

# Tailoring Digestibility of Food Proteins and Emulsions: a Consumer Sex-Based in Vitro Perspective on Animal and Plant-Based Model Food Systems

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This study examines the sex-based differences in the digestibility of animal- and plant-derived proteins to engineer targeted nutritional solutions optimized for male and female physiology, ultimately informing the development of personalized dietary recommendations and tailored food products designed to promote enhanced health and well-being. An *in vitro* digestion model, replicating key physiological differences between male and female gastrointestinal tracts, was used to assess the differential release of amino acids and bioactive peptides from bovine milk and oat-based milk alternatives. Results reveal distinct digestive trajectories significantly influenced by the simulated sex-specific environment. This influences nutrient bioaccessibility and suggests the potential for engineering techno-functional foods that better meet consumer needs for health and well-being. By understanding these nuances, targeted food development strategies can be implemented to optimize product formulations, ensuring technical feasibility and maximizing the nutritional benefits of plant-based and animal-derived protein sources for diverse consumer populations.

## 1. Introduction

The global shift towards sustainable and health-conscious diets has intensified the scrutiny of protein sources, particularly the comparison between animal-derived and plant-based options (Willett et al. 2019; Nirmal et al., n.d.; Chen, Chaudhary, and Mathys 2022; Kieran and Dolan 2024; Verkerk 2019; Liu, Shen, and Wang 2024; Lee et al. 2016). Beyond the consideration of essential amino acid profiles, a comprehensive understanding of protein digestibility and the release of bioactive peptides is crucial (Turgeon and Rioux 2011; Santos-Sánchez et al. 2024; Krul et al. 2024; Sousa et al. 2023). This understanding becomes even more nuanced when considering the inherent physiological differences between male and female consumers.

Accumulating evidence suggests that the human gastrointestinal tract operates differently based on sex and various inter-individual characteristics. Documented variations between the sexes in parameters such as gastric acidity, enzyme activity, and transit times can significantly alter protein digestion (Lajterer, Shani Levi, and Lesmes 2022; Vinarov et al. 2021; Walther et al. 2019; Freire et al. 2011; Gandhi et al. 2004), impacting the bioaccessibility of essential amino acids and the formation of potentially bioactive peptides. These sex-based differences are often overlooked in nutritional research, leading to generalized dietary recommendations that may not be optimal for all individuals. The present study addresses this gap by investigating the *in vitro* digestibility of bovine milk and oat protein, simulating both male and female digestive conditions. The main goal is to elucidate the differential proteolytic pathways and bioactive peptide release, thereby providing a foundation for more personalized dietary guidance.

## 2. Materials and Methods

Bovine milk and oat-based drink samples were prepared and standardized for protein content. *In vitro* digestion was performed using a sex-specific gastrointestinal model that incorporates distinct physiological parameters for males and females, including differences in gastric acidity, pepsin and pancreatic enzyme concentrations,

and gastric emptying rates (Lajterer, Shani Levi, and Lesmes 2022). Gastric pH was set within a range of -4.5 1.7 for males and 2.1-5.0 for females, reflecting the generally higher gastric acidity in women. Pepsin concentrations in simulated gastric fluid were 2,000 U/mL for males and 1,600 U/mL for females. Gastric emptying time was also adjusted, with the female model set to 1.5 times longer than the male model, in accordance with reports of slower gastric transit in women. In the intestinal phase, chymotrypsin concentrations were slightly lower for females (22.5 U/mL SDF) compared to males (25 U/mL SDF). Regarding bile salts, sodium glycodeoxycholate was maintained at 5 mM for both sexes, while taurocholic acid was set at 10 mM for females and 5 mM for males, reflecting a higher concentration in women. Other parameters were kept similar unless otherwise specified. These distinctions were critical for capturing the nuanced differences in protein digestion and nutrient bioaccessibility between sexes. Protein breakdown was monitored by SDS-PAGE, while peptide and amino acid release were quantified using appropriate chromatographic and spectrophotometric assays. Zeta potential and particle size measurements were conducted to assess colloidal changes during digestion. All experiments were performed in triplicate to ensure reproducibility.

### 3. Results and Discussion

#### 3.1 Differential Proteolysis of Milk and Oat-based milk alternative beverages

To gain initial insights into the differential digestion of bovine milk (Milk) and oat drink (OD) in simulated male and female digestive environments, Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was employed. This technique allows for visualization of protein degradation patterns and the appearance of peptide fragments throughout the *in vitro* digestion process. Digesta samples were meticulously prepared to ensure equal protein loading (20  $\mu$ g) based on titration data and accounted for volume changes during digestion. Samples were resolved using both standard acrylamide gels (focusing on 10-180 kDa range) and Tris-Tricine gels (optimized for 1.7-40 kDa), providing a comprehensive view of both larger protein breakdown and smaller peptide generation. Gels were stained using Coomassie brilliant blue R250.

The resulting SDS-PAGE profiles revealed striking differences in the proteolytic patterns between male and female digestion models for both milk and oat drink (Figure 1).

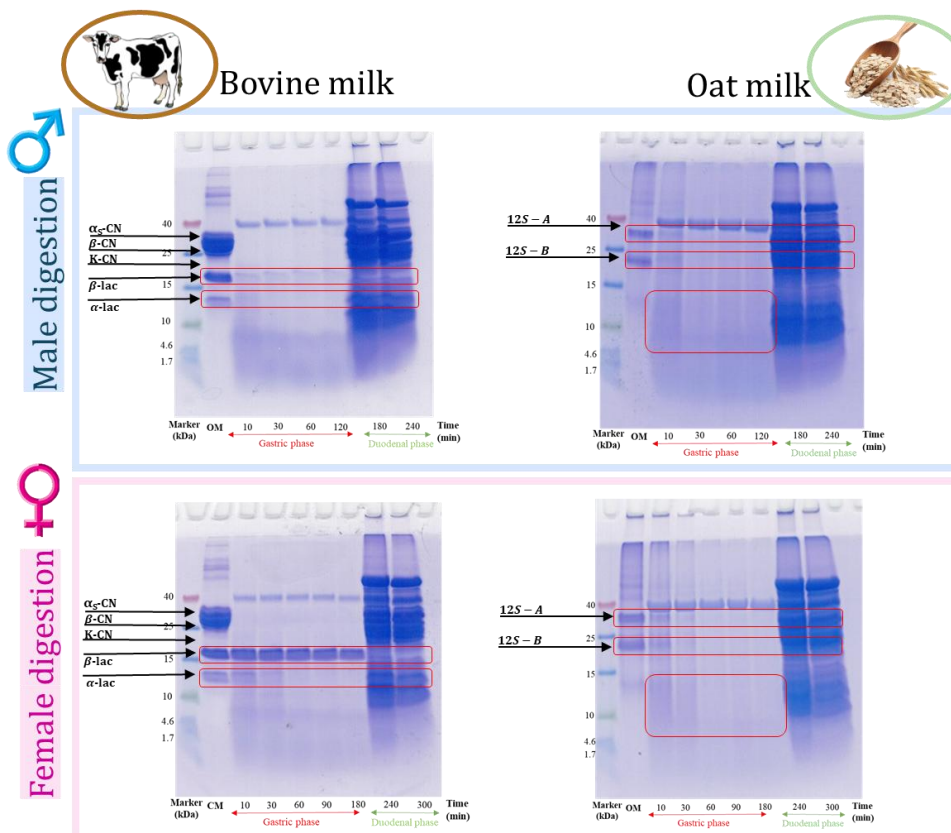


Figure 1. Qualitative analysis of protein breakdown by SDS-PAGE which shows sex-specific ( $\delta/\eta$ ) simulated digestion breakdown of milk and oat mill alternative drink.

In the gastric phase, milk proteins in the male digestion model exhibited a more rapid degradation, as evidenced by the faster disappearance of the prominent whey protein bands and the concurrent appearance of lower molecular weight peptides. In contrast, female gastric digestion of milk showed a slower and more gradual breakdown of these whey proteins. This observation aligns with known differences in gastric physiology, particularly the higher basal acidity and faster emptying rates in males, which facilitate more efficient pepsin activity (Lajterer, Shani Levi, and Lesmes 2022). Unlike milk, OD exhibited less pronounced overall sex-specific differences; however, in the gastric phase, SDS-PAGE analysis revealed a tendency towards a higher abundance of smaller peptide fragments in the male digesta compared to the female digesta.

Following the assessment of protein breakdown via SDS-PAGE, the study further investigated the colloidal behavior of the milk and oat drink systems by characterizing their surface charge properties using zeta potential measurements. Zeta potential ( $\zeta$ -potential) measurements were conducted to assess the surface charge properties of the colloidal systems formed during the *in vitro* digestion of milk and OD. This technique, based on electrophoretic light scattering, provides insights into the stability and interactions of food particles in the digestive environment. Initial measurements of milk showed a uniform sub-micron droplet size distribution and zeta potentials of approximately -30 mV, irrespective of consumer sex.

The study observed significant changes in the zeta potential values for both milk and OD throughout the digestion process. In the gastric phase, the zeta potential of milk exhibited notable sex-specific differences. Males showed a charge reversal to a significantly higher positive value (+10.7 mV) compared to females (+4.9 mV) ( $p < 0.05$ ). This charge reversal, coupled with increased aggregation and larger particle sizes, can be attributed to the dynamics of gastric acidity and the isoelectric point (pI) of the protein emulsifiers (Jean et al. 2006; Wang et al. 2018; Li et al. 2022; Ye et al. 2016). Specifically, the transition from the elevated gastric pH upon ingestion, where proteins are negatively charged, to lower pH values during gastric digestion (below the pI of most dairy proteins,  $4.5 < \text{pH} < 5.5$ ) results in a positive charge (Dupont, Croguennec, and Pochet 2018). The lower positive zeta potential values observed in females (+4.9 mV) may indicate less electrostatic repulsion and a higher tendency for flocculation. This aligns with previous observations of whey gastric flocculation or clotting during dynamic gastric digestion emulsifiers (Jean et al. 2006; Wang et al. 2018; Li et al. 2022; Ye et al. 2016). However, these sex-specific differences were abolished upon 2 hours of intestinal digestion, consistent with other reports on emulsion breakdown (Hunter and Senefeld 2024; Perez, Shani Levi, and Lesmes 2025).

### 3.2 Isolated Protein Results: Gel Electrophoresis, Particle Size, and Bioactive Peptides

SDS-PAGE analysis of the isolated skimmed milk powder (SMP) and oat protein isolate (IOP) digests revealed qualitatively similar protein breakdown patterns between male and female digestion models, suggesting a convergence in proteolytic pathways when matrix effects are minimized. Moreover, measurements indicated distinct particle size variations, hinting at potential differences in peptide release and bioaccessibility (Figure 2).

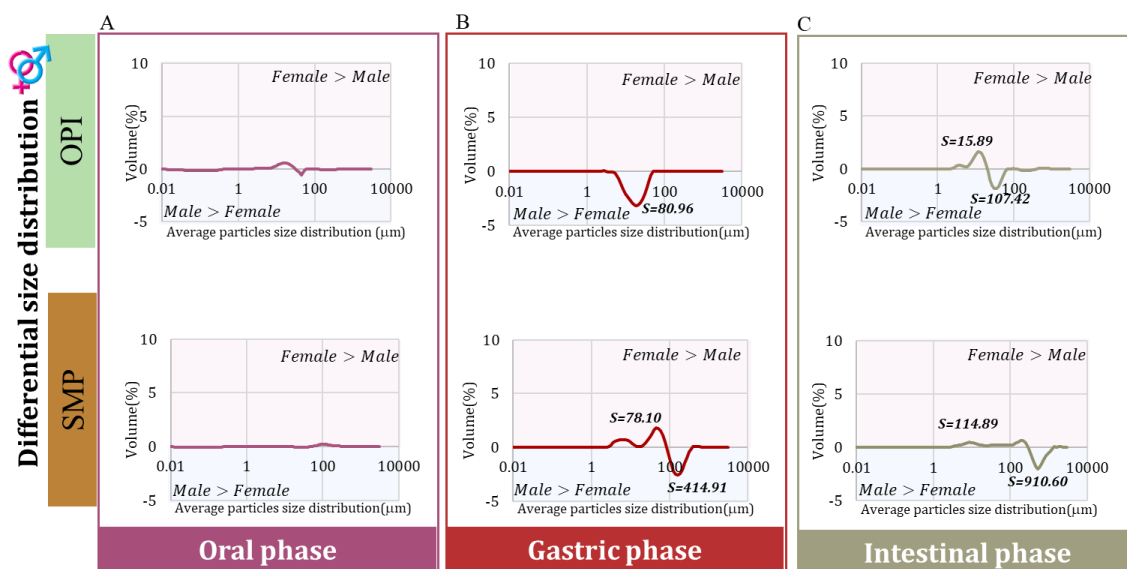
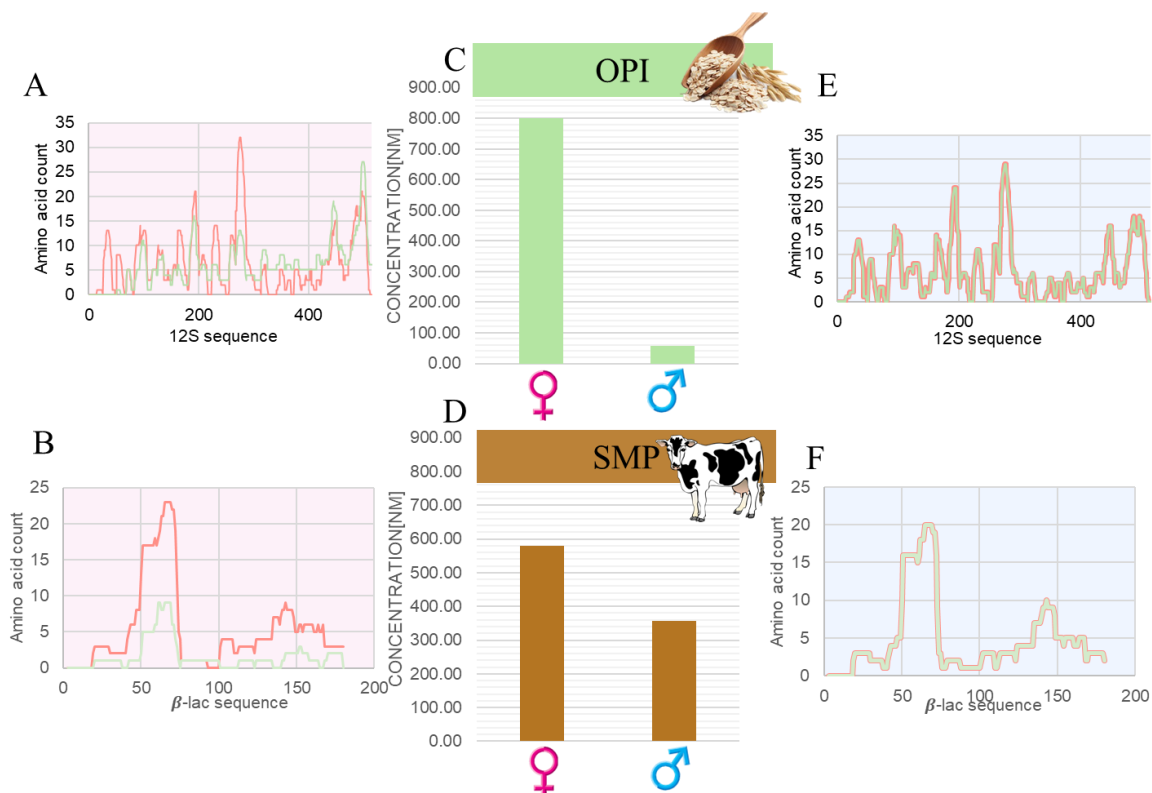


Figure 2. Differential droplet size distribution between males and females found during oral [A], gastric [B] and intestinal [C] *in vitro* digestion of oat protein isolate (OPI) or skim milk powder (SMP), highlighting sex-based variations. The curves depict the magnitude and direction of particle size variation between female and male simulated digestion phases. Positive values denote larger particle sizes in female digesta; negative values in male digesta.

The gastric phase exhibits notable sex-dependent variations in particle size, influencing downstream proteolysis and bioaccessibility. Regarding OPI, larger particle sizes were observed under male conditions. This correlates with lower molecular weight peptides are observed in gel samples (Figure 1) and lower extraction of EEAs (Figure 3C) compared to the female digests, which suggest a less digestive breakdown for OPI is happening during the male digestive modelling. In addition, Figure 3 A+E shows a significant decrease in the amino acid count of the main protein 12S globulin, between the stomach and intestine in females compared to males. Conversely, SMP exhibited an opposite digestion pattern when compared to OPI between males and females. Males had high overall effectiveness in the digestion of SMP, compared to females, as evident in the gels (Figure 1).

Furthermore, males achieved a higher concentration of EAAs from milk proteins than from the plant-based source (OPI) (Figure 3D). Consequently, proteomic analysis identified  $\beta$ -Lactoglobulin-derived peptides with reported DPP-IV inhibitory activity, selectively produced during male SMP digestion, which may be due to the favourable activity that allows greater peptide release from its surface. In stark contrast, the bioaccessible peptide VAPFPEVFGKEKVNE, an alignment to a reported osteoanabolic peptide, was uniquely recovered from female SMP digests, potentially linking to bone health benefits particularly relevant for women. This divergence suggests that these populations are able to gain different nutrients and health benefits based on a diet. These nuanced differences underscore the impact of sex on the generation of bioactive peptides and bioaccessible amino acids from isolated proteins, calling for further investigation into tailored nutritional strategies.



**Figure 3.** Sex-dependent differences in peptide profiles and amino acid bioaccessibility following *in vitro* digestion of isolated skimmed milk powder (SMP) and oat protein isolate (OPI). Breakdown patterns of peptides generated during the breakdown of  $\beta$ -lactoglobulin (B+F) from whey or 12S globulin from oats (A+E). Concentration of total essential amino acids in intestinal digests of OPI (C) and SMP (D), with bars indicating female and male digestion conditions.

#### 4. Conclusion

This research emphasizes the importance of considering sex as a key factor in protein digestion, particularly for animal versus plant sources. Distinct digestive patterns suggest females may process plant proteins more effectively, while males may better utilize animal proteins, resulting in differing bioaccessible compounds. Given the rising prominence of plant-centric diets, acknowledging these sex-related variations is essential for personalized nutrition and innovative food development.

Future studies can optimize functional foods based on protein digestive performance depending on sex. This will allow evidence-based development in the food industry and encourage personalized nutrition.

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