Equol as phytoestrogen metabolite in animal products ! How and what is the interest for the consumer ?

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Table of contents

- The context in which the project was born
- The *PhytoHealth* project
- First results
  - Equol in Belgian milks ?
  - Impact of skimming & microfiltration ?
- Conclusions and perspectives

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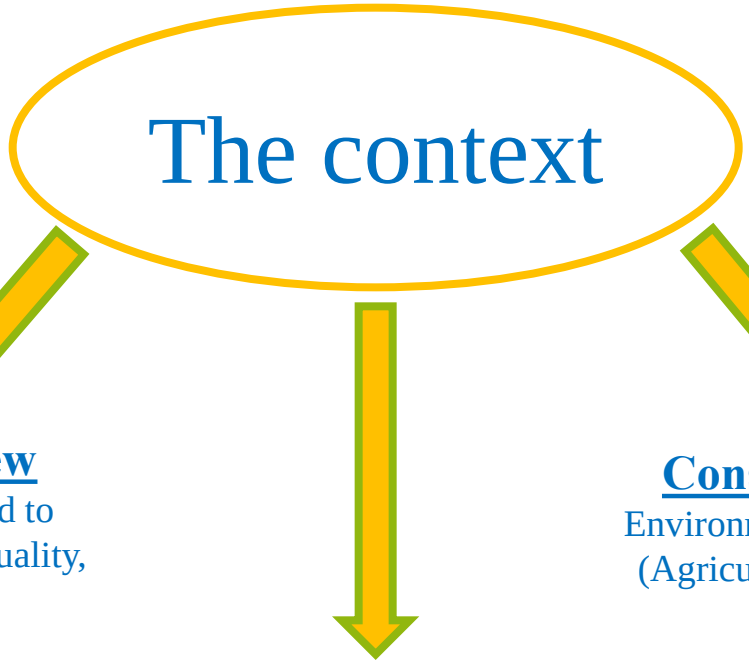
❖ Introduction



Producer's point of view
Economic crisis → Farmers need to secure their products in terms of quality, quantity and costs

Industrial's point of view
Manufacturers are interested by new differentiated quality products which could be checked before being sold

Consumer's point of view
Environment, health and animal welfare (Agriculture perception improvement)

→ Why not enrich animal products with phytoestrogens ?

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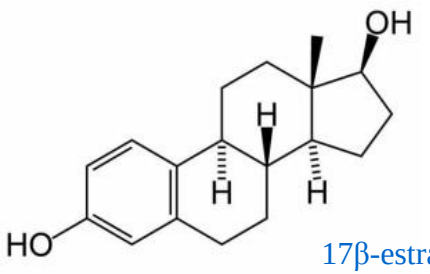

17β-estradiol

➢ Phytoestrogens*

- They are polyphenolic compounds present in plants and which contribute to the plants development.
- They are structurally or functionally similar to mammalian estrogens which allows them to have estrogenic activity.
- They are essentially present in Leguminosae (soja, clover, etc.).

Causing infertility in livestock and possible impaired reproductive processes in humans.

Acting as antioxidants and/or anti-inflammatories. Reducing atherosclerosis, osteoporosis, severity and frequency menopausal symptoms, etc.

* M. Mostrom, T.J. Evans. Veterinary Toxicology –Basic and Clinical Principles (2nd). Ramesh C. Gupta (ed.), Netherlands, 2012, Chapter 76.

* Rapport Afssa : Sécurité et bénéfices des phyto-estrogènes apportés par l'alimentation – Recommandations, 2005.

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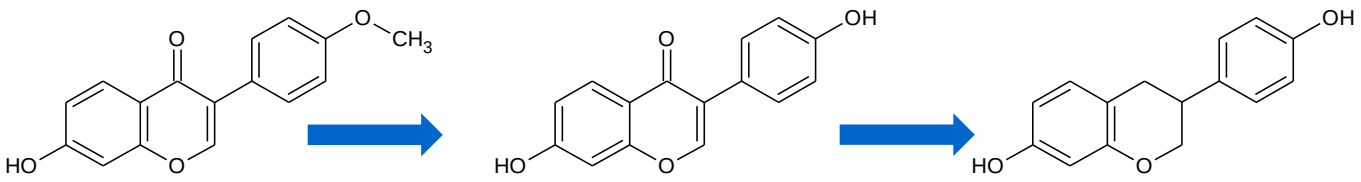
❖ Introduction

➢ Equol*:



Formononetin → Daidzein → S-(-)-Equol

- Microbial metabolite produced by specific intestinal bacteria.
- Beneficial effects :

  - **Acting as antioxidants,**
  - **Reducing atherosclerosis, osteoporosis, severity and frequency menopausal symptoms,**
  - **Helping to prevent the onset of prostate cancer and to decrease bone resorption in postmenopausal women.**

- Equol-producers → 1/3 of Western population & 2/3 of Asian population.

* M. Mostrom, T.J. Evans. Veterinary Toxicology –Basic and Clinical Principles (2nd). Ramesh C. Gupta (ed.), Netherlands, 2012, Chapter 76.

* P. Kalač, 2013. Czech J. Anim. Sci., 58 (7), 296-303.

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PhytoHealth : *Phytoestrogens-rich plants to improve the biosynthesis of equol*

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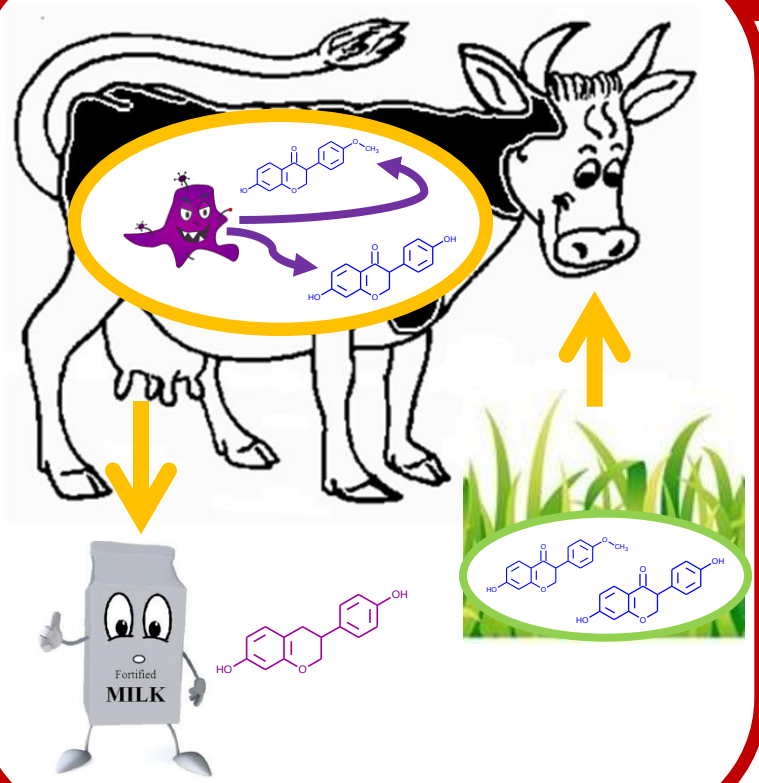

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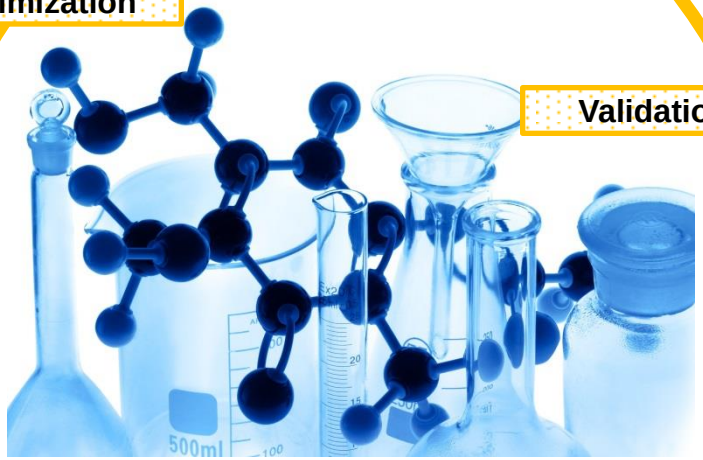
Optimization

Validation



Development of analytical methods

Routine use

Selection

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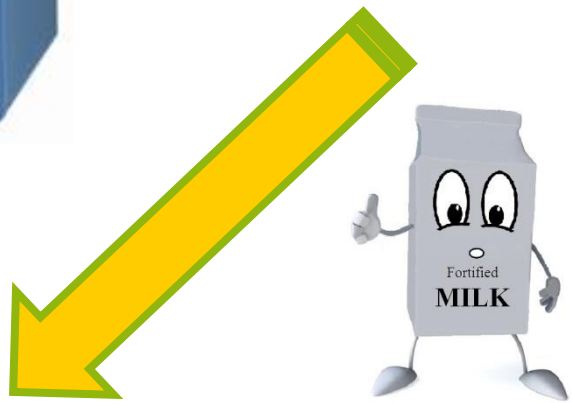
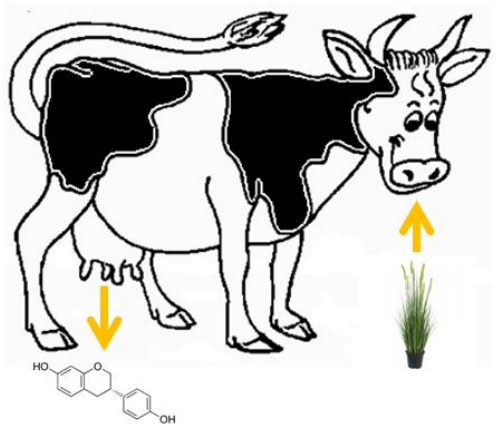
➢ *PhytoHealth :*

UPLC®-MS/MS

Development of fast and easy to use ***analysis methods*** to detect phytoestrogens and their microbial metabolites in different samples



For a ***better understanding of phytoestrogens metabolization*** in dairy cows in order to create a controlled production where the ***milk*** would be **naturally *enriched* in equol**.



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❖ What is the equol concentration in Belgian milks ?

Results of a first screening…

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❖ What is the equol concentration in Belgian milks ?

➢ Optimization and validation of Ultra-Performance Liquid Chromatography method with tandem mass spectrometry *

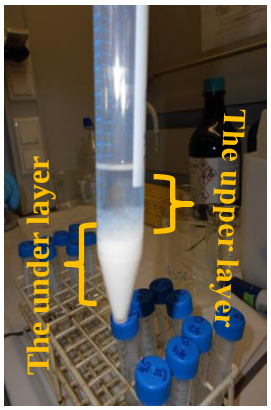
Random sampling & storage at -18°C
(*max. 2 months*)

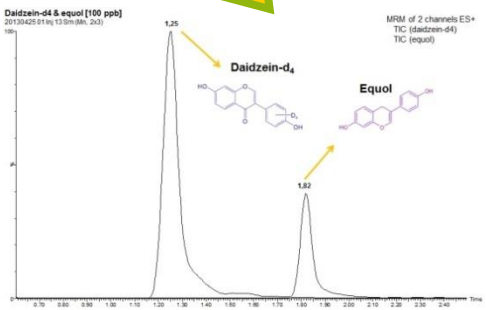
Enzymatic hydrolysis
(*β-glucuronidase, 2 h & 37°C*)

Double extraction Liq./Liq.
(*Hexane → remove fat & ethyl acetate → recover interest compounds*)

Evaporation & reconstitution in methanol 80%

UPLC®-MS/MS analysis





* Sep2013 : Validation d'une méthode d'analyse quantitative de l'équol dans le lait par UPLC-MSMS. *(http://orbi.ulg.ac.be/handle/2268/149435)*

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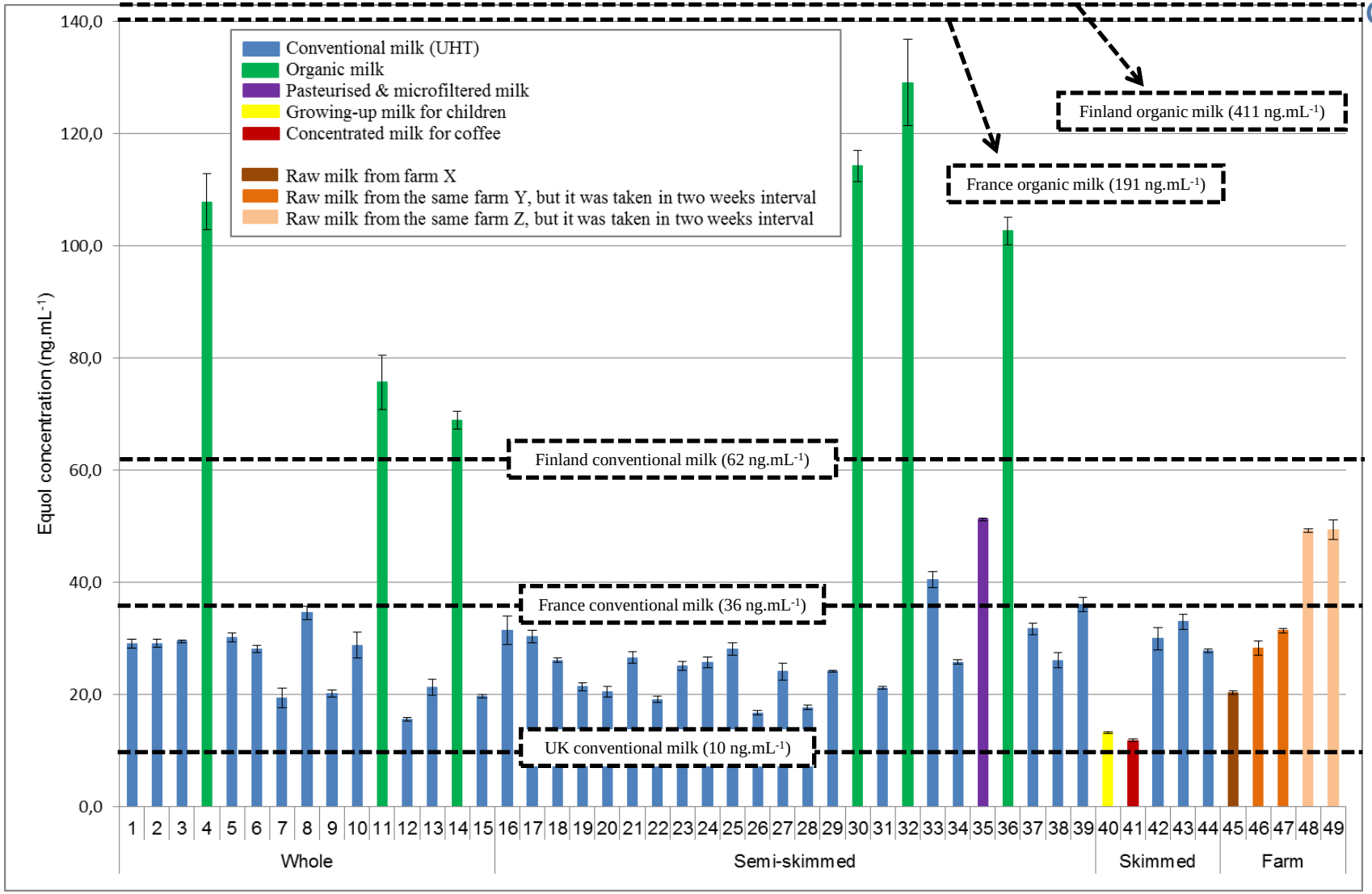
❖ What is the equol concentration in Belgian milks ?



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--- *Kalač P., 2011. Food Chemistry, 125, 307-317.*

* Journées3R2013: Détermination de la teneur en équol dans les laits commercialisés en Wallonie (Belgique)

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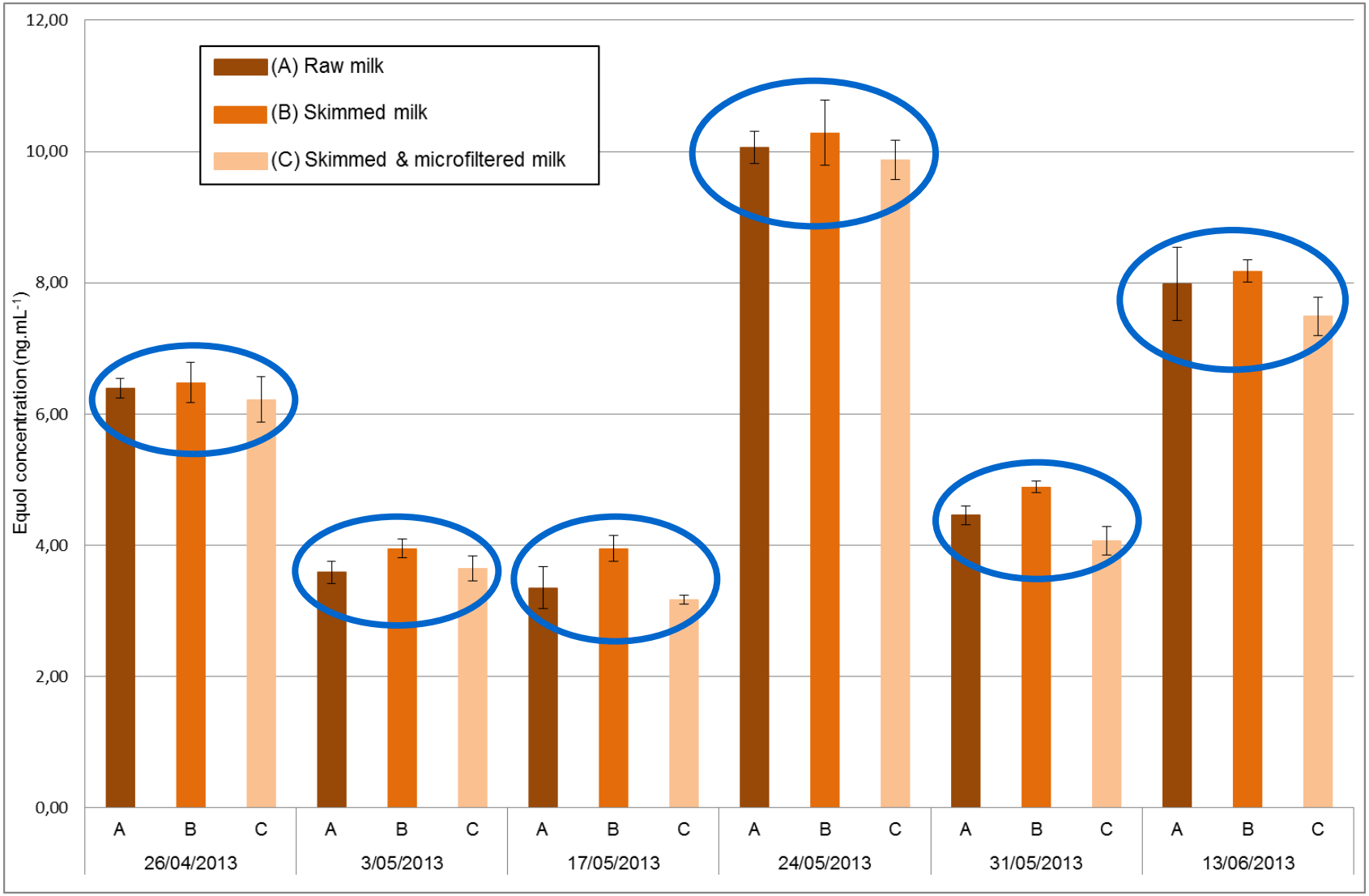
❖ What is the impact of skimming and microfiltration process on equol concentration in milk ?



* Journées3R2013: Détermination de la teneur en équol dans les laits commercialisés en Wallonie (Belgique)

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❖ Conclusion and perspectives

Development of analytical methods for a better understanding of phytoestrogens metabolization in dairy cows in order to naturally enriched milk with beneficial compounds for human health.

- Equol was always present in milk.
- No difference between whole, semi-skimmed and skimmed milks.
- Equol content was higher in organic milks than conventional milks. *(? agricultural practice influence ?)*
- Variability between countries and between farms.
- Skimming and microfiltration had no impact on equol concentration in milks.

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❖ Conclusion and perspectives

- Development, optimization and validation of analytical methods to quantify equol and its phytoestrogens precursors in several matrices.

- Metabolic and zootechnical trials *(dairy cows)*.

- Same approach for laying hens.

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Thank you for your attention

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