Solvent-Free Recovery of Functional Caseins from Dairy Side-Streams for Circular One-Health Biomaterials

Rogério Medeiros*[1,2], João Pedro Leite[3], Mónica V. Loureiro[6], Ana Paula Serro[4,5], Helena Cristina Vasconcelos[2], Raquel Galante[1]

rogeriomedeiros@cimpa.pt

[1]Centro de Inovação em Materiais e Produtos Avançados (CIMPA), Lagoa, Açores, Portugal
[2]University of the Azores – Faculty of Science and Technology, Ponta Delgada, Açores, Portugal
[3]Instituto de Inovação Tecnológica dos Açores (INOVA), Ponta Delgada, Açores, Portugal
[4]Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Science
[5]Centro de Química Estrutural (CQE), Institute of Molecular Sciences, Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa
[6]CERENA, Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa

Background:

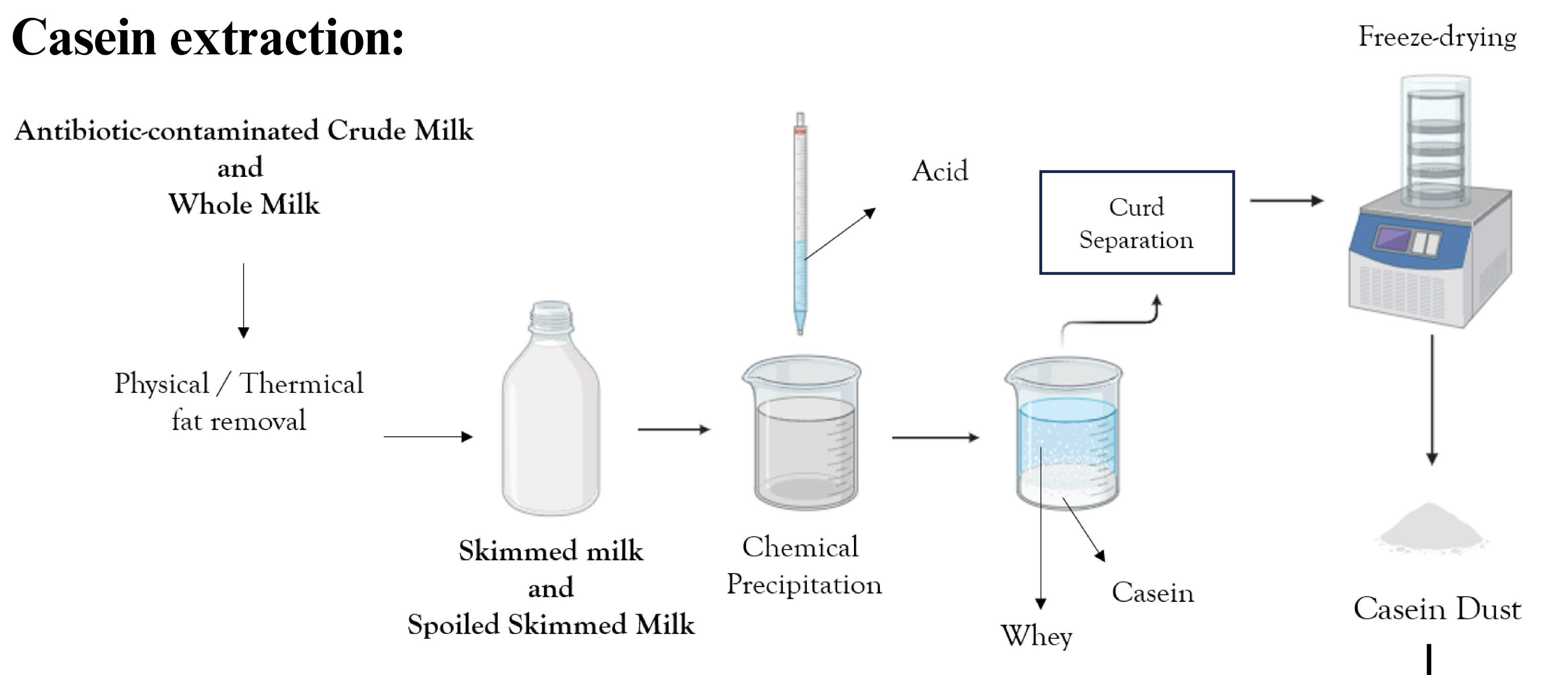
Worldwide dairy output has risen steadily for more than a decade[1], yet almost one-fifth of the milk handeled in the European supply chain never reaches consumers[2]. Significant losses arise from (i) withdrawal milk containing antibiotic residues[3], (ii) clinical and sub-clinical mastitis that drives somatic-cell counts and bacterial loads beyound regulatory limits[4], and (iii) spoilage during storage, transport or heat treatment[2]. Milk waste, however, is an untapped feed-stock rich in casein (~80 % of total protein[5]) and in growth factors such as EGF, IGF and bFGF, all biomolecules with prove roles in tissue repair[6]. Casein is intrinsically biocompatible[7], biodegradable[8], non-toxic and inexpensive[9]; its selective recovery from waste streams therefore offers a sustainable route to high-value biomaterials.

Main Goal:

Explore a solvent-free, acid-precipitation process to recover casein from four types of milk: skimmed milk (SM), whole milk (WM), antibiotic-contaminated crude milk (ACCM) and spolied skimmed milk (SSM) and benchamark the resulting "eco-caseins" against commercial bovine casein (CCM).

Methods:

Casein extraction:



Characterization:
Yield quantification;
Emulsifying and foaming capacity;
FTIR spectra;
DSC and TGA analysis.

Results and Discussion:

Extraction Yields

Milk Type	Milk (L)	Yield (%)	Average (%)	STD
Skimmed Milk	1	97,67	94,93	3,25
	1	95,80		
	1	91,33		
Spoiled Skimmed Milk	2	47,17	62,37	13,16
	2	70,00		
	2	69,93		
Whole Milk	1	191,60	187,99	4,53
	1	182,90		
	1	189,47		
Antibiotic-contaminated Milk	1	157,17	129,48	51,76
	1	161,50		
	1	69,77		

Table 1: Extraction yields of the "eco-caseins" from 4 types of milk: skimmed milk (SM), spoiled skimmed milk (SSM), whole milk (WM) and antibiotic-contaminated crude milk (ACCM).

- Fat in milk increased the yield;
- Potential protein degradation in spoiled milk;
- Potential de-fating problems in ACCM extration process.

FTIR spectra

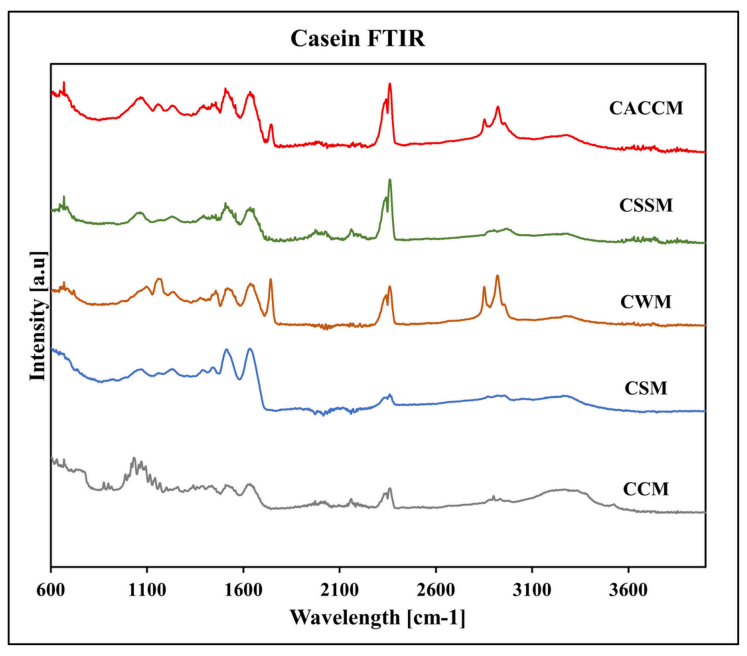


Figure 3: Absorbance FTIR spectra of the "eco-caseins".

- All spectra showed amide I (1655 cm^{-1}) and amide II (1545 cm^{-1}) bands.
- CWM and CACCM also displayed fat peaks near 3000 cm^{-1} → residual fat content.

Emulsifying capacity

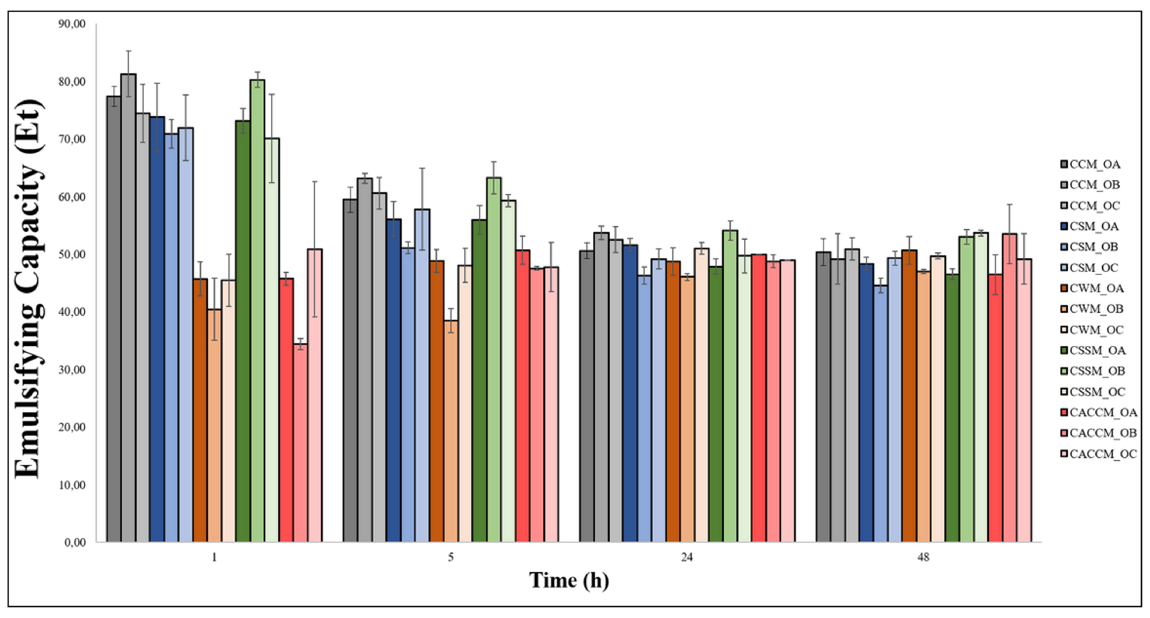


Figure 1: Emulsifying capacity of the "eco-caseins" in sweet almonds oil (OA), olive oil (OB) and sunflower oil (OC).

- SM and SSM-derived caseins match the emulsifying profile of control;
- Residual lipids depress performance for CWM and CACCM;
- All emulsions reach a steady layer after 24 h, independent of casein source.

Differential Scanning Calorimetry

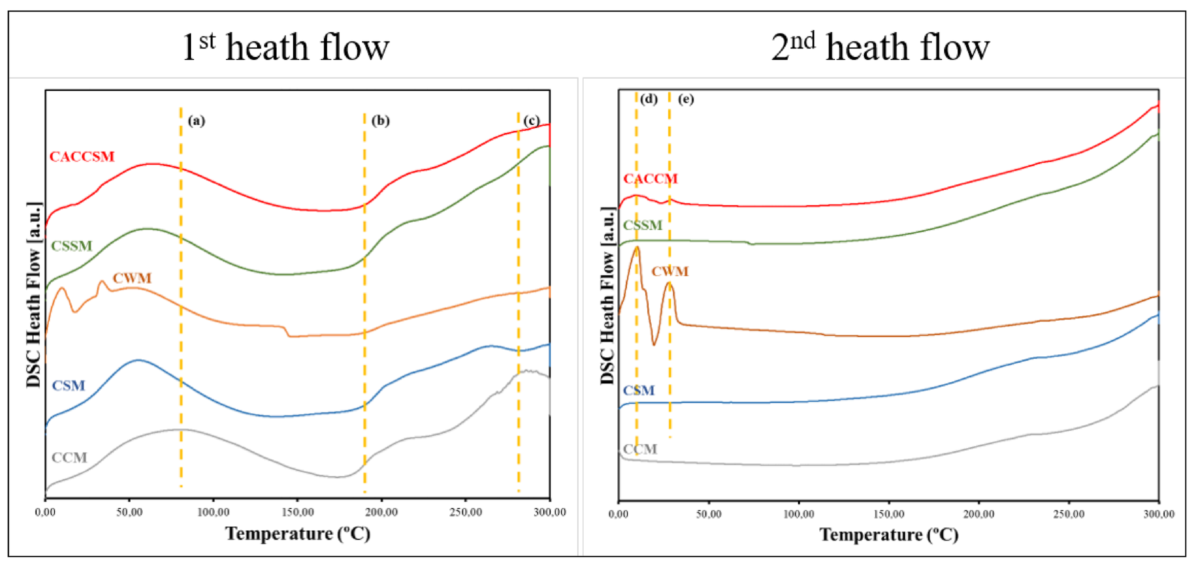


Figure 4: Differential Scanning Calorimetry (DSC) of the "eco-caseins".

- The peaks **(a)**, **(b)** and **(c)** might be related to casein denaturation[10] → only notourious in CCM;
- In the second heating, the peaks **(d)** and **(e)** are observed in the CWM and CACCM, near the temperatures of fat melting transitions seen in the first heating.

Foaming Capacity

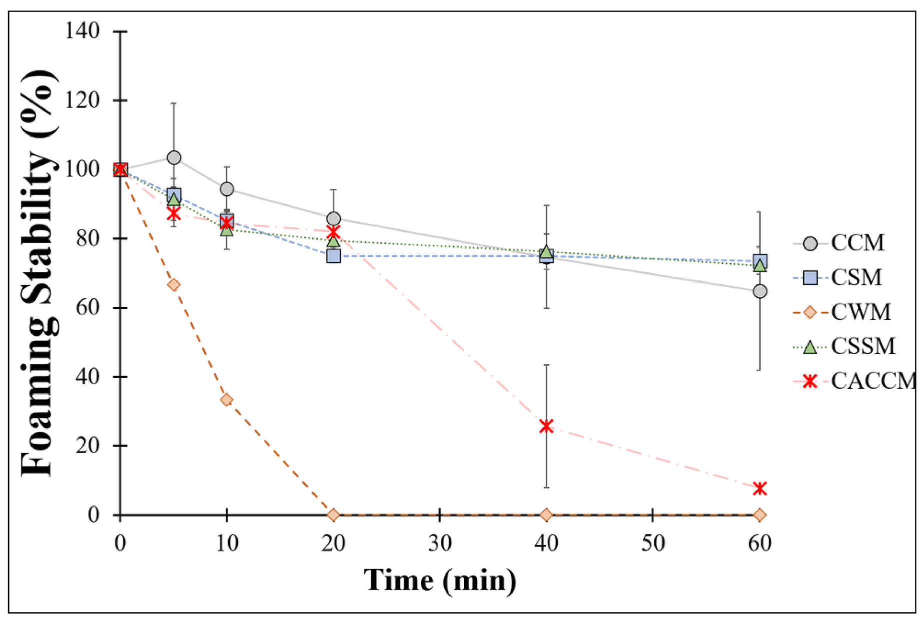


Figure 2: Foaming properties of the "eco-caseins" in phosphate buffer saline solution.

- Caseins obtained from fat-rich milks exhibited poor foaming capacity;
- CSM and CSSM produced abundant, stable foam similar with the commercial control.

Thermogravimetric Analysis

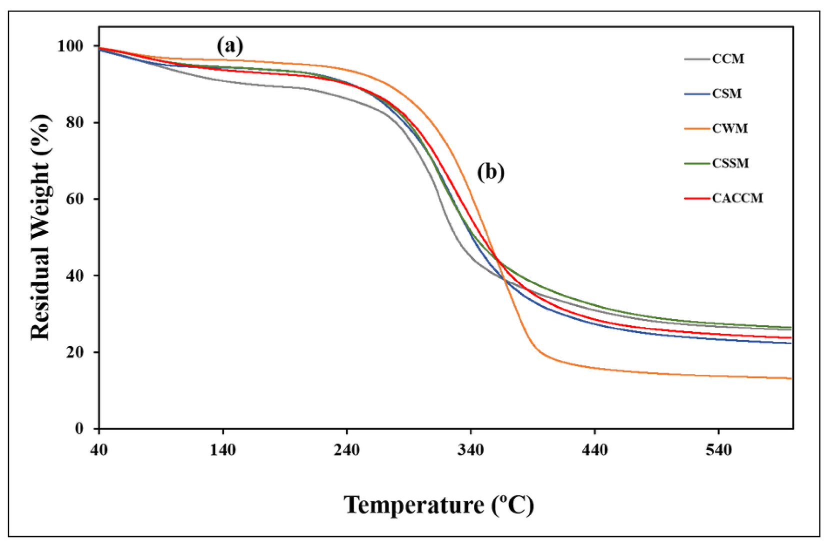


Figure 5: Thermogram of the "eco-casein" samples.

- All samples showed water evaporation **(a)**. CCM lost the most; CWM, the least.
- Regarding degradation **(b)**, CSSM had the lowest stability. CWM showed the highest Ti, Tmax, and residue.
- CACCM had similar thermal stability to CSM → contamination didn't significantly affect degradation behavior.

References:

[1] Antoshchenkova, V. and Y.J.E.a. Kravchenko, Current trends in milk production and consumption in the world in the conditions of globalization. 2022. 32(2): p. 7-14.
[2] FAO. Global food losses and food waste – Extent, causes and prevention. 2011 [cited 2024 05/09/2024]; Available from: https://www.fao.org/4/mb060e/mb060e00.pdf.
[3] Dikmetas, D.N., et al., From waste to remedy: Extraction and utilization of food waste-derived bioactive components in wound healing. Trends in Food Science & Technology, 2024. 145: p. 104347.
[4] Ogola, H., A. Shitandi, and J.J.J.o.v.s. Nanua, Effect of mastitis on raw milk compositional quality. 2007. 8(3): p. 237.
[5] Wilbanks, D.J., et al., Comparison of micellar casein isolate and nonfat dry milk for use in the production of high-protein cultured milk products. Journal of Dairy Science, 2023. 106(1): p. 61-74.
[6] Zhang, Y., et al., Growth factors, as biological macromolecules in bioactivity enhancing of electrospun wound dressings for diabetic wound healing: A review. Int J Biol Macromol, 2021. 193(Pt A): p. 205-218.
[7] Wang, X., et al., Production of a novel non-toxic γ-PGA/casein composite hydrogel using MTG and optimization by response surface methodology. Grain & Oil Science and Technology, 2021. 4(2): p. 71-80.
[8] Tůmová, E., et al., Development of Organic and Biodegradable Insulating Material for ETICS. Procedia Engineering, 2017. 195: p. 81-87.
[9] Sangeetha, J. and J. Philip, The interaction, stability and response to an external stimulus of iron oxide nanoparticle–casein nanocomplexes. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2012. 406: p. 52-60.
[10] Pugliese, A., Paciulli, M., Chiavaro, E. et al. Application of differential scanning calorimetry to freeze-dried milk and milk fractions. J Therm Anal Calorim 137, 703–709 (2019).

Conclusions:

- CSM and CACCM yield solvent-free "eco-casein"'s;
- Structure and stability of waste stream casein's match commercial casein (FTIR, DSC, TGA show native profiles);
- Functionality is adequate for biomaterials → SSM casein equal commercial emulsifying/foaming: CACCM still form stable 24 emulsions.
- CACCM shows no drug-protein alteration and retains biomedical utility, supporting One-Health valorization.

Funding:

Fundação para a Ciência e a Tecnologia

The authors gratefully acknowledge the financial support of Fundação para a Ciência e a Tecnologia through project MILK 4 WOUND CARE - 2022.03408.PTDC for making this research possible.

Bolsa de Doutoramento, M3.1.a/F/006/2023, financiada pelo Fundo Regional da Ciência e Tecnologia, Governo Regional dos Açores (Programa PRO-SCIENTIA).