ELSEVIER

Food Chemistry 72 (2001) 521–524

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Use of high pressure liquid chromatography (HPLC) for the determination of cda-tocopherol levels in forage (silage/grass) samples collected from different regions in Ireland

A. Lynch [a], J.P. Kerry [a,*], D.J. Buckley [a], P.A. Morrissey [a], C. Lopez-Bote [b]

[a] *Department of Food Science and Technology, University College Cork - National University of Ireland, Cork, Ireland*
[b] *Dto de Producci Animal, Facultad de Veterinaria, Universidad Complutense, 28040, Madrid, Spain*

Received 15 March 2000; received in revised form 8 October 2000; accepted 8 October 2000

Abstract

First, second and third cut grass silage samples were collected from eight regions around Ireland (184 samples in total) and analysed for vitamin E content. Fresh grass samples were also collected at one site in Co. Cork and analysed for α-tocopherol content. The concentration of α-tocopherol [μg/g dry matter (DM)] was determined using high performance liquid chromatography analysis. A wide variation in α-tocopherol levels in silage samples was found. α-Tocopherol levels in first, second and third cut silage samples ranged from 4.9 to 20.8, 4.4 to 13.0 and 1.3 to 3.9 μg/g DM, respectively. The mean values of α-tocopherol in first, second and third cut silage samples were 11.3±0.9, 9.7±0.7 and 2.3±0.5 μg/g DM, respectively. There was no significant difference in α-tocopherol levels between first and second cut silage samples. However, third cut silage samples had significantly ($P < 0.05$) less α-tocopherol compared to first and second cut samples. There was no decrease in α-tocopherol levels in silage samples following storage at −20°C for 2 months. Fresh grass samples were classified as pasture grass, meadow grass (consisting of 20% *Bromus* spp and 80% *Holcus* spp, hedgerow grasses (predominately *Dactylis glomerata* L.) and white or red clover. Pasture grass had significantly ($P < 0.05$) higher α-tocopherol levels than other grass types analysed. Meadow grass had significantly ($P < 0.05$) higher α-tocopherol levels than clover or hedgerow grasses. α-Tocopherol concentration in the different grass types decreased in the order: pasture grass > meadow grass > hedgerow > white clover > red clover.

Keywords: Silage; Grass; Vitamin E; HPLC; Variation

1. Introduction

Vitamin E is a generic name used to describe a group of eight compounds with similar activities which are extracted as oils from plant material (Buckley & Morrissey, 1992). α-Tocopherol is one of the most active forms of vitamin E and accounts for almost all vitamin E activity in living tissue (Buckley & Morrissey). The most widely accepted function of vitamin E is as its role as a major chain-breaking anti-oxidant. α-Tocopherol is capable of quenching free radicals and thus, protects phospholipids and cholesterol against oxidation and subsequent breakdown to potentially harmful, chemically reactive products (Gray, Gomma & Buckley 1996). α-Tocopherol has been demonstrated to have beneficial effects on the oxidative and colour stability of red meats, such as beef (Sanders, Morgan, Wulf, Tatum, Williams & Smith 1997) and lamb (Guidera, Kerry, Buckley, Lynch & Morrissey 1997a,b) through dietary supplementation. However, these studies involved the addition of commercially available artificial preparations of α-tocopheryl acetate to grain-based concentrates.

Very little research has been reported to date on the occurrence of α-tocopherol levels in grass and silage feeds for ruminants. Because of this lack of knowledge, it is difficult to gauge the nutritional impact that these feeds may have on general ruminant health and final meat quality. In the Irish situation, beef cattle are generally grazed outdoors from March to November and overwintered indoors where grass silage is the main forage offered accounting for 25% of the annual consumption of feed dry matter (O'Kiely, Power & O'Donnell 1993).

* Corresponding author. Tel.: +353-21-4903789; fax: +353-21-4276318.
E-mail address: joe.kerry@ucc.ie (J.P. Kerry).

0308-8146/01/$ - see front matter
PII: S0308-8146(00)00283-1

The objective of this study was to determine the range of α-tocopherol concentrations naturally present in silage samples collected from different regions within Ireland and assessment of factors such as seasonal cutting times and grass composition on α-tocopherol levels using high performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Chemicals

All chemicals were 'AnalaR' or HPLC grade and were obtained from Wardle Chemicals Ltd., Cheshire, UK; British Drug House, Poole, Dorset, UK; Sigma Chemical Co., Poole, Dorset, UK; Alkem Chemicals Ltd., Co. Cork, Ireland and Rathburn Chemicals, Co. Cork, Ireland.

2.2. Sample collection

Samples of silage produced during the 1996 season were collected from eight regions within Ireland (counties Carlow, Cavan, Cork, Donegal, Galway, Laois, Offaly and Roscommon). Silage samples were categorised as first (May–June), second (July–August) and third (September–early October) cut silage samples. Silage sampling locations within each region were well distributed. All samples were collected, packed in sealable freezer bags and transported to UCC within 48 h of sampling, vacuum packaged on arrival in the laboratory and stored at −20°C for up to a maximum of two months prior to analysis. First cut samples from one source was analysed immediately after sampling and again after a period of 2 months to determine the effects of storage at −20°C on α-tocopherol levels. Five different types of grass were collected from one region location (Co. Cork) during the month of July, to determine whether the type of grass used in silage making might have an effect on the final silage α-tocopherol level.

2.3. Sample preparation for HPLC Analysis

Subdued light (partial or complete elimination) was used at all stages of the sample preparation during these studies. Silage (100 g) was dried in an oven at 40°C for 48 h. The dried silage was weighed to determine the % moisture and stored in a dessication chamber. Fresh silage (5 g) was chopped finely using plastic knives and weighed into a cellulose extraction thimble and fine white sand was added to absorb excess moisture. The thimble was then plugged with cotton wool. Anti-bumping granules were added to a 250 ml flat-bottomed flask with a Quik-Fit neck. The sample was placed in a Soxhlet extractor, which was connected to the flask and a condenser. Acetone (100 ml) was poured into the extractor and the sample was refluxed for 3 h on a hot-plate at maximum heat. The use of heated acetone for extraction was found not to impact on α-tocopherol levels detected. The sample was filtered through Whatman No. 1 filter paper into a 250 ml round-bottomed flask and rotary evaporated at 40°C. The sample was then re-suspended by adding 5×2 ml aliquots of hexane to which butylatedhydroxy toluene (0.05%, w/v) had been added. Aliquots (2 ml) were stored in screw-cap test-tubes, covered in aluminium foil and stored in the dark overnight at room temperature and then centrifuged at 2500 rpm for 10 min. The supernatant was syringed through a 0.45 μm HV filter (Nihon Millipore Ltd., Yonezawa, Japan) prior to analysis by HPLC. α-Tocopherol content was assessed using a modification (column was washed for 60 min after each sample) of the method of Sheehy, Morrissey and Flynn (1994) and quantified by HPLC using a Waters model S10 pump, a Waters 717 autosampler, a Machery-Nagel Nucleosil 5 C_{18} (250×0.4 mm) reverse phase column and a Waters model 486 UV-visible wavelength detector (Millipore Corporation, Milford, MA 01757, USA) set at 292 nm. The mobile phase was methanol:water (97:3) at a flow rate of 2 ml/min. Data were recorded and evaluated using a Millipore Millenium 2010 chromatography management system (Millipore Corporation, Milford, MA 01757, USA). Each silage sample was sub-sampled in triplicate and each sub-sample was analysed in duplicate. The concentration of α-tocopherol was then calculated on a dry matter basis, using the % moisture determined after drying for 48 h. The HPLC method outlined is a standardised method and conforms to those standards laid down by the National Institute of Standards and Technology (NIST, Gaithesburg, USA).

2.4. Statistical analysis

The analysis of variance was separated into four parts, one which analysed first and second silage cuts only and treated the region of origin as a random factor and a second which analysed first, second and third cuts from three regions (counties Cork, Laois and Offaly) which provided samples for all three cutting times. The third analysis of variance involved the effect of storage time on the α-tocopherol content of first cut silage from one region and the fourth examined the effect of grass type on α-tocopherol content in fresh grass samples. The analysis was carried out using SPSS 8.0 for windows (SPSS, Chicago, IL, USA) software package.

3. Results and discussion

All data relating to the level of α-tocopherol contained in first, second and third cut silage samples from eight different regions in Ireland are shown in Table 1.

Table 1
α-Tocopherol levels (μg/g DM) in first and second cut silage samples collected from eight regions in Ireland

Cutting times	Number of samples	α-Tocopherol level (μg/g DM)	
		Range	Mean±S.E.M.
First	117	0.8–49.9	20.77±0.78
Second	59	0.8–20.6	24.01±1.16

In general, a wide range of α-tocopherol levels was determined in first and second cut samples from all regions. Mean α-tocopherol levels in first, second and third cut silage samples from three regions (counties Cork, Laois and Offaly) are shown in Table 2. Overall, the region of origin did not have a significant effect on α-tocopherol levels detected. There were no significant differences between α-tocopherol levels detected in first and second cut samples. Third cut silage samples had significantly ($P < 0.05$) lower α-tocopherol levels than either first or second cuts from the same regions. McDowell et al. (1996) reported that the vitamin E content in forage is affected by the stage of maturity at the time of forage cutting. The data presented in this study would concur with this finding and further suggests that the major differences in α-tocopherol levels observed might also be due to seasonal growth rate. The effect of interaction between region of origin and number of cuts was also not significant. However, there were significant ($P < 0.001$) differences in α-tocopherol levels determined between all three cutting times. Because α-tocopherol concentrations present in fresh forage can theoretically result in muscle saturation with α-tocopherol, the correct nutritional requirements in finishing diets supplied to meat producing animals may vary widely due to the recent nutritional history of these animals (Liu, Lannari & Schaefer, 1995). Arnold et al. (1993) found that cattle grazed on good quality pasture had high α-tocopherol concentrations in skeletal muscle. This finding might also apply to silage quality. Therefore, first and second cut silage samples analysed during this study would seem to be better sources of quality forage, in terms of α-tocopherol content, than third cut silage. There was no significant decrease in the level of α-tocopherol detected in first cut silage samples taken from one region in Ireland, after storage under vacuum at −20°C for 2 months (Table 3). Therefore, α-tocopherol levels in silage samples remained stable during short-term frozen storage.

Table 2
α-Tocopherol levels (μg/g DM) in first, second and third cut silage samples collected from three regions in Ireland

Silage cut	Number of samples	α-Tocopherol (μg/g DM)	
		Range	Mean±S.E.M.
First	36	2.00–31.42	9.75±0.94
Second	19	1.53–16.30	9.80±1.07
Third	13	0.33–5.19	2.32±0.50

During this study, rock type (limestone, granite, shale or sandstone) and soil characteristics (loamy, silty, clay or heavy) were also provided with each silage sample collected. However, neither rock type nor soil type had any significant influence on the distribution of α-tocopherol levels in the silage samples (data not presented).

The levels of α-tocopherol found in five types of grass collected at one regional location (Co. Cork) in July, 1996 are shown in Table 4. There was a significant ($P < 0.001$) difference in α-tocopherol levels detected in the grass types sampled. Pasture grass had significantly ($P < 0.05$) higher α-tocopherol levels than all other grass types. Meadow grass (*Bromus spp* and *Holcus spp*) also had significantly ($P < 0.05$) higher α-tocopherol levels than either hedgerow (*Dactylis glomerata L.*) or clover grasses. There were no significant differences detected between α-tocopherol levels determined in white clover, red clover and hedgerow grass. α-Tocopherol levels decreased in the grasses sampled in the order: pasture > meadow > hedgerow grasses > white clover > red clover and the α-tocopherol levels determined were 90.0±1.4, 64.7±0.8, 28.7±0.1, 7.5±0.2 and 6.5±0.2 μg/g DM, respectively. α-Tocopherol levels in pasture and meadow grass were significantly ($P < 0.001$) higher than those detected in silage samples. McDowell et al. (1996) reported that ensiled forages contain only a fraction of the amount of vitamin E found in freshly cut forages. Therefore, the grass type chosen for the manufacture of silage might be a factor which could be manipulated to ensure higher vitamin E levels in ensiled forage for winter feeding to ruminants.

Table 3
Effect of storage (−20°C for 2 months) on α-tocopherol levels (μg/g DM) in first cut silage samples collected from one region in Ireland

Storage time	Number of samples	α-Tocopherol (μg/g DM)	
		Range	Mean±S.E.M.
0 months	6	5.31–10.31	8.36±0.92
2 months	6	7.50–8.39	7.88±0.05

Table 4
Comparison of α-tocopherol levels (μg/g DM) in five different grass types collected at one regional site within Ireland

Grass type	Number of samples	α-Tocopherol (μg/g DM)	
		Range	Mean±S.E.M.
Pasture	6	13.92–15.17	14.48±0.36
meadow	6	7.56–8.43	7.98±0.25
Hedgerow	6	0.57–8.47	3.46±2.51
White Clover	6	0.44–2.29	1.40±0.39
Red Clover	6	0.59–1.76	1.01±0.21

While this study was conducted over a single season and is therefore limited with respect to annual variations of α-tocopherol in silage and grass samples, the data generated during this study has shown that factors such as seasonality of cutting, grass composition and herbage freshness are all factors which have some impact on the α-tocopherol content of forage. Consideration of these and other factors could allow natural manipulation of ruminant diets to increase uptake of the natural antioxidant ?-tocopherol by meat producing animals, thereby enhancing colour and oxidative stability of fresh meats.

Acknowledgements

The help and co-operation of John Kennedy, Teagasc offices, Portlaoise, Co. Laois and of the Macra Na feirme Young Farmers Chairmen from counties Carlow, Cavan, Cork, Donegal, Galway, Offaly and Roscommon is gratefully appreciated. The research described in this manuscript was entirely funded from internal UCC resources.

References

Arnold, R. N., Arp, S. C., Scheller, K. K., Williams, S. N., & Schaefer, D. M. (1993). Tissue equilibration and sub-cellular distribution of vitamin E relative to myoglobin and lipid oxidation in displayed beef. *Journal of Animal Science, 71*, 105–118.

Buckley, D. J., & Morrissey, P. A. (1992). *Vitamin E, meat quality; "animal production highlights" roche — vitamins, fine chemicals*. Basel, Switzerland: Hoffmann-La Roche Ltd, 4002.

Gray, J. I., & Gomma, E. A., & Buckley, D. J. (1996). Oxidative quality and shelf Life of Meats. *Meat Science, 43*, S111–S123.

Guidera, J., Kerry, J. P., Buckley, D. J., Lynch, P. B., & Morrissey, P. A. (1997a). The effect of dietary α-tocopheryl acetate supplementation on muscle α-tocopherol levels and lamb quality. *Irish Journal of Agricultural and Food Research, 36*, 241–247.

Guidera, J., Kerry, J. P., Buckley, D. J., Lynch, P. B., & Morrissey, P. A. (1997b). The effect of dietary vitamin E supplementation on the quality of fresh and frozen lamb meat. *Meat Science, 44*, 33–43.

Liu, Q., Lannari, M. C., & Schaefer, D. M. (1995). A review of dietary vitamin E supplementation for improvement of beef quality. *Journal of Animal Science, 73*, 3131–3140.

McDowell, L. R., Williams, S. N., Hidiroglou, N., Njeru, C. A., Hill, G. M., Ochoa, L., & Wilkinson, N. S. (1996). Vitamin E supplementation for the ruminant. *Anim. Feed Sci. Technol, 60*, 273–296.

O'Kiely, P., Power, R., & O'Donnell, C. (1993). Grass conservation, storage and feeding in Ireland. *Farm and Food, 3*(1), 4–7.

Sanders, S. K., Morgan, J. B., Wulf, D. M., Tatum, J. D., Williams, S. N., & Smith, G. C. (1997). Vitamin E supplementation of cattle and shelf-life of beef for the Japanese market. *Jounal of Animal Science, 75*, 2634–2640.

Sheehy, P. J. A., Morrissey, P. A., & Flynn, A. (1994). Consumption of thermally-oxidised sunflower oil by chicks reduces α-tocopherol status and increases suscetibility of tissues to lipid oxidation. *British Journal of Nutrition, 71*, 53–56.