Effect of the sainfoin preservation on the secondary compounds and antioxidant activity

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Introduction: The polyphenols, carotenoids and tocopherols are secondary compounds in forages. These compounds are abundant in fresh forages and increase the antioxidant (AO) activity (Debier and Larondelle, 2005), being able to improve the milk and meat quality (Kumar, 2015). Fresh sainfoin (*Onobrychis viciifolia*) is a perennial legume with high nutritional value and medium to high secondary compounds content (Rufino-Moya et al., 2019). However, it is needed to preserve the sainfoin because two thirds of its annual production is obtained with the first cut of spring. The aim of this study was to evaluate how the preservation method affected the contents of these secondary compounds and AO activity.

Materials and methods: Fifteen samples of sainfoin were collected at the late bloom stage and divided into samples for fresh forage (n=5), hay (n=5) and silage (n=5) (for details see Rufino-Moya et al., 2019). The samples for hay were naturally sun-dried, whereas the samples destined to make silage were wilted one day, chopped (3-5 cm) and vacuum-ensiled in plastic bags for 82 days. The total polyphenols were extracted (Makkar, 2000) and quantified (Julkunen-Titto, 1985). The carotenoids and tocopherols extracted (Fu et al., 2011) were determined by chromatography (Chauveau-Duriot et al., 2010). The AO activity (Roncero-Ramos et al (2017) was assessed through the free radical scavenging activity (DPPH and ABTS assays) and the ferric reducing antioxidant power (FRAP). Data were analysed using SAS v 9.1. with a general linear model (5 replicates each method) considering the method of preservation as fixed effect. The LSMeans and their associated standard errors and differences between means were obtained using the Tukey correction.

Results: The method of preservation affected all the parameters ($P<0.001$) (Table 1). Fresh sainfoin and hay had greater polyphenol content than the silage ($P<0.001$). Fresh sainfoin had highest contents of xanthophylls and all-E-β-carotene. The hay had the greatest γ-tocopherol, whereas the silage had the greatest 13z- and 9z-β-carotenes. Regarding the AO activity, the silage had the lowest values in the three parameters ($P<0.001$). The hay had greater ABST, similar DPPH and lower FRAP than the fresh sainfoin.

Table 1. Effect of the preservation method on secondary compounds and antioxidant activity in sainfoin

	Fresh	Hay	Silage	s.e.	P-value
Polyphenols, eq-g tannic acid/kg DM	44.6a	47.1a	34.1b	5.6	0.0001
Neoxanthin, μg/g DM	35.1a	14.9b	N.D.	0.23	0.0001
Violaxanthin, μg/g DM	12.5a	6.4b	N.D.	0.19	0.0001
Zeaxanthin, μg/g DM	17.9a	11.2b	20.7a	0.28	0.0006
Lutein, μg/g DM	164a	103b	66c	1.3	0.0001
13 z-β-carotene, μg/g DM	4.87b	1.45c	11.9a	0.08	0.0001
9 z-β-carotene, μg/g DM	11.6b	4.4c	14.0a	0.1	0.0001
All-E-β-carotene, μg/g DM	101a	41.3c	52.6b	0.7	0.0001
α-tocopherol, μg/g DM	123a	61b	119a	1	0.0001
γ-tocopherol, μg/g DM	8.0c	11.4a	9.6b	0.1	0.0002
ABTS, eq-μmol trolox /g DM	311b	336a	247c	3	0.0001
DPPH, eq-μmol trolox /g DM	264a	264a	140b	2	0.0001
FRAP, eq-μmol trolox /g DM	282a	290b	189c	3	0.0001

Within a parameter, means with different letter differ at $P<0.05$.

Conclusion: the preservation method greatly affected the contents of all the parameters. Further studies are required to assess the effects of all these changes on the deposition in the tissues of the animals and evaluate the impact on the quality of the final product.

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Polyphenols, carotenoids and tocopherols are abundant secondary compounds in fresh forages that increase the antioxidant (AO) activity, and improve milk and meat quality.
Sainfoin (*Onobrychis viicifolia*) is preserved to optimise its use as feedstuff

OBJECTIVE

The preservation method affected the contents of these secondary compounds and AO activity?

MATERIAL Y METHODS

Sampling

Late bloom stage
15 samples

- Fresh: lyophilised (n=5)

- Hay: sun-dried (n=5)

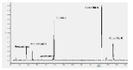
- Silage: wilted, chopped & vacuum – packed 82 d (n=5)

Chemical analyses

Polyphenols

Total polyphenols (Makkar, 2000; Julkunen-Tiitto, 1985)
Spectrophotometry

Carotenoids and tocopherols



Xanthophylls: Neoxanthin, violaxanthin, lutein, zeaxanthin
Carotenes: all-E-, 13Z-, 9Z-β-carotene
Tocopherols: α-, γ-, δ-tocopherol
UPLC

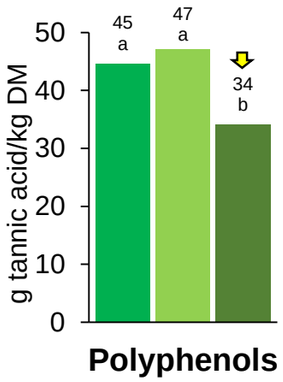
(Fu *et al*, 2011)

Antioxidant activity

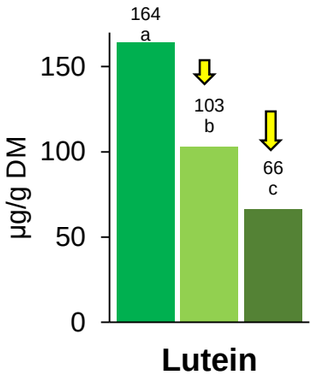
DPPH, ABTS (Roncero-Ramos et al., 2017)
FRAP (Morales & Jiménez-Pérez, 2001)
Spectrophotometry

RESULTS

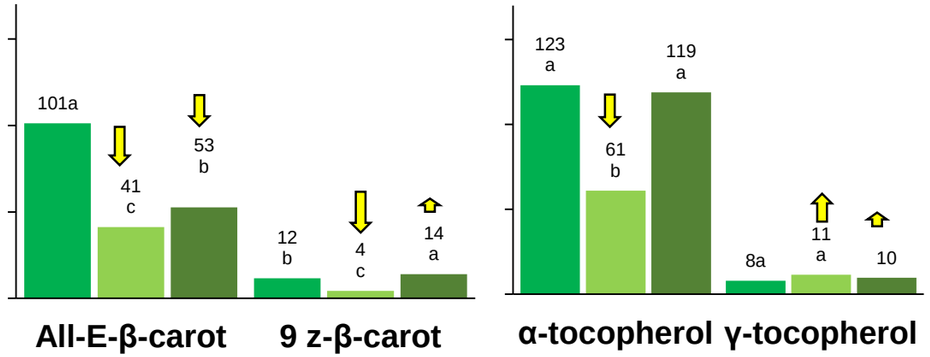
Total polyphenols



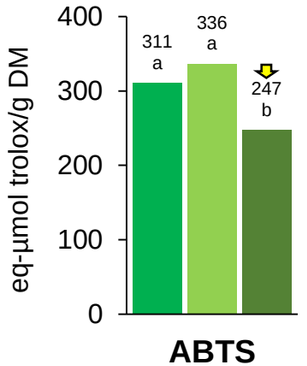
Xanthophylls



Carotenes & tocopherols



AO activity



CONCLUSIONS

The preservation method **greatly** affected the contents of all secondary compounds. Further studies are required to evaluate the impact of these changes on the quality of the final product