We described here a sensitive and highly selective high-performance liquid chromatography (HPLC) method for determination of vitamin K1 and vitamins K2 (MK-4 to MK-10) in fermented milk and fresh cheese products. The different steps of the method have been optimized to be able to quantify vitamin K in small amounts (until 0.05 µg/100 g) in dairy products. Chromatography was performed by reverse phase separation on a RP-18e column followed by a post-column zinc reduction to facilitate fluorescent detection. The chromatography conditions were optimized to improve vitamin K resolution. A study was performed by two laboratories (DANONE Research Analytical Support and AQUANAL – Laboratoire Aquitaine Analyses). The results were statistically analyzed and confirm the method validity.

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**INTRODUCTION**

At least two naturally-occurring forms of vitamin K have been identified:
- vitamin K1 (phylloquinone) widely distributed in green leafy vegetables and plant oils,
- vitamin K2 (menaquinones) derived from bacteria and animals.

Menaquinones comprise a family of molecules distinguished from phylloquinone by unsaturated side-chains of isoprenoids units varying in length from 1 to 14 repeats.

**PROTOCOL**

Phylloquinones and menaquinones are extracted by enzymatic treatment using a lipase solution adapted to the quantity of dairy product analyzed. Then two steps of solvent extraction (alcoholic reagent and hexane) are needed before quantification with reverse-phase HPLC and fluorescent detection after post-column reduction with metallic zinc.

**RESULTS**

Both laboratories performed independent replicate analyses of fermented milk. A study on supplemented fresh cheese samples was carried out by AQUANAL and demonstrated a recovery about 100%. As only 3 standards of the 8 vitamins K detected are commercially available (K1, MK4 and MK7), the concentration is expressed in µg/100g equivalent MK4 for MK4 to MK6 and equivalent MK7 for MK7 to MK10. The end result is expressed as the sum of all these molecules.

**CONCLUSION**

The proposed HPLC method for determining vitamin K in dairy products is:
- highly selective
- reproducible
- reliable
- accurate