Several studies have demonstrated the potential of using milk extracts enriched in growth factors as therapeutic compositions for health-related applications such as skin disorders, gut and bone health [1]. Contradictory results are reported in the literature on the stability of growth factors through the gastrointestinal tract. This represents an important challenge since such health products are generally intended to be administered with four computer-controlled chambers simulating the physiological conditions of the stomach, duodenum, jejunum and ileum [Fig. 1]. The simulated parameters included the body temperature, pH, gastric and intestinal content mixing with peristaltic movements, secretions and absorption of water and small molecules.

The aim of the present study was to investigate the effect of in vitro gastrointestinal digestion using TIM-I on a whey protein extract (WPE) enriched in growth factors by measuring the degradation of proteins and growth factors (TGF-β2, IGF-I) during digestion. The effect of WPE and their digested fractions on the intracellular glutathione concentration in Jurkat T cells was also determined.

**MATERIALS AND METHODS**

Digestion protocol and sampling:
- An experimental lot of WPE (83.3 % proteins) was supplied by Advitech inc. WPE was dispersed in water (25 g/300 mL) then added to the stomach compartment of TIM-I. The digestions were performed with physicochemical analysis) and without bile (cells culture), both using the protocol simulating the adult conditions after the intake of a semi-solid meal with a slow gastrointestinal passage [3]. During digestion, samples were collected at 1, 3 and 5 h in stomach, duodenum, jejunum and ileum, and at every hour in jejunal and ileal fluids and effluent [Fig. 1].

Characterisation of the WPE and digested samples:
- Total protein content was determined by Dumas combustion method (Leco) while the content in undigested whey proteins were quantified by RP-HPLC using a Resource™ RPC 1 mL column from GE Healthcare Life Sciences.
- Both TGF-β2 and IGF-I were quantified by ELISA.
- Intracellular glutathione concentration was determined in Jurkat T cells in the presence of culture medium only (control), WPE or digested samples using a Glutathione Kit (Sigma); optimal concentration of samples (500 µg/mL) was determined from preliminary assays.

**RESULTS & DISCUSSION**

After 1h of digestion, 47% of the initial protein content of WPE remained in the stomach while at the end of the digestion (5h), 74% and 26% were found in the jejunum and ileal fluids (theoretically bioavailable), and in the effluent.

**Table 1:** Whey proteins measured in the initial WPE solution and the different compartments of TIM-I during digestion (n = 2).

<table>
<thead>
<tr>
<th>Digestion time (h)</th>
<th>Total protein (mg/mL)</th>
<th>α-TGFB</th>
<th>β-TGFB</th>
<th>αIGF-I</th>
<th>βIGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.9 ± 0.8</td>
<td>44 ± 1</td>
<td>31 ± 1</td>
<td>31 ± 1</td>
<td>325 ± 25</td>
</tr>
<tr>
<td>1</td>
<td>3.3 ± 2.5</td>
<td>21 ± 1</td>
<td>33 ± 1</td>
<td>22 ± 1</td>
<td>55 ± 11</td>
</tr>
<tr>
<td>3</td>
<td>1.4 ± 1</td>
<td>30 ± 1</td>
<td>44</td>
<td>7.3 ± 1</td>
<td>42 ± 15</td>
</tr>
<tr>
<td>5</td>
<td>0.0 ± 0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Figure 4:** TGF-β2 content in the different compartments of TIM-I during digestion of WPE (n = 2).

The initial amount of IGF-I in WPE (59.2 pg/mg protein) decreased in the stomach during time of digestion with values of 78.4%, 46% and 0% after 1, 3 and 5h respectively, suggesting that IGF-I is gradually degraded in the stomach.

After 5h of digestion, 12.5% of IGF-I is detected in the duodenum suggesting that a part of this growth factor remain intact in the intestine. The low amount of IGF-I detected in the intestinal compartments could be related either to its digestion by the pancreatic enzymes or its interaction with peptides released during the digestion of WPE and interfering with the detection method (ELISA).

Unlike to IGF-I, the initial amount of TGF-β2 (50.2 ng/mg protein) in WPE is totally detected in the different TIM-I compartments up to 3h of digestion, the highest amount (58.8%) being found in the effluent.

**CONCLUSION**

- Individual whey protein concentrations measured in the stomach decreased gradually over time, indicating that the proteins were digested in the stomach or simply transferred in the duodenal compartment, where they are rapidly digested by pancreatic enzymes.
- The efficiency of whey proteins digestion by pancreatic enzymes is confirmed by the absence of intact protein in all other TIM-I compartments up to 5h of digestion.

**REFERENCES**


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