In Vitro Gastric Digestion of Heat-Induced Aggregates of β-lactoglobulin

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Food processing – beneficial and undesirable effects on protein digestibility and its nutritive value

A fundamental understanding of structural changes – food products of consistent nutritional quality
Native β-Lg – resistant to gastric digestion

Heating, high pressure and urea – denaturation and unfolding
Denaturation and aggregation of β-Lg

Series of changes in the structure of β-Lg & formation of aggregates

- Non native monomers (Reactive)
- Native dimer
- Native monomers
- Non native monomers (Reactive)

5min
- Non native dimers
- Non native trimers
- Non native tetramers

60 min
- Non native pentamers

120 min
- Non native hexamers
- Large aggregates

Heating time
Objective

Understand the digestion behavior of heat induced aggregates of β-Lg
Methodology

Heating of aqueous solutions of β-lactoglobulin (5mg/ml)

In vitro pepsin digestion assay
(3.2 mg/ml of Pepsin in 35mM NaCl and 0.084 N HCl at pH 1.2)

SDS-PAGE (reduced, non-reduced), Native-PAGE, 2D electrophoresis
SDS-PAGE profile & quantification of in vitro gastric digestion of β-Lg heated at 90 °C for 5, 60 & 120 min E:S ratio 3:1

Digestion Time (min)

%β-Lg remaining

Digestion Time (min)

Native β-Lg
β-Lg heated 90 °C for 5 min
β-Lg heated 90 °C for 60 min
β-Lg heated 90 °C for 120 min
Native and SDS-PAGE (non-reduced) profile of *in vitro* gastric digestion of β-Lg heated at 90 °C for 60 min E:S ratio 3:1

Complete digestion of non-native aggregates within 1 min
Native PAGE profile of *in vitro* gastric digestion of β-Lg heated at 90°C for 5 & 120 min E:S ratio 1:6

- Dimers – resistant to digestion (5 min heating) and digested fast (120 min heating)
- Large aggregates – digested fast
SDS-PAGE (non-reduced) profile of *in vitro* gastric digestion of β-Lg heated at 90°C for 5 & 120 min E:S ratio 1:6

Intermediate Mol. Wt. species – digested slowly with the formation of low molecular weight species
SDS-PAGE profile & quantification of in vitro gastric digestion of β-Lg heated at 90 C for 5, 60 & 120 min. E:S ratio 1:6

- E:S ratio(1:6) - Slow rate of digestion and generation of low molecular weight peptides
2D electrophoresis of heated β-Lg (90°C for 5 min) and digested for 10 min

Intermediate Mol.Wt. species – peptides linked to β-Lg through disulfide bonds
In vitro gastric digestibility of β-Lg aggregates – depends on the E:S ratio and type of aggregates

- High Mol. Wt. aggregates - digested fast
- Dimers – some are resistant to digestion and some are digested slowly
- Intermediate species – digested slowly
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