
# 13th NIZO Dairy Conference: Innovations in Milk Proteins

## 17-20 October 2023, Papendal, Arnhem, The Netherlands

Κατά τη διάρκεια του 13ου Διεθνές Συνεδρίου «NIZO Dairy Conference: Innovations in Milk Proteins» που πραγματοποιήθηκε στις 17-20 Οκτωβρίου στο Άρνεμ της Ολλανδίας, η υποψήφια διδάκτορας του Τομέα μας Χαρίτου Γαρουφαλιά βραβεύτηκε με το 1ο βραβείο καλύτερης σύντομης προφορικής παρουσίασης με αναρτημένη ανακοίνωση (€500, 1 year subscription to International Dairy Journal). Η βραβευθείσα παρουσίασε την εργασία με τίτλο:

**«Exploring the potential of ethanol-treated whey proteins to form nanoparticles, stabilize Pickering emulsions and modify *in vitro* lipid digestion».**

Με συγγραφείς τους: Garoufalia Charitou, Panagiota Tsafrakidou, Charikleia Kyrkou, Athina Lazaridou, Alexandra-Maria Michaelidou, Thomas Moschakis*

Το συνέδριο διοργανώθηκε από την εταιρία NIZO, η οποία αποτελεί κορυφαία εταιρία παγκοσμίως στην έρευνα και την καινοτομία τροφίμων με ιστορία 75 χρόνων, καθώς και τον εκδοτικό οίκο Elsevier. Η οργανωτική επιτροπή αποτελούνταν από διακεκριμένους επιστήμονες της εταιρίας NIZO και πιο συγκεκριμένα τους René Floris, Fred van de Velde, Celine Brattinga. Τέλος, η επιστημονική επιτροπή αποτελούνταν από τους Fred van de Velde (NIZO Food Research), René Floris (NIZO Food Research), Thom Huppertz (FrieslandCampina, Wageningen University) και Peter de Jong (NIZO Food Research, VHL University of Applied Sciences).

Το εν λόγω συνέδριο αφορούσε την έρευνα και καινοτομία στον τομέα των πρωτεϊνών γάλακτος, αποσκοπώντας να αναδείξει τα πιο πρόσφατα ερευνητικά ευρήματα σχετικά με τις πρωτεΐνες του γάλακτος, να τα συνδυάσει με τα λειτουργικά τους χαρακτηριστικά και να φέρει σε επαφή την ακαδημαϊκή ερεύνα με την βιομηχανία γάλακτος. Επιπλέον, κάλυψε διαφορετικές περιοχές της έρευνας αλλά και των εφαρμογών των πρωτεϊνών του γάλακτος μέσω των παρακάτω θεματικών ενοτήτων:

- Nutrition, digestion, and health aspects of milk proteins, Protein structure, stability, and interactions
- Process-product interactions affecting milk protein functionality
- Milk protein ingredient manufacture, including microbial production and functionality
- Influence of milk proteins in product structure and stability
- Milk proteins produced by precision fermentation

Στο συνέδριο παρουσιάστηκαν 6 keynote lectures, 21 προφορικές ανακοινώσεις, 6 short oral presentations (Young Scientist Award), και 50 αναρτημένες ανακοινώσεις.

https://www.nizodairyconference.com/


# Exploring the potential of ethanol-treated whey proteins to form nanoparticles, stabilize Pickering emulsions and modify *in vitro* lipid digestion

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## Introduction

Pickering emulsions, which are stabilized by solid nanoparticles, exhibit exceptional stability against coalescence and flocculation, possibly enhancing resistance during lipid digestion and thus benefit human health. Meanwhile, whey protein micro- and nano-particles have received particular attention for enhancing food functionality. Recently, it has been shown that whey proteins treated with ethanol can form nanoparticles, which may possess different properties compared to their counterparts prepared by heat (Geroukas et al., 2022). Thus, these innovative particles represent a promising candidate for stable Pickering emulsion formation.

## Objective

This study investigates the ability of nanoparticles, formed from whey proteins treated with ethanol, to stabilize Pickering emulsions and their effect on *in vitro* lipid digestion.

## Materials and methods

Preparation of whey protein (WPI) particles


Preparation of oil-in-water emulsions (o/w)


*In vitro* lipid digestion of oil-in-water emulsions (o/w)

An *in vitro* lipid digestion protocol was applied (Tzavaras et al., 2023). The emulsions were diluted, resulting in final emulsions containing 0.50 w/w oil. The samples were kept at 37 °C for 10 minutes, and the pH was set to 7.0. Subsequently, bile extract, CaCl2, and pancreatic lipase suspension were added. An automatic pH-stat titration unit was used to monitor and keep pH at 7.0 during the lipid digestion process by titrating with a 0.05 M NaOH solution to neutralize any free fatty acids (FFAs) that were released. The %FFA released was calculated by dividing the moles of NaOH needed to neutralize the FFAs by the moles of FFA that could potentially be produced through complete digestion of triacylglycerols.

## Results and Discussion

Characterization of whey nanoparticles pre-treated with ethanol

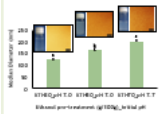


Fig 1. Comparison of the median diameter of nanoparticles produced from ethanol pre-treated WPI (10-70% w/w) after the removal of ethanol. In black boxes, CLSM micrographs and photographs illustrating of nanoparticles' dispersions are presented.

- ✓ Nanoparticles from ethanol-treated whey proteins were formed after the removal of ethanol.
- ✓ The diameter of the particles exhibited small variations (100-200 nm).

Sunflower o/w emulsions stabilized by WPI and WPI particles pre-treated with ethanol

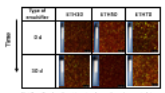


Fig 3. Confocal micrographs of o/w emulsions stabilized by WPI and WPI nanoparticles prepared by pre-treatment with different amounts of ethanol.

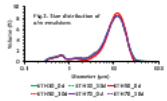


Fig 5. Size distribution of o/w emulsions.

In vitro lipid digestion of sunflower o/w emulsions

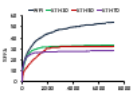


Fig 6. Time dependence of FFA release (%FFA) from sunflower o/w emulsions stabilized by WPI and WPI nanoparticles pre-treated with 30, 50 and 70% ETOH (0.05 w/w).

- ✓ Oil-in water emulsions were prepared using very low concentration of WPI nanoparticles pre-treated with ethanol that remained stable upon 30 days storage.
- ✓ The particles-stabilized emulsions showed a considerably reduced level of lipid digestion in comparison to those stabilized by WPI.

## References

Geroukas, T., Dimitriou, G., Magoni, C., Aiello, A., Moschakis, T., & Papagiannopoulos, A. (2022). Formation of nanoparticles from ethanol-denatured whey proteins. *Food Structure*, *33*(June), 100257.

Tzavaras M.V., Kanakaris T., Schullers E., Biliaderis C.G. (2023). In vitro lipid digestion of edible nanocrystal-stabilized o/w emulsions. *Food & Function*, *1* (January), 17–29.

## Conclusions

Nanoparticles with a median diameter of 100-200 nm were prepared from WPI pre-treated with ethanol and were utilized to stabilize o/w emulsions. The study showed that at very low particle concentration (0.05 w/w), these emulsions remained stable for 30 days. Moreover, compared to conventional WPI-prepared emulsions, they exhibited enhanced stability against lipid digestion.

## Acknowledgments

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