**Undesirable compound development in ultra-high temperature (UHT) processed high protein liquid food systems**

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As it currently stands, UHT treated, animal-based protein isolates are deemed the ‘gold standard’ in terms of desirable flavour attributes. However, the same cannot be said for legume protein isolates with consumer acceptance being viewed as one of the biggest challenges industry faces, despite the growing interest in milk alternatives in recent years1,2. Many publications have identified the volatile compound hexanal as the main protagonist of malodorous flavour due to its undesirable organoleptic attributes and low detection threshold. Therefore, it is imperative to identify and track the development of hexanal and understand any molecular interactions between the volatile and various protein systems. Initially this should be performed in a whey protein system to develop a baseline and allow further research into the refinement of legume protein isolates with the goal of bringing organoleptic attributes closer to that of whey protein isolate.

As such, this study makes use of *β*-lactoglobulin, as a major fraction of whey protein isolate, for use in interaction studies. Samples containing a fixed amount *β*-lactoglobulin (25µM) and varying concentrations of hexanal (50, 100 and 150µM) were formulated. UV-vis spectrophotometer (UV-vis), Fourier-transform infrared-spectroscopy (FT-IR), Circular dichroism (CD), Intrinsic fluorescence spectra analysis were performed, and binding sites were proposed using molecular docking simulations. Furthermore, a shelf-life study was undertaken on whey protein isolate (WPI) (4% w/w) model systems which were heat treated using ultra high temperature processing (UHT) at a temperature of 140˚C and packaged aseptically for a shelf-life study at 22˚C. Gas chromatography-mass spectrometry (GC-MS) analysis was completed to track hexanal development.

UV-vis analysis identified a maximum absorption peak occurring at 278 nm due to the tyrosine and tryptophan residues of the protein. However, very little interaction occurred in response to increasing the hexanal concentration, with all samples maintaining a uniform peak wavelength with only slight increases to absorbance in the presence of hexanal. Both CD and FTIR analyses show the addition of hexanal to the β-lactoglobulin complex has made conformational changes to the protein structure with increasing hexanal concentrations causing a reduction in both mean residue ellipticity and absorbance. Furthermore, FTIR spectra revealed that a peak shift from 1635cm-1 to 1639cm-1 in the Amide I region was induced by hexanal binding to β-lactoglobulin. Fluorescence quenching was also observed with a maximum fluorescence intensity of 2.4 x 106 which was gradually decreased upon increasing concentration of hexanal, a shift in peak wavelength from 338nm to 336nm was also identified as a function of increasing hexanal concentration. Moreover, a shelf-life study, tracking hexanal concentration over storage time showed that hexanal steadily decreased in concentration for the initial period of storage (days 0-7) with a reduction from 0.2 to 0.01ppm. A subsequent increase was observed from day 7, with a peak concentration reading at day 25 of 1.3ppm followed by a gradual reduction which continued for the duration of the study up to day 121, with a final concentration of 0.4ppm. This work demonstrates that binding between hexanal and proteins present in UHT formulations may be an important factor in the development of malodourous flavor during long term storage.

**References:**

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