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Antioxidant capacity of different cheeses: Affecting factors and prediction by near infrared spectroscopy

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ABSTRACT

In this study, we analyzed antioxidant capacity of 224 cheese samples prepared using 16 varied mixtures of milk from cows, ewes, and goats, in 2 manufacturing seasons (winter and summer), and over 6 mo of ripening. Antioxidant capacity was evaluated using the spectrophotometric 2,2-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid) (ABTS) method. Total antioxidant capacity was significantly correlated with season of manufacturing and time of ripening but not with animal species providing the milk. Moreover, statistically significant correlations between the total antioxidant capacity and retinol (r = 0.399), fat percentage (r = 0.308), protein percentage (r = 0.366), K (r = 0.385), Mg (r = 0.312), Na (r = 0.432), and P (0.272) were observed. We evaluated the use of near infrared spectroscopy technology, together with the use of a remote reflectance fiber-optic probe, to predict the antioxidant capacity of cheese samples. The model generated allowed us to predict antioxidant capacity in unknown cheeses of different compositions and ripening times.
Key words: cow, ewe, and goat milks, ripening, season, cheese composition

INTRODUCTION

The oxidative stability of milk and dairy products is of concern to the dairy industry because oxidation processes in milk can result in strong off-flavors and a deterioration of its nutritional quality (Dimick and Kilara, 1983), and also because dairy products can be beneficial for the oxidative defense in consumers through several mechanisms (Bounous, 2000; Steijns and van Hooijdonk, 2000).

The antioxidant capacity of milk and dairy products is the result of a complex equilibrium. Milk antioxidants play important roles in preventing lipid peroxidation and maintaining milk quality (Lindmark-Månsson and Åkesson, 2000). In this sense, milk and milk fractions (e.g., skim milk, whey, casein and lactoferrin) have been found to have antioxidant properties (Colbert and Decker, 1991; Cervato et al., 1999; Steijns and van Hooijdonk, 2000). Moreover, carotenoids, tocopherols, ascorbate, ureate, and other low-weight compounds (Clausen et al., 2010; de Renobales et al., 2012), together with enzymes such as glutathione peroxidase, play an important role in preventing milk lipid oxidation (Lindmark-Månsson et al., 2001). In contrast, other compounds such as polyunsaturated lipids and metals render milk more vulnerable to oxidation (Focant et al., 1998).

The composition of the feed partly influences the composition of milk; hence, certain feeding regimens could modify total antioxidant capacity. Pasture-based diets, which are seasonal, are associated with higher levels of xanthophyll, retinol, and α-tocopherol in goat, ewe, and cow milks (Rubino et al., 2000; Lucas et al., 2006a, 2008b). Recent studies carried out with sheep milk have demonstrated that Trolox equivalent antioxidant capacity (**TEAC**) is primarily associated with milk caseins, but it has also been reported that grazing increases TEAC significantly due to the presence of low-molecular-weight components in whey (de Renobales et al., 2012), such as phenolic compounds, as observed in the milk of grazing goats (De Feo et al., 2006). High contents of phenols in milk have been shown to improve the oxidative stability of dairy products (O'Connell and Fox, 2001). Nevertheless, grazing-based diets are correlated with higher levels of PUFA (Mel'uchova et al., 2008; D'Urso et al., 2008). Total vitamin contents also depend on the synthetic compounds added as supplements to the diet (Meglia et al., 2006), whereas the supplementation of dairy ruminant diets with vegetable

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oils, oilseeds, fish oil, or marine algae increases the proportions of MUFA and PUFA, as reviewed recently by Shingfield et al. (2013).

Work addressing the antioxidant capacity of cheeses is somewhat scarce but there is increasing interest in this field. Although total antioxidant capacity is determined by the cheese-making process itself (Lucas et al., 2006b), it is mainly correlated with fat-soluble vitamins (Lucas et al., 2006b, 2008b). Moreover, TEAC depends on ripening time and is related to the rate of formation of soluble antioxidant peptides as proteolysis progresses (Gupta et al., 2009; Perna et al., 2015).

The primary aim of the present work was to study the factors affecting the antioxidant capacity of cheeses prepared with cow, ewe, and goat milks at different proportions, with ripening times of up to 6 mo and using milk collected in winter or summer to establish the effect of species, ripening, and seasonality, as well as to determine parameters, such as fat composition, protein and peptide contents, mineral composition, and fat-soluble vitamins, with which the antioxidant capacity was correlated. To accomplish this, 224 cheeses were prepared, controlled, and analyzed. A second aim was to assess the application of near infrared spectroscopy (**NIRS**) to the prediction of the antioxidant capacity in cheeses of variable composition. Application of the NIRS technique to the determination of the antioxidant capacity in cheeses allows conventional analytical methods to be replaced by techniques that are multiparametric, less costly, faster, and do not involve sample destruction.

MATERIALS AND METHODS

Samples

To perform the present study, 224 cheeses of known composition; that is, cheeses with known, varying proportions of milk from cows, ewes, and goats, with percentages ranging between 0 and 100% (Table 1), were prepared following the procedure described by González-Martín et al. (2011b) and controlled. To do this, raw bovine, ovine, and caprine milks were collected directly from farms in winter and then 16 different compositions were collected in winter and again in summer. Cheeses were monitored over 6 mo and individual cheese samples were analyzed at 0, 1, 2, 3, 4, 5, and 6 mo. In total, 112 winter cheeses and 112 summer cheeses were analyzed.

TEAC Analysis

Total antioxidant capacity was determined by the ABTS method, which is based on the reduction of the 2,2-azinobis(3-ethylenebenzothiazoline-6-sulfonic acid) radical cation. Scavenging of the $ABTS^{\bullet+}$ radical was monitored by the decrease in absorbance at 734 nm by spectrophotometry (Chen et al., 2003). The ABTS method was modified from that described by Re et al. (1999). The water-soluble vitamin E analog Trolox (6-hydroxy-2,5,7,8-tetramethylchorman-2-carboxylic acid) was used as standard. Samples for analysis were prepared by diluting 2.5 mg of ground cheese in 10 mL of water. After stirring in a water bath at 40°C, the mixture was centrifuged at 20°C for 30 min (3,000 × *g*) and the supernatant was recovered and brought up to a final volume of 10 mL.

To prepare the ABTS radical cation, an ABTS solution was oxidized in water by treatment with potassium persulfate (molar ratio = 1:0.35) for 12 to 16 h in the dark, and then diluted in a 2-mL cuvette with 0.1 *M* potassium phosphate buffer, pH 7.4, before the assays, giving an absorbance of 0.7 ± 0.02 at 734 nm. A suitable amount of sample (20 μL) was added to the reagent and the mixture was incubated at 25°C. Absorbance was recorded every minute for 10 min using a Shimadzu spectrophotometer (Shimadzu UV-1603, Duisburg, Germany). Appropriate solvent blanks were run in each assay and each sample were analyzed in triplicate. The percentage of inhibition of absorbance at 734 nm was calculated and plotted as a function of the concentration of Trolox, resulting in the TEAC (μmol of Trolox/mg of cheese).

Chemical Composition

Crude protein composition (Kjeldahl method, N × 6.38) was analyzed using the AOAC International (1995) method, and total fat content was determined using the method of Geber van Gulik (ISO, 1975). All

Table 1. Composition (%) of the reference cheeses elaborated (winter and summer milk)

Cow milk	Ewe milk	Goat milk
100	0	0
0	100	0
75	25	0
50	50	0
25	75	0
0	0	100
25	0	75
50	0	50
75	0	25
0	25	75
0	50	50
0	75	25
33	33	33
10	45	45
45	10	45
45	45	10