J. Dairy Sci. 101:1–6
https://doi.org/10.3168/jds.2017-14187
© American Dairy Science Association®, 2018.

Technical note: Development and validation of an HPLC method for the quantification of tocopherols in different types of commercial cow milk

G. Niero,*[1] M. Penasa,* J. Berard,†‡ M. Kreuzer,† M. Cassandro,* and M. De Marchi*
*Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy
†ETH Zurich, Institute of Agricultural Sciences, Universitätstrasse 2, 8092 Zurich, Switzerland
‡ETH Zurich, AgroVet-Strickhof, Eschikon 27, 8315 Lindau, Switzerland

ABSTRACT

In the present study, a methanol-fluorescence-based HPLC method was validated for its use to quantify α-tocopherol and γ-tocopherol in raw milk, whole UHT milk, partially skimmed UHT milk, whole pasteurized milk, and partially skimmed pasteurized milk. Repeatability and reproducibility, calculated as relative standard deviation of 10 measurements within the same day and 30 measurements across 3 d, respectively, were always below 5% for both tocopherols concentrations and retention times. Recovery was assessed through 3 spiking levels and it ranged from 89 to 107%. The method was able to detect the expected declines in tocopherols in milk exposed to UHT or skimming treatments. Vitamin E, calculated as the sum of α-tocopherol and γ-tocopherol, was similar in whole pasteurized and raw milk, averaging 1.57 and 1.56 mg/L, respectively, followed by whole UHT (1.33 mg/L), partially skimmed pasteurized (0.77 mg/L), and partially skimmed UHT milk (0.61 mg/L).
Key words: milk, tocopherol, UHT, skimming

Technical Note

Tocopherols are among the major fat-soluble antioxidants and they exist in nature in 4 variants, namely α-, β-, γ-, and δ-tocopherol (**T**); together with tocotrienols, they belong to vitamin E compounds (Ju et al., 2010; Shehata et al., 2015). Both in vivo and in vitro studies demonstrated that tocopherols have direct and indirect antioxidant activity, and human epidemiological studies showed an association between low vitamin E status and an increased risk of cancer and heart diseases (Galli et al., 2017). Tocopherols cannot be synthesized in human organisms, and therefore dietary sources are essential to reach the recommended daily requirement. Among the animal-derived foods, cow milk and milk products can be sources of these compounds (Guo et al., 2014) and they are worthy of scientific investigation for 2 main reasons. First, tocopherols are frequently used as additives in the diet of dairy cows to cover their requirements and to improve milk quality, health status, fertility, and productive performance (Baldi et al., 2000). Second, from a technological point of view, tocopherols play an important role in maintaining an oxidative stability of milk and dairy products, especially to protect and preserve oxidative stability of PUFA in milk fat (Havemose et al., 2006). Nevertheless, processing techniques, such as skimming and heating, may adversely affect the bioavailability of these compounds (Manzi and Pizzoferrato, 2010; Guneser and Yuceer, 2012). In the literature, several alkaline saponification procedures for milk tocopherols extraction, as well as HPLC protocols for tocopherols separation and detection, have been described. The majority of these procedures involve invasive and time-consuming sample pretreatment, such as the use of a rotovapor (Renzi et al., 2005), which might affect accuracy of the results. The chromatographic conditions are mainly based on toxic and expensive mobile phases, such as heptane, hexane (Ellis et al., 2007), acetonitrile, dichloromethane (Chauveau-Duriot et al., 2010), or triethylamine (Ramalho et al., 2012). Finally, UV and visible wavelengths are often used for detection, resulting in a weak signal-to-noise ratio and a low sensibility (Renzi et al., 2005; Chauveau-Duriot et al., 2010; Guneser and Yuceer, 2012). The present work aimed to develop a fast milk sample preparation protocol avoiding the previously noted disadvantages and to validate a cost-effective HPLC separation program coupled with sensible fluorescence detection for quantification of tocopherols in different types of commercial cow milk.

Received November 23, 2017.
Accepted April 3, 2018.
[1] Corresponding author: giovanni.niero@studenti.unipd.it

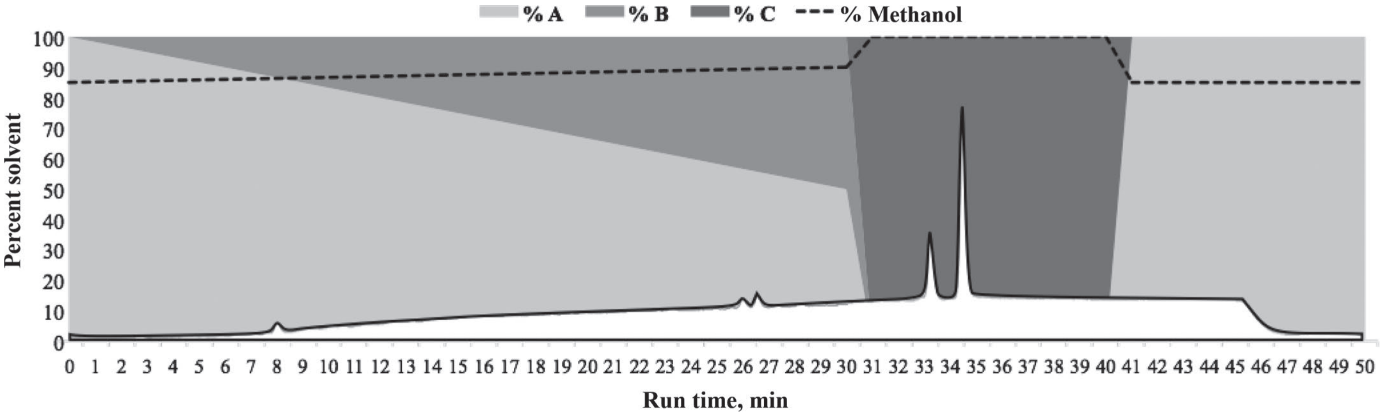

Figure 1. Elution program applied for the separation of milk tocopherols in HPLC (solvent A = 85% methanol, solvent B = 95% methanol, solvent C = 100% methanol), overlaid with the chromatogram obtained from raw milk.

A raw milk (**RM**) sample was purchased in a vending machine (Zurich, Switzerland). Four milk samples of the same dairy brand, including whole UHT milk (**WUM**), partially skimmed UHT milk (**SUM**), whole pasteurized milk (**WM**), and partially skimmed pasteurized milk (**SM**), were purchased in a single local commercial store (Zurich, Switzerland). According to the nutritional labels, the fat contents were 3.7% (RM), 3.9% (WM and WUM), and 2.5% (SM and SUM). All samples were kept at 4°C and warmed at room temperature before analysis. Aliquots of 10 mL of milk were poured into screw-top glass vessels, and added to 1 g of ascorbate, 10 mL of methanol, and 10 mL of 0.18 *M* KOH in methanol and water (1:2). The resulting mixture was cooked in a water bath at 100°C for 1 h in hermetically closed vessels. Samples were cooled at room temperature and rinsed with 35% ethanol up to a volume of 100 mL. An aliquot of 10 mL of this mixture was added to 3 mL of hexane and toluene (1:1), and gently shaken for 5 min to promote T extraction. Samples were centrifuged for 5 min at 2,500 × *g* at room temperature and 2 mL of the top organic phase were dried under nitrogen. Finally, samples were resuspended in 1 mL of methanol.

Milk tocopherols were detected and quantified through a quaternary pump HPLC device (Waters Alliance 2695; Waters Corporation, Milford, MA), equipped with a fluorescence detector (Waters Multi Fluorescence Detector 2475) and a reverse phase C18 column (Nucleodur PolarTec 250 mm, 3-μm particle size; Macherey-Nagel, Düren, Germany). Samples were kept at 10°C in a refrigerated autosampler. Gradient elution was carried out with a mixture of 3 solvents (A, B, and C), which consisted of 85% (A) and 95% (B) of methanol in water and 100% (C) methanol. The elution program depicted in the background of Figure 1, was adjusted following column usage recommendations. The elution consisted of a linear gradient from 100 to 50% A and from 0 to 50% B in 30 min, a linear gradient from 50 to 0% A, from 50 to 0% B, and from 0 to 100% C in 1 min, an isocratic elution at 100% C for 9 min, and a linear return to the starting condition within 1 min. Injection volume was 25 μL. Before injecting the following sample, the column was re-equilibrated under the starting conditions for 9 min. The flow rate was 0.4 mL/min, the column temperature was kept at 30°C, and the detection was carried out in fluorescence with an excitation wavelength of 295 nm and an emission wavelength of 330 nm. Overall, 25 milk samples can be treated and prepared simultaneously in 4 h, and the following HPLC analyses will last for about 20 h. As a result, 25 milk samples can be analyzed in 24 h with the method described.

Standard solutions were prepared in methanol and quantification of each chromatographic peak was obtained with 5-point calibration curves ($R^2 \geq 0.99$). For α-T the lowest standard concentration was 0.20 mg/L, followed by 2.00, 3.00, 4.00, and 5.00 mg/L. For γ-T the lowest standard concentration was 0.02 mg/L, followed by 0.20, 0.40, 1.00, and 2.00 mg/L. Results were corrected by the recovery rate of δ-T, used as internal standard in a final concentration of 1.00 mg/L, to cope with the underestimation due to the losses of target molecules.

Recovery of the method was assessed through 3 spiking levels of the starting milk. In particular, 0.01 and 0.0025 mg, 0.02 and 0.005 mg, and 0.04 and 0.01 mg of α-T and γ-T standards were dissolved in 10 mL of methanol for spiking 1, 2 and 3, respectively. The obtained solutions were added to milk as described in the first step of milk sample preparation procedure and treated following the same protocol. The aliquots

Download English Version:

https://daneshyari.com/en/article/8500857

Download Persian Version:

https://daneshyari.com/article/8500857

Daneshyari.com