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Production of a functionalized film for non-food applications from casein-based powders obtained from expired pasteurized milk

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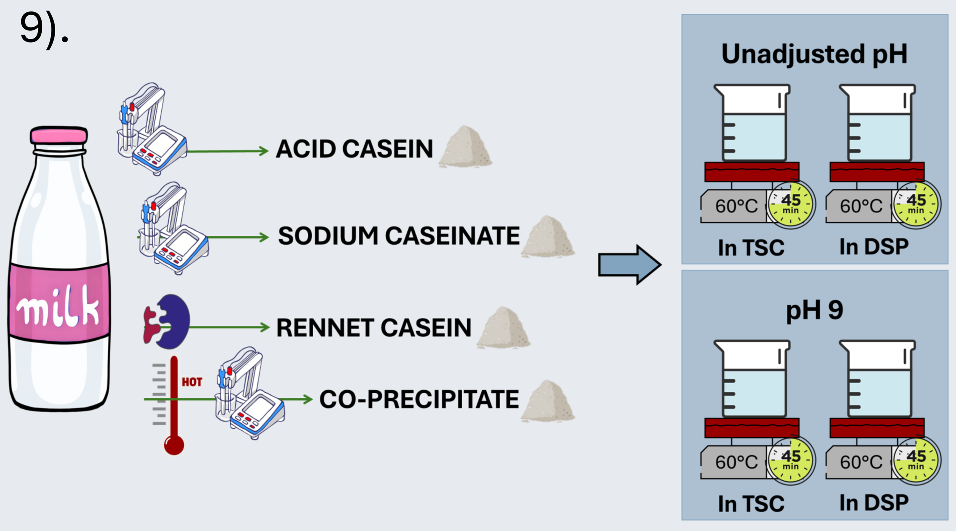
BACKGROUND

Pasteurized milk has a short shelf life, and one past its **expiry date**, it is classified as a special category 3 waste (Reg. CE 1069/2009). However, it still contains about 26 g/L of casein that can be recovered for non-food uses, such as packaging films [1]. **Casein** can be obtained as acid casein, rennet casein, co-precipitate or caseinates. Some of these powders often show **poor solubility** and rehydration, which can be improved by adjusting pH, temperature, or by adding calcium chelator [2]. Among them, sodium caseinate (NaCas) is water-soluble and suitable as film-forming biopolymers. **NaCas films** offer good barriers to non-polar substances but are hydrophilic and brittle, often requiring plasticizers or reinforcement. Protein crosslinking before film formation may improve strength of the three-dimensional network and modify functional properties. In this study, two reagent-free chemical approaches were tested to promote low-level **casein crosslinking** in NaCas film-forming solutions: (i) photo-oxidation leading to di-tyrosine (di-Tyr) formation [3]; (ii) β-elimination reactions leading to lysinoalanine (LAL) formation [4].

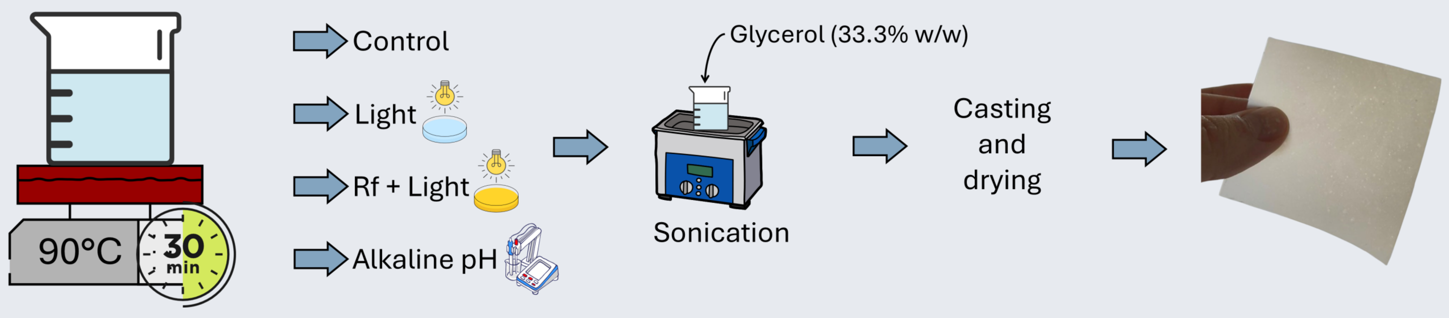
MATERIALS AND METHODS

- Recovery of casein-based powders from expired pasteurized semi-skimmed milk and solubilization of the powders through heat treatment at 60 °C for 45 minutes in 30 mM trisodium citrate (TSC) and 112 mM disodium phosphate (DSP). Powders were reconstituted at 3.5% and 10% protein, under two pH conditions (unadjusted and pH 9).



- **Determination of insolubility index.** Calculated as % (w/w) ratio between the pellet weight obtained after centrifugation (36 *g* for 10 min) and the weight of 1.5 mL of sample before centrifugation.

- NaCas (10% protein in MilliQ water) was used to prepare film-forming solutions under four conditions: (i) heating at 90 °C for 30 min (Control); (ii) exposition to LED light for 60 min then heating (90°C for 30 min) (Light); (iii) riboflavin addition followed by LED illumination (60 min) and heating (90°C for 30 min) (Rf + Light); (iv) pH adjustment to 9 with 1 M NaOH and heating (90°C for 30 min) (Alkaline pH).



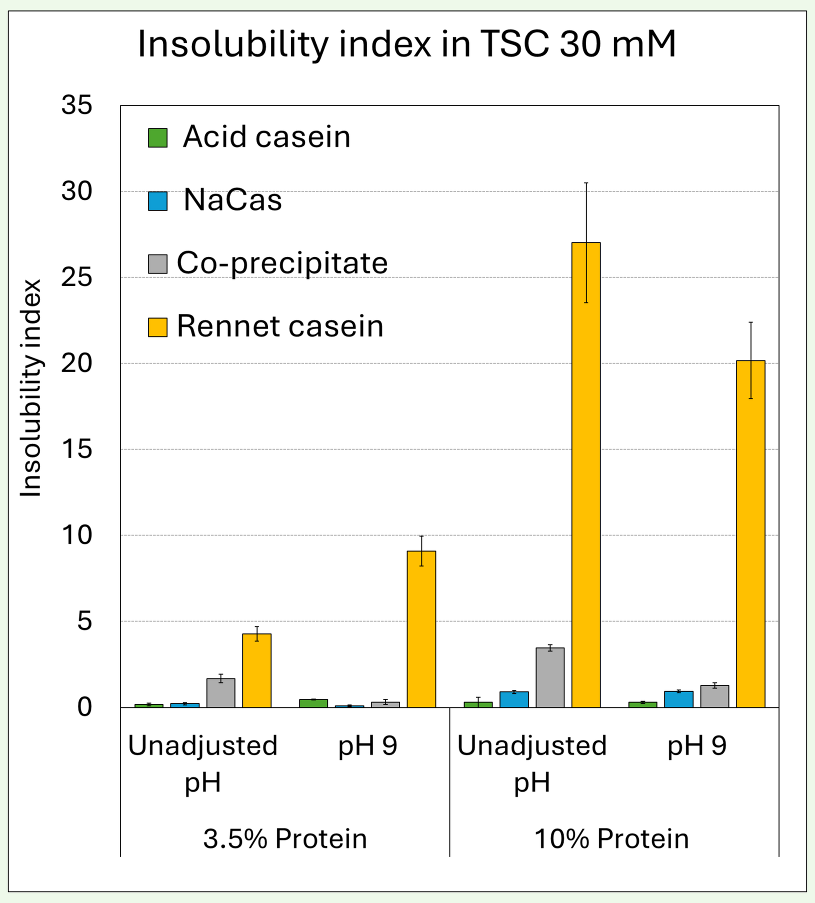
- **Determination of LAL and di-Tyr** formation in film-forming solutions by LC-MS and HPLC-FL, respectively.
- **Determination of protein aggregates** in film-forming solutions by reducing SDS-PAGE.
- **Mechanical properties** characterization of films.

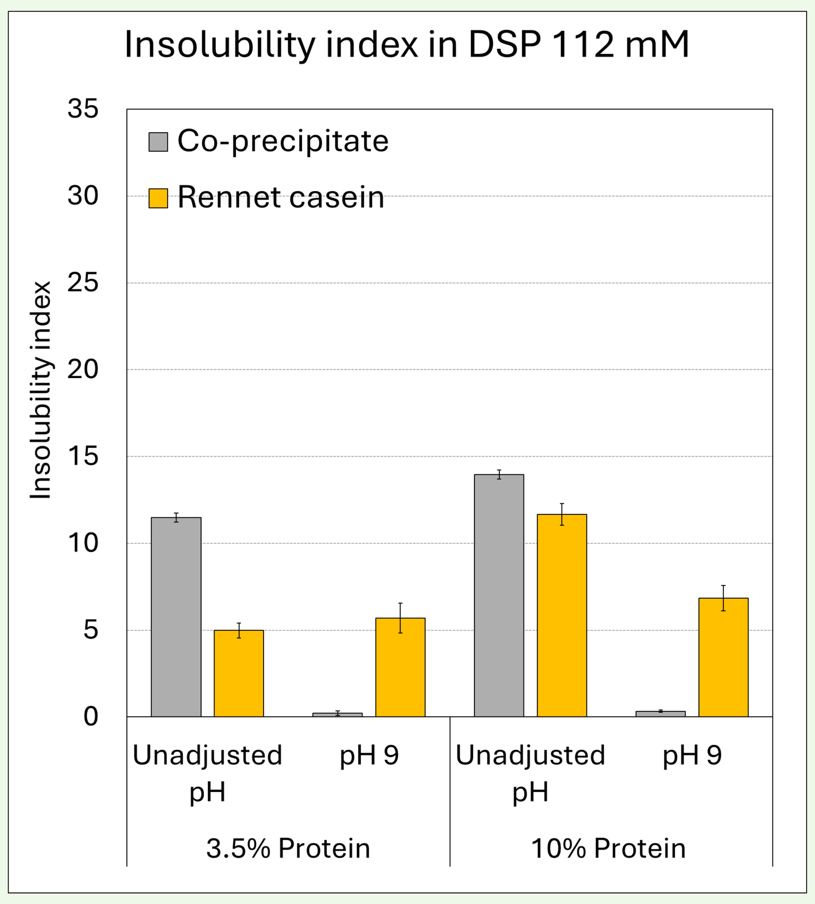
RESULTS and DISCUSSION

CASEIN-BASED POWDERS SOLUBILITY ASSESSMENT

The solubility of the powders was significantly affected by both pH and powder type:

- Acid casein and sodium caseinate: the insolubility index was always <1%, regardless of pH and reconstituted protein concentration;
- Co-precipitate: the solubility was strongly influenced by pH, with lower insolubility at alkaline pH;
- Rennet casein: showed the highest insolubility index in TSC among powders. The insolubility index at 10% protein improved in DSP *vs* TSC.





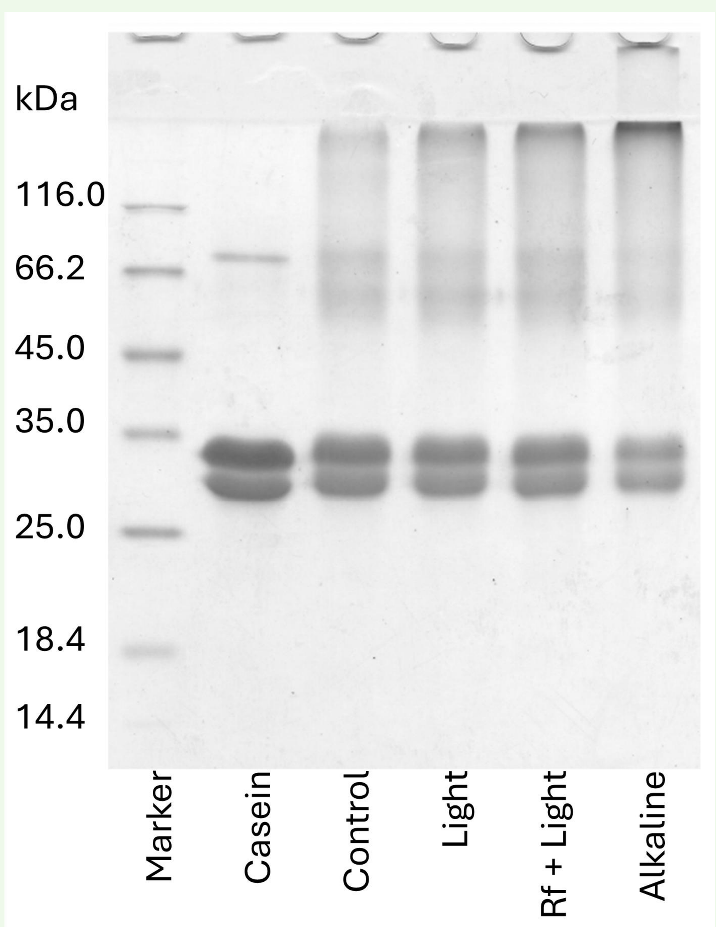
NaCas FILM-FORMING SOLUTIONS CHARACTERIZATION

NaCAS solution treatment	LAL formation (μmol/g protein)	di-Tyr formation (μmol/g protein)
Control	3.88 ± 0.1	0 ± 0
Light	4.06 ± 0.1	0.01 ± 0
Rf + Light	3.46 ± 0.05	1.39 ± 0.02
Alkaline pH	15.86 ± 0.3	0 ± 0

Cross-link formation.

All NaCas solutions contained LAL, as all samples were subjected to the heat treatment. At alkaline pH, LAL was ~4 times higher then at unadjusted pH.

di-Tyr was absent in control and Alkaline pH samples, while light exposure alone induced only trace amounts. The addition of riboflavin increased di-Tyr formation confirming its role as a photo-sensitizer.



Protein aggregates formation.

Control NaCas solution already showed aggregates. Alkaline-thermal treatment further enhanced protein aggregation, with a strong decrease in monomers and accumulation of high-molecular-weight aggregates.

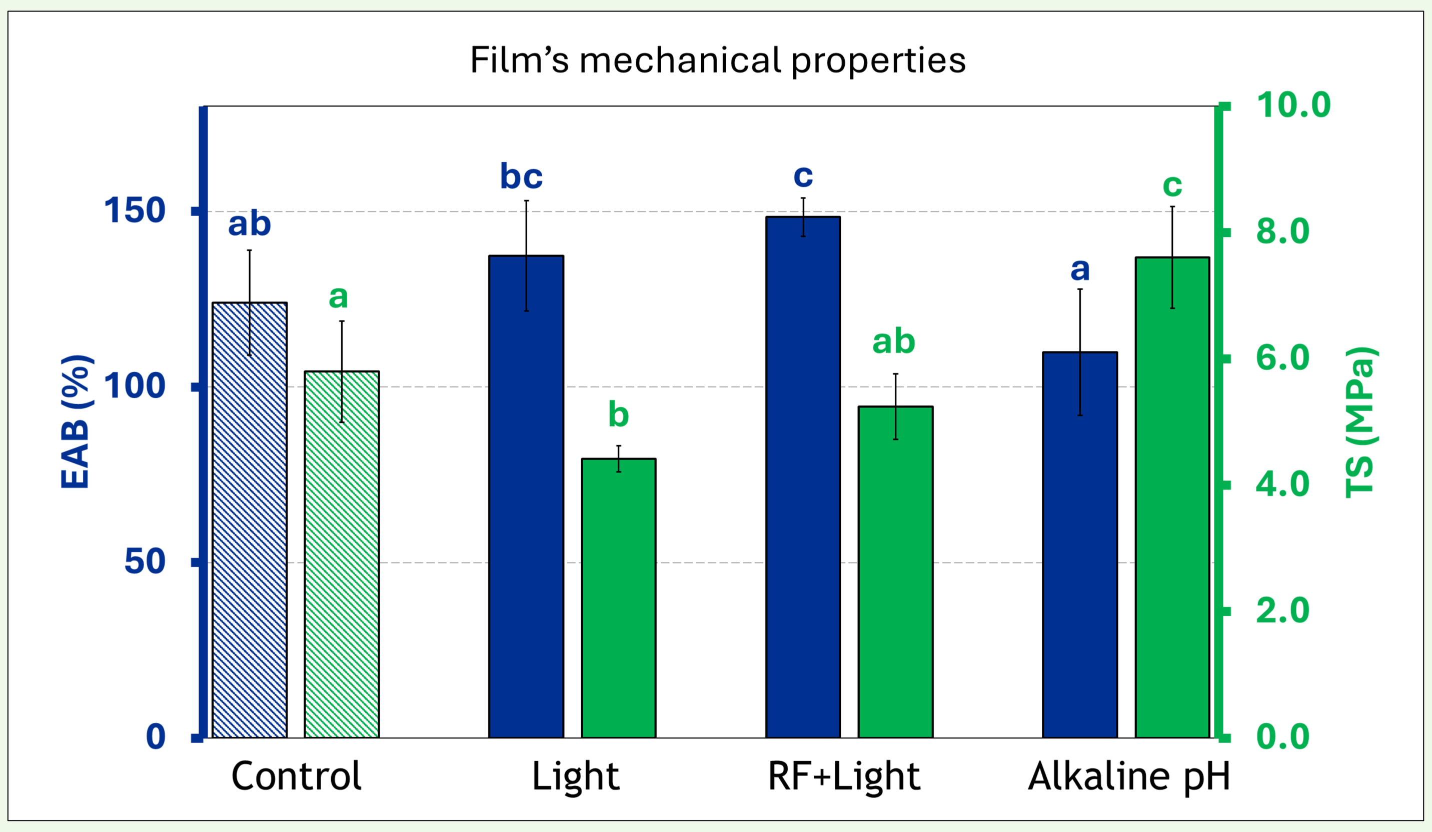
The contribution of di-Tyr to aggregation was minimal, as its levels were much lower than LAL and did not lead to additional aggregate formation.

CHARACTERIZATION OF NaCas FILMS

Mechanical properties.

Films derived from alkaline-thermal treated casein resulted in the highest tensile strength (TS) and the lowest elongation at break (EAB), consistent with the presence of extensive LAL-mediated crosslinking.

Films obtained from light-treated casein showed reduced tensile strength and increased elongation at break despite the minimal di-Tyr formation. The addition of riboflavin slightly improved both properties, though the effect remined limited.



CONCLUSION

This study confirmed that casein type is a key factor for its solubilization. Acid casein and sodium caseinate were highly soluble, while co-precipitate dissolved only at alkaline pH. Rennet casein remained poorly soluble except at 3.5% protein in TSC or 10% protein in DSP. Only NaCas films were functionalized using two green strategies, which enhanced their strength and elasticity, highlighting their potential for sustainable packaging films.

Packaging prototype using Na-cas film:

REFERENCES: **(1)** Gerna et al. "Strategies for exploiting milk protein properties in making films and coatings for food packaging: A review." *Foods* 12.6 (2023): 1271. **(2)** Crowley, et al. "Rehydration characteristics of milk protein concentrate powders." *Journal of Food Engineering* 149 (2015): 105-113. **(3)** Fuentes-Lemus et al. "α-and β-casein aggregation induced by riboflavin-sensitized photo-oxidation occurs via di-tyrosine cross-links and is oxygen concentration dependent." *Food chemistry* 256 (2018): 119-128. **(4)** Cattaneo, Masotti, Pellegrino. "Chemical modifications of casein occurring during industrial manufacturing of milk protein powders." European food research and technology 235.2 (2012): 315-323.

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