

# DETERMINATION OF VITAMIN K DERIVATIVES IN FERMENTED MILK PRODUCTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND POST-COLUMN ZINC REDUCTION

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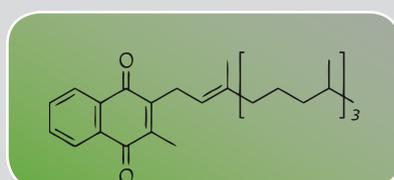
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## INTRODUCTION

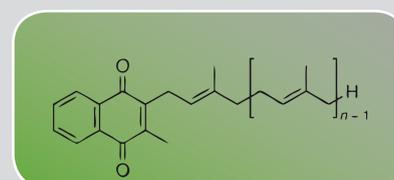
At least two naturally-occurring forms of vitamin K have been identified :

- **vitamin K1 (phyloquinone)** 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 phyloquinone by unsaturated side-chains of isoprenoids units varying in length from 1 to 14 repeats.



Vitamin K1—Phylloquinone



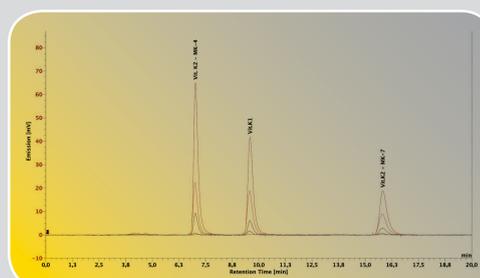
Vitamin K2 – Menaquinones-n

We described here a sensitive and highly selective high-performance liquid chromatography (HPLC) method for determination of vitamin K1 and vitamin K2 (MK-4 to MK-10) in fermented milk and fresh cheese products.

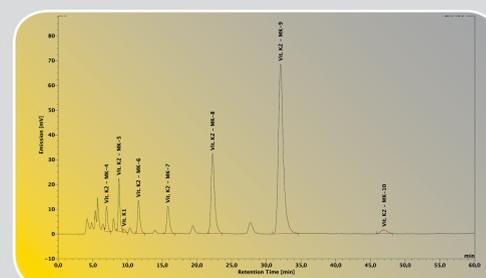
The different steps of the method have been optimized to be able to quantify vitamin K in small amounts (until 0.05 µg/100 g) in dairy products. Chromatography was performed by reverse phase separation on a RP-18e column followed by a post-column zinc reduction to facilitate fluorescent detection. The chromatography conditions were also 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.

## PROTOCOL

Phylloquinone and menaquinones are extracted by enzymatic treatment using a lipase solution adapted to the quantity of dairy product analysed. 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.



4 levels standard profiles



Vitamin K profile in fermented milk

## 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.

Intercomparison study on fermented milk samples :

11 fermented milk samples were analysed. Figure 1 shows correlation between the values obtained for MK-7, MK-8 and MK-9. The MK-7, MK-8 and MK-9 vitamins were chosen for this comparison because their concentration ranges were the largest. The data showed good correlation between the two laboratories for MK-7 (Fig 1a,  $y = 0.9522x$ ,  $R^2 = 0.9954$ ), MK-8 (fig 1b,  $y = 1.0069x$ ,  $R^2 = 0.9933$ ) and MK-9 (Fig 1c,  $y = 1.023x$ ,  $R^2 = 0.9889$ ), respectively.

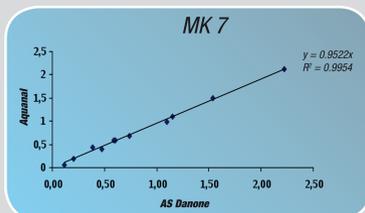


Figure 1a

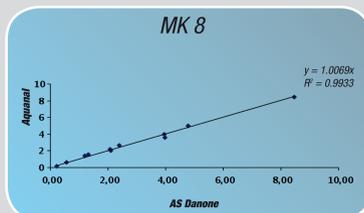


Figure 1b

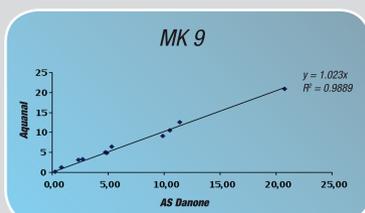


Figure 1c

Figure 1: Comparison of the results obtained by Aquanal and AS Danone on fermented milk samples (µg/100g)

Study on fresh cheese samples :

Analyses were performed on 6 samples supplemented with natto powder. The theoretical concentration of MK-7 in these supplemented samples is calculated according to the known concentration of MK-7 in natto powder specification. Figure 2 shows the correlation between the MK-7 theoretical values and the results obtained by Aquanal.

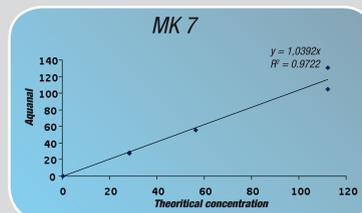
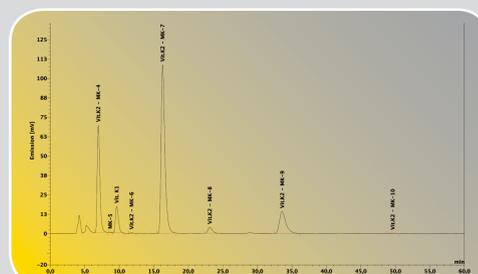


Figure 2 : Comparison of the results obtained by Aquanal and the theoretical MK-7 concentration on fresh cheeses supplemented with natto powder (µg/100g)

The results obtained showed a good correlation with the theoretical MK-7 concentration ( $y = 1.0392x$ ,  $R^2 = 0.9722$ ). These results suggested that the recovery of MK-7 on fresh cheese is about 100% using this method.



Vitamin K profile in fresh cheese supplemented with vitamin K1, MK4 and MK7

## CONCLUSION

The proposed HPLC method for determining vitamin K in dairy products is :

- highly selective • reproducible • reliable • accurate