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Quantitative measurement of vitamin K_2 (menaquinones) in various fermented dairy products using a reliable high-performance liquid chromatography method

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ABSTRACT

We evaluated menaquinone contents in a large set of 62 fermented dairy products samples by using a new liquid chromatography method for accurate quantification of lipo-soluble vitamin K_2, including distribution of individual menaquinones. The method used a simple and rapid purification step to remove matrix components in various fermented dairy products 3 times faster than a reference preparation step. Moreover, the chromatography elution time was significantly shortened and resolution and efficiency were optimized. We observed wide diversity of vitamin K_2 contents in the set of fermented dairy products, from undetectable to 1,100 ng/g of product, and a remarkable diversity of menaquinone forms among products. These observations relate to the main microorganism species currently in the different fermented product technologies. The major form in this large set of fermented dairy products was menaquinone (MK)-9, and contents of MK-9 and MK-8 forms were correlated, that of MK-9 being around 4 times that of MK-8, suggesting that microorganisms able to produce MK-9 also produce MK-8. This was not the case for the other menaquinones, which were produced independently of each other. Finally, no obvious link was established between MK-9 content and fat content or pH of the fermented dairy products.
Key words: vitamin K_2, menaquinone, fermented milk, cheese

INTRODUCTION

Vitamin K_2 (menaquinone) is a natural form of vitamin K that occurs in food. Vitamin K_2 is mainly present in fermented food (e.g., natto, fresh fermented dairy product, cheese), whereas the other form of vitamin K, vitamin K_1 (phylloquinone), is abundant in leafy green vegetables (e.g., cabbage, spinach, lettuce). It is generally presumed that vitamin K_2 is produced by microorganisms. Vitamin K_2 refers to a series of naphthoquinones with a variable side chain that is usually between 4 and 10 isoprene units long. The compounds in the series are referred to as menaquinone (**MK**)-n, where n denotes the number of isoprene units.

Vitamin K_2 exhibits significant health benefits, particularly for cardiovascular (Beulens et al., 2009) and bone health (Schurgers et al., 2007). The daily intake dose recommended by European Food Safety Authority (EFSA, 1990) is 75 μg for adults and 12 μg for children from 6 mo to 4 yr. Some studies suggest that the bioavailability of vitamin K_2 is related to the length of the side chain, with medium-length menaquinone (e.g., MK-7) being more bioavailable than short-chain menaquinone (e.g., MK-4; Schurgers and Vermeer, 2000). Therefore, we assumed that not only the total amount of vitamin K_2 but also the distribution of vitamin K_2 forms could be of interest in supporting or enhancing health benefits.

Although the presence of menaquinones in food is well known and demonstrations of its health benefit are increasing, publications on reliable and accurate quantification methods (and efficient quantitative extraction methods) from food matrices are scarce.

The dosage of phylloquinone has been studied and quantified in different foodstuffs (Booth et al., 1993). A European standard method (ISO, 2004; NF EN 14148) exists to determine vitamin K_1 by HPLC, but no official method exists for the measurement of vitamin K_2. We propose an optimized technique of dosage and quantification of vitamin K_2 adapted to fermented dairy products. Using this method, we quantified total vitamin K_2 and evaluated the distribution of side-chain forms MK-6 to MK-10 in fermented dairy products obtained by different dairy technologies (e.g., thermophilic cheeses, mesophilic cheeses, fresh fermented milks).

MATERIALS AND METHODS

Fermented Dairy Samples

Sixty-two fermented dairy samples were purchased in late 2010 from several retail stores in France, Germany,

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Table 1. Fermented dairy samples in this study

Sample category and ID	Country (production country, if different)	Shelf life date (month/day/year)	Analysis date (month/day/year)	DM (g/100 g)	Fat (g/100 g)	pH
Blue cheese						
BlueC1	France	11/09/2010	11/09/2010	66.8	41.0	5.57
BlueC2	France	11/08/2010	11/08/2010	51.7	29.0	6.44
BlueC3	France	12/13/2010	11/30/2010	54.3	35.0	7.19
BlueC4	England	Unknown	12/15/2010	65.7	39.5	6.47
Caerphilly						
HardC-Caer	England	11/30/2010	11/30/2010	58.2	32.8	4.57
Cheddar						
HardC-Ched1	England	12/28/2010	12/03/2010	65.4	35.0	4.81
HardC-Ched2	England	12/02/2010	12/02/2010	62.3	33.0	5.08
HardC-Ched3	England	12/08/2010	12/03/2010	64.1	35.0	5.01
HardC-Ched4	England	11/22/2010	11/22/2010	61.1	32.8	5.01
HardC-Ched5	England	01/08/2011	12/03/2010	67.1	36.0	5.20
HardC-Ched6	England	12/31/2010	12/03/2010	66.0	36.0	5.22
Cheshire						
HardC-Ches	England	12/10/2010	12/03/2010	58.3	31.5	4.36
Comté						
HardC-Co1	France	11/12/2010	10/29/2010	65.2	34.5	5.57
HardC-Co2	France	12/13/2010	10/29/2010	65.2	34.5	5.68
Emmental						
HardC-Em1	France	12/06/2010	10/29/2010	61.3	30.5	5.59
HardC-Em2	France	12/28/2010	10/29/2010	61.0	29.5	5.52
Leicester						
HardC-Leic	England	12/01/2010	12/01/2010	63.0	33.0	4.97
Mesophilic fermented milk						
MFM1	France	10/31/2010	10/29/2010	16.3	9.5	4.34
MFM2	France	11/14/2010	10/29/2010	20.7	8.5	4.72
MFM3	France	11/11/2010	10/29/2010	17.2	2.8	4.51
MFM4	France	12/09/2010	11/30/2010	18.0	3.5	4.52
MFM5	France	12/05/2010	11/30/2010	24.0	10.0	4.57
MFM6	France	12/06/2010	11/30/2010	22.8	0.3	4.33
MFM7	France	05/01/2011	11/30/2010	18.6	0.3	3.84
MFM8	France	12/03/2010	11/30/2010	16.9	6.0	4.29
MFM9	Germany	12/12/2010	12/10/2010	25.0	10.0	4.37
MFM10	Germany	01/05/2011	12/15/2010	11.2	<0.3	4.09
MFM11	Germany	12/09/2010	12/09/2010	19.2	4.0	4.28
MFM12	Poland	11/26/2010	11/26/2010	9.3	0.5	4.40
MFM13	Poland	12/06/2010	12/06/2010	26.5	ND[1]	4.36
MFM14	Poland	12/10/2010	12/10/2010	11.9	1.5	4.52
MFM15	Poland	12/05/2010	12/03/2010	11.3	1.3	4.24
MFM16	Poland	12/11/2010	12/10/2010	25.1	4.0	4.34
MFM17	Poland	12/05/2010	12/03/2010	29.2	8.0	4.39
MFM18	Poland	12/06/2010	12/06/2010	21.9	6.5	5.05
MFM19	Poland	12/09/2010	12/09/2010	20.6	7.0	4.50
MFM20	Poland	12/16/2010	12/15/2010	26.8	18.0	4.36
MFM21	Poland	12/16/2010	12/15/2010	24.4	0.5	4.41
Mozzarella cheese						
Mozz	France (Germany)	11/28/2010	11/26/2010	43.7	23.5	5.79
Semihard cheese						
SemiHC1	France (the Netherlands)	01/24/2011	10/29/2010	57.8	29.5	5.04
SemiHC2	France (the Netherlands)	Unknown	10/29/2010	60.9	29.5	5.25
SemiHC3	France	11/30/2010	10/29/2010	55.7	27.5	5.41
SemiHC4	France (the Netherlands)	01/14/2011	10/29/2010	57.1	24.0	5.20
SemiHC5	France	11/16/2010	10/29/2010	51.3	26.0	5.05
SemiHC6	France (the Netherlands)	12/27/2010	10/29/2010	58.5	27.0	5.43
SemiHC7	Denmark	Unknown	12/15/2010	58.8	29.0	5.96
SemiHC8	Denmark	Unknown	12/15/2010	56.4	29.0	6.11
SemiHC9	Denmark	12/07/2010	12/07/2010	53.7	25.1	6.41
SemiHC10	Poland	01/31/2011	12/15/2010	56.8	27.0	5.31
SemiHC11	Poland	11/30/2010	11/30/2010	59.3	27.8	6.12
Soft cheese						
SoftC1	France	11/22/2010	11/09/2010	51.2	25.0	6.62
SoftC2	France	11/16/2010	11/09/2010	54.4	27.0	6.60
SoftC3	France	12/12/2010	11/30/2010	65.6	34.0	5.45

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