples.

Results and discussion

In fresh and wilted samples the total CT concentration was 34.2 and 29.2 g/kg DM, respectively. The mDP in the extract was lower than in the whole plant samples (6.8 *vs.* 10.4) and was also lower in wilted compared to fresh samples (7.1 *vs.* 10.2). These values are low compared to those reported by Azuhnwi et al. (2013) who found 5-times longer tannin polymers in the same cultivar. In the whole plant as well as the extract, the PD were with 70% dominant compared to the PC with 30%. Moreover, the PC:PD ratio in terminal units decreased from 69:31 to 53:47 and from 51:49 to 32:68 in fresh compared to wilted samples, in the whole plant and extract samples, respectively. An increase in PD proportion by wilting could reflect a modification of hydroxylation pattern since PD have one more hydroxyl group (-OH) than PC. Koupai-Abyazani et al. (1993) have demonstrated a change in the degree of hydroxylation of the polymers constituents with development. In their study there were, for instance, more PD in the leaves of sainfoin at stage 2 (leaflets separated but folded) than in stage 1, young leaves (leaflets not separated). This change in the percentage of terminal units can be explained by a greater EGC content (P=0.06) and lower contents of EC, C and GC in wilted compared to fresh samples (Table 1). However, no GC was found in the terminal units of the whole plant whereas it was found in the extract, suggesting a very low quantity of GC in the plant which cannot be detected by this method. Finally, no difference was found in extension units between wilted and fresh samples. It has just been noticed that C content is really low (Table 1), which is consistent with previous findings of Koupai-Abyazani et al. (1993).

Conclusion

The CT content of tanniniferous plants is influenced by many factors such as genetic, environmental, agronomic and technologic factors (Azuhnwi et al., 2011; Manolaraki, 2011). The present results demonstrate that also wilting affects the size of tannins polymers and the hydroxylation pattern. We hypothesize that these changes in mDP and CT composition lead to differences in CT reactivity which ultimately might affect biohydrogenation of PUFA in the rumen.

Table 1. Percentages of individual flavan-3-ols (GC, gallocatechin; EGC, epigallocatechin; C, catechin; EC, epicatechin) and prodelphinidin (PD)/procyanidin (PC) ratio in terminal units and extension units in fresh and wilted samples of sainfoin after thiolysis. Thiolysis was performed either directly on the lyophilised plant material (plant) or after extraction (extract) with acetone:water (7:3; v/v)

Plant	Stadium	% of flavan-3-ols in terminal units					% of flavan-3-ols in extension units				
fractions	of harvest	GC	EGC	C	EC	PC : PD	GC	EGC	C	EC	PC : PD
Plant	Fresh	0	31	23	45	69 : 31	14	60	4	22	26 : 74
	Wilted	0	47	17	36	53 : 47	18	56	4	22	26 : 74
Extract	Fresh	11	38	19	32	51 : 49	10	62	3	25	28 : 72
	Wilted	4	64	12	20	32 : 68	10	58	3	29	32 : 68

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References

Azuhnwi, B. N., Boller, B., Martens, M., Dohme-Meier, F. , Ampuero, S., Günter, S., Kreuzer, M., Hess, H. D. (2011): Morphology, tannin concentration and forage value of 15 Swiss accessions of sainfoin (*Onobrychis viciifolia* Scop.) as influenced by harvest time and cultivation site. *Grass and Forage Science*. **66:** 474-487

Azuhnwi, B. N., Boller, B., Dohme-Meier, F. , Hess, H. D., Kreuzer, M., Stringano, E., Mueller-Harvey, I. (2013): Exploring variation in proanthocyanidin composition and content of sainfoin (*Onobrychis viciifolia*). *Journal of the Science of Food and Agriculture*. DOI: 10.1002/jsfa.6119

Cabiddu, A., Molle, G., Decandia, M., Spada, S., Fiori, M., Piredda, G., Addis, M. (2009): Responses to condensed tannins of flowering sulla (*Hedysarum coronarium L.*) grazed by dairy sheep. Part 2: effects on milk fatty acid profile. *Livestock Science*. **123:** 230-240

Gea, A., Stringano, E., Brown, R.H., Mueller-Harvey, I. (2011): *In situ* analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high throughput germplasm screening. *Journal of agricultural and food chemistry*. **59:** 495-503

Koupai-Abyazani, M.R., McCallum, J., Muir, A.D., Bohm, B.A., Towers, G.H.N., Gruber, M.Y. (1993): Developmental changes in the composition of proanthocyanidins from leaves of sainfoin (Onobrychis viciifolia Scop.) as determined by HPLC analysis. *Journal of agricultural and food chemistry*. **41:** 1066-1070

Manolaraki, F.(2011): Propriétés anthelminthiques du sainfoin (*Onobrychis viciifoliae*): Analyse des facteurs de variations et du rôle des composés phénoliques impliqués. Dissertation. Université de Toulouse. France

Schreurs, N.M., Lane, G.A., Tavendale, M.H., Barry, T.N., McNabb, W.C. (2008): Pastoral flavour in meat products from ruminants fed fresh forages and its amelioration by forage condensed tannins. *Animal Feed Science and Technology*. **146:** 193-221

Vasta, V., Priolo, A., Scerra, M., Hallett, K.G., Wood, J.D., Doran, O. (2009): Δ 9 desaturase protein expression and fatty acid composition of *longissimus dorsi* muscle in lambs fed green herbage or concentrate with or without added tannins. *Meat Science*. **82:** 357-364.