Molecular characterisation of interactions between β-lactoglobulin and hexanal – an off flavour compound

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Introduction:

Our human senses, especially smell, play a vital role in food selection. Food technologists often manipulate these senses by adjusting the volatile compounds that create different food odours. Aromatic volatile aldehyde compounds are of particular interest due to their significant detectability by human olfaction due to their extremely low detection threshold limits. In the milk industry, whey proteins, which constitute of approximately 20% of the total milk protein and are rich in amino acids, are used to improve dietary protein content and food manufacturing. Most whey protein comprises β-lactoglobulin, a globular structure with numerous binding locations (Wang & Heinonen, 2017).

Volatile aldehydes originate from lipid catalysation, leading to off flavours often disliked by consumers. Hexanal, a common aldehyde in whey protein with a grassy odour, is frequently associated with such off-flavours. Interactions between hexanal and β-lactoglobulin are crucial for understanding the connection between flavour compounds and biological matrices. Recent studies have indicated that aroma compounds bind hydrophobically to β-lactoglobulin at near neutral pH (Guichard et al., 2023). However, there is still a plethora of confusion surrounding the specific binding locations.

This research aims to explore the binding nature of hexanal and β-lactoglobulin using molecular modelling and multispectral techniques. The study's findings will provide insights into a potential novel binding location and enhance our understanding of how β-lactoglobulin and hexanal interact.

Objectives:

- To investigate the β-lactoglobulin's secondary structure components with hexanal addition.
- To identify the fluorescent nature of the β-lactoglobulin complexation with hexanal addition.
- To confirm the type of binding (physical or chemical) β-lactoglobulin undertakes with the addition of hexanal.
- To propose a theoretical model which identifies hexanal's novel location within the β-lactoglobulin matrix.

Methods:

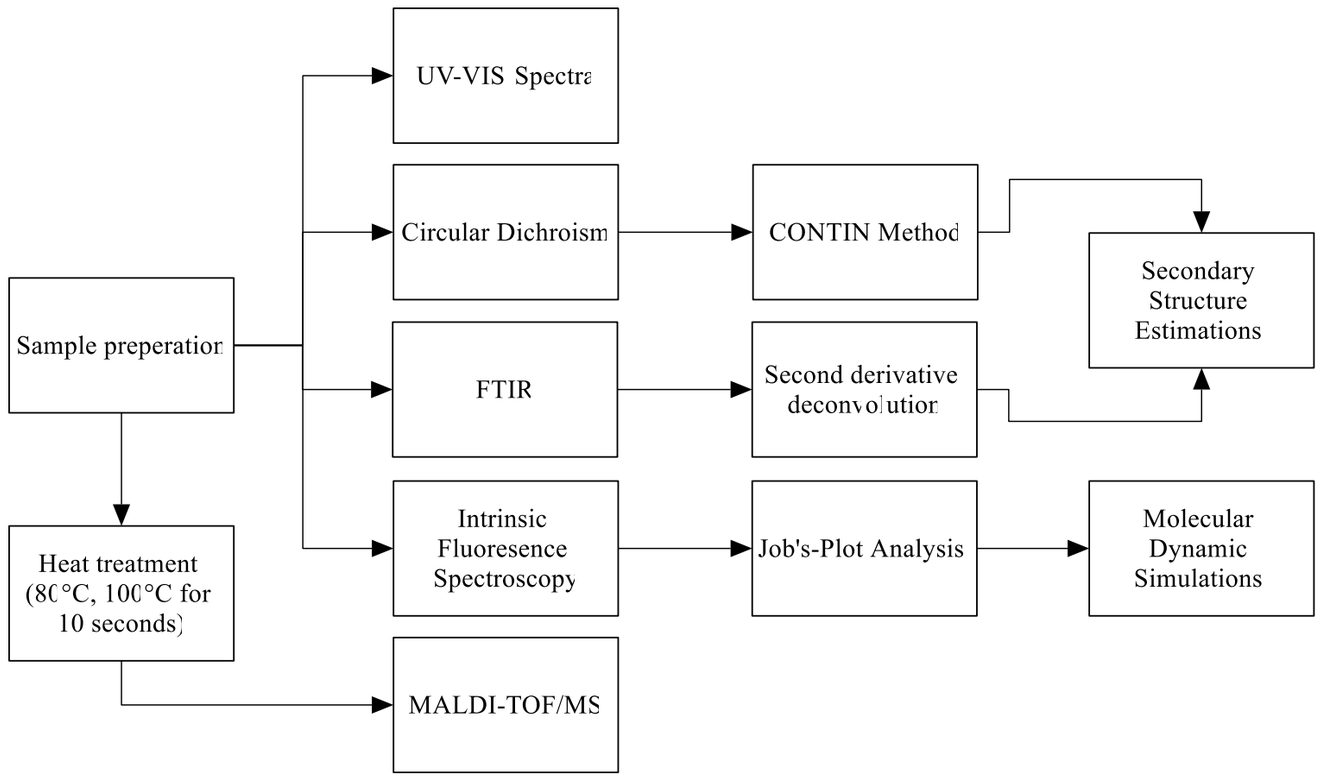

Figure 1. Project methodology flowchart

Results:

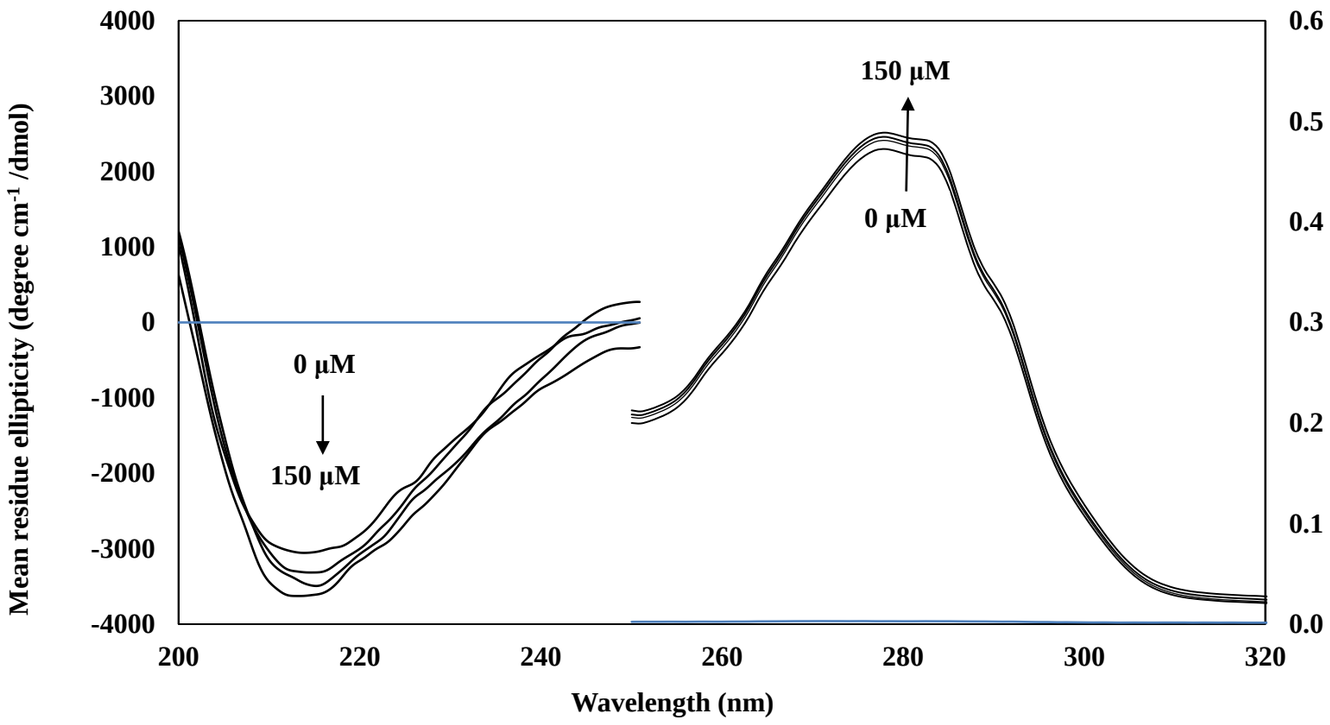

Figure 2. UV and CD Spectra of β-lactoglobulin (25µM) with increasing concentrations of hexanal (50, 100, 150µM) exhibiting increasing absorbance and decreasing MRE respectively; Hexanal does not absorb at the bottom and middle of the UV and CD scale respectively (blue line)

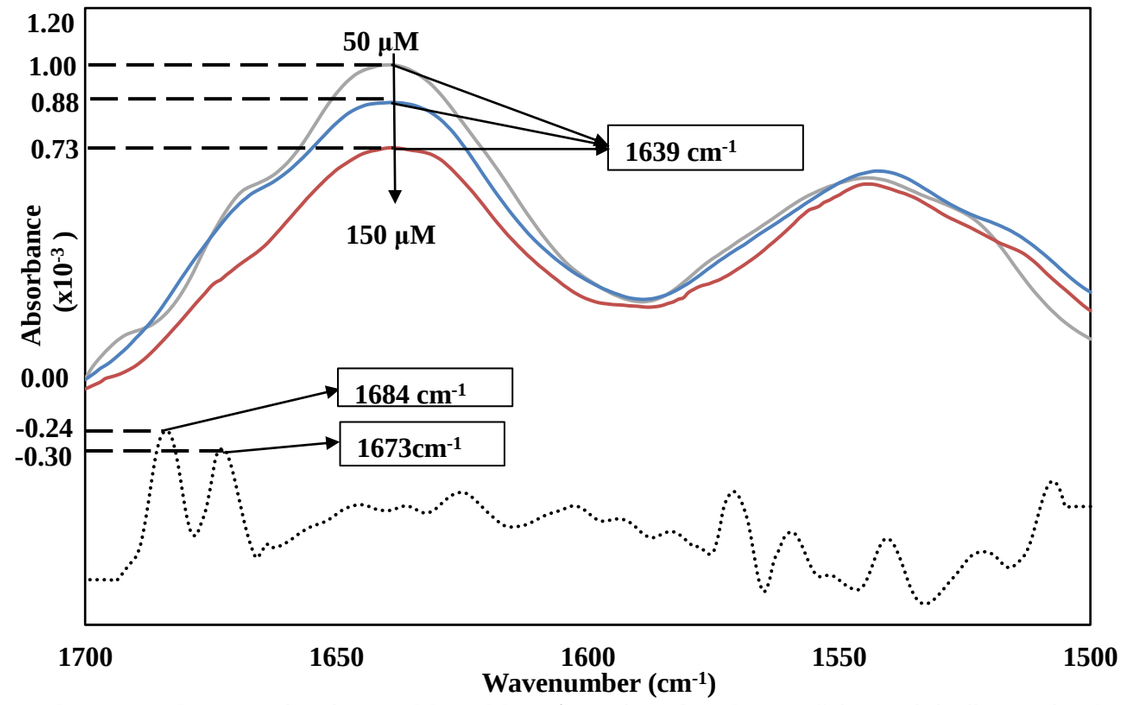

Figure 3. FTIR Spectra in the 1700-1500cm^{-1} region for 25µM β-lactoglobulin and 50µM hexanal (Grey line) 25µM β-lactoglobulin and 100µM hexanal (Blue line) 25µM β-lactoglobulin and 150µM hexanal (Orange line) and β-lactoglobulin and hexanal (150µM) complex after subtraction of the protein contribution (dotted line)

Discussion:

- Covalent interactions were ruled out with ultraviolet spectroscopy (UV-VIS) yielding minimal spectral differences between the native β-lactoglobulin and hexanal-treated samples, indicating no covalent bonds, but potential minor absorbance changes due to aromatic amino acid modifications. Furthermore, MALDI-TOF-MS analysis revealed that heat treatment did not propagate molecular weight shifts between the native protein and hexanal suggesting covalent interactions did not occur.
- Circular dichroism (CD) and infrared spectroscopy (FTIR) demonstrated that hexanal addition to β-lactoglobulin led to decreased α-helical structures, increased β-sheet content, amide I peak displacement, and decreased absorbance. These alterations were tied to hexanal concentration and were echoed across both spectroscopic assessments, with the amide II region remaining unchanged. Notably, increases in β-sheet and unordered structures, and decreases in α-helix and β-turns were significant at 50µM hexanal. Further changes, primarily in α-helix and unordered structures, were identified at higher hexanal concentrations, indicating a clear interaction between hexanal and β-lactoglobulin..
- Fluorescence quenching analysis, focusing on tryptophan and tyrosine residues, showed binding interactions between hexanal and β-lactoglobulin. A decrease in maximum fluorescence intensity and a slight shift in peak locations were observed with increased hexanal concentration, indicating a change in the tryptophan residues' environment. The Beckett equation model, showing a strong fit (r^2 value of 0.986), demonstrated that the interaction between hexanal and β-lactoglobulin is strong and efficient, with hexanal binding altering the protein environment, but not directly interacting.
- Through the Job plot method, β-lactoglobulin and hexanal exhibit a 1:1.5 binding ratio, evidenced by a peak at a 0.6M hexanal molar fraction. This result aligns with molecular dynamic simulations, suggesting that hexanal molecules bind both in the calyx of β-lactoglobulin monomers and at their interface, supporting previous research.
- Molecular docking simulations identified three binding sites for hexanal within the β-lactoglobulin complex, consistent with earlier analysis. The stability of hexanal is due to interactions with several amino acids. Thermodynamic calculations showed increased stability of the protein-ligand complex. These results align with prior studies and provide explanations for observed protein structures.

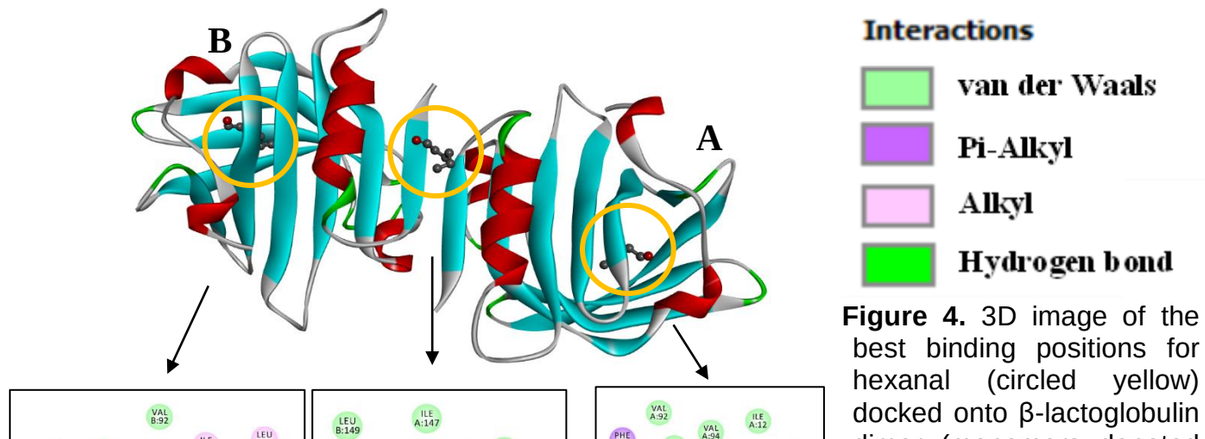

Figure 4. 3D image of the best binding positions for hexanal (circled yellow) docked onto β-lactoglobulin dimer (monomers denoted as A and B) along with their respective 2D representation of the binding sites as indicated by the arrows.

Conclusions

- Non-covalent interactions between hexanal and β-lactoglobulin were suggested by minimal shifts in UV-VIS analysis and MALDI-TOF-MS.
- Circular dichroism and FTIR analysis showed changes in the protein's secondary structure with increased hexanal concentration, with reduced α-helical and β-sheets and increased β-turns and unordered structures.
- Fluorescence spectroscopy indicated a strong binding capacity and intensity between hexanal and the protein, with shifts and quenching observed.
- Further investigation using molecular dynamics simulations confirmed two binding locations in each of the hydrophobic parts of the calyx and one binding location at the protein's dimeric form interface.
- This foundational study helps understand the interactions between volatile compounds and whey proteins, setting a reference point for future research.

References:

Guichard, E., Ayed, C., & Salles, C. (2023). Retention and release of aroma and taste compounds, influence on perception. In Flavor (pp. 3–27). Elsevier. https://doi.org/10.1016/B978-0-323-89903-1.00006-2

Wang, B., & Heinonen, M. (2017). Protein–Tannin Interactions of Tryptic Digests of α-Lactalbumin and Procyanidins. Journal of Agricultural and Food Chemistry, 65(1), 148–155. https://doi.org/10.1021/acs.jafc.6b04256

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