HYDROLYSATES IN EASY TO DIGEST FORMULAS

*In vitro evaluation of digestion kinetics*

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DIGESTION KINETICS OF WHEY PROTEIN HYDROLYSATE

In infants minor gastroenterological problems often occur\(^1\). Most common problems are constipation, diarrhea, vomiting, regurgitation, failure to thrive and prolonged crying fits (colic). These symptoms were found in 55% of infants 0-6 months\(^1\).

Hydrolysates are pre-digested proteins, and can therefore assist the digestion process. To substantiate this effect, an experiment has been performed, in which the kinetics of Domo’s EtD whey hydrolysate were evaluated compared to those of the intact protein from which it has been developed.

**Experimental set-up**

The digestion of intact whey protein (Hiprotal Casein Whey 80) and a whey hydrolysate prepared from this whey protein source (Hyvital Whey EtD 120) was monitored in an in vitro digestion model based on the physiological situation in infants aged 0-6 months (see figure).

Samples were taken at subsequent intervals and analyzed for terminal NH\(_2\) (OPA), SDS-Page, molecular weight distribution and fingerprint.

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![Digestion Kinetics Diagram](image-url)
Extent of protein degradation higher for whey hydrolysate

The extent of degradation of the whey hydrolysate was higher during the whole gastric and intestinal phase (t=160) when compared to the intact proteins. Overall extent of digestion (t=160) was equal for both products, as shown by the kinetic data, MWD and fingerprint analysis. The fact that the degree of degradation of the hydrolysate was higher up to 160min, suggests that the digestion process of the whey hydrolysate is easier (requires less effort) compared to that of intact whey protein. Moreover, this further degradation may result in a faster gastro-intestinal transit.

Figure: Terminal NH2 (µmol/g protein) in intact whey protein (Hiprotal Casein Whey 80) and whey protein hydrolysate (Hyvital whey EtD 120) samples taken at several time points during in vitro digestion.

Footnote: Potential consumer benefits are not to be considered as health claims. They should be considered as potential leads that might be developed into health claims complying with the local legal requirements". 

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DIGESTION KINETICS - RESULTS

**SDS-PAGE**

SDS-PAGE shows that there is no intact protein left at the end of digest.

Molecular weight distribution

MWD is shifted towards smaller MW for Hyvital Whey EtD120 compared to intact whey up to t=100. MW fraction <1000D is higher for Hyvital Whey EtD120 compared to intact whey protein up to t=100.

Peptide fingerprint

At the end (t=160) of the in-vitro digestion, fingerprints show equal peptide profiles for both intact whey and hydrolyzed whey, suggesting that no new/novel peptides were formed.

**Figure:** SDS-PAGE of intact whey protein (Hiprotal Casein Whey 80) and whey protein hydrolysate (Hyvital Whey EtD 120) samples taken at several timepoints during in vitro digestion.

Lane
1. Hiprotal Casein Whey 80
2. Hyvital EtD 120
3. Hiprotal Casein Whey 80 stomach start
4. Hyvital EtD 120 stomach start
5. Hiprotal Casein Whey 80 stomach end
6. Hyvital EtD 120 stomach end
7. Hiprotal Casein Whey 80 intestine start
8. Hyvital EtD 120 intestine start
9. Hiprotal Casein Whey 80 intestine end
10. Hyvital EtD 120 intestine end

**Figure:** MWD of intact whey (Hiprotal Casein Whey 80) and whey hydrolysate (Hyvital Whey EtD 120) samples taken at several timepoints during in vitro digestion.

**Figure:** Fingerprints of intact whey protein (top) and whey hydrolysate (bottom) during in vitro digestion.
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**Figure:** Fingerprints of intact whey protein (top) and whey hydrolysate (bottom) during in vitro digestion.
DIGESTION KINETICS - CONCLUSION

**Higher degree of degradation may decrease GI-transit time**

During the gastric and intestinal digestion, the whey hydrolysate was further digested compared to the intact whey protein. This may suggest that the whey protein hydrolysate will result in a shorter gastric emptying time. Gastric emptying seems to be an important factor for digestion and satiety\(^1\)-\(^4\). The faster the emptying of the stomach, the earlier protein or peptides will reach the duodenum, and the faster satiety hormones will be produced.

**Possible advantages pre-digested proteins**

- Assistance of the still developing digestion system of the infant
- Improved gastric transit due to smaller particles and lower viscosity
- Less gastro-esophageal reflux
- Quicker absorption of amino acids and small peptides in small intestine
- Quicker release of satiety inducing hormones (more satisfied baby)
- Requiring less effort of still developing digestion system of the infant
- Higher tolerance for these smaller fragments
- Reduction of cramps, gas, crying

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Marjan Gros, 2014
Supporting literature

In full-term neonates and infants, gastric emptying is faster when a protein hydrolysate is fed as compared with formulas containing native cow’s milk protein. Mihatsch et al. found a markedly shorter gastrointestinal transit time (9.8 h) in preterm infants receiving a protein hydrolysate formula compared with that of those receiving standard formula (19 h). The formula in this study contained an ultra-filtered mixture of hydrolysed whey and hydrolysed casein (60:40) (MW: 75%<1500Da, 15% free amino acids).

A possible explanation for this is effect is that hydrolysis might have reduced the activity of milk protein derived opioid peptides (e.g. β-casomorphins, released during casein digestion), which are known to reduce bowel activity and increase transit time. Moreover, hydrolysis of the protein increases the osmolarity and osmotic effects might have reduced gastro-intestinal transit time.

References


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EU legislation on health claims

In EU, no health claims other than those related to reduced risk of cow’s milk allergy are allowed on IF (0-6 months), even in case that enough scientific data would be available. So no health claims with regard to digestibility can be made on IF.

For health claims on FOF, the claims Regulation (EC) No 1924/2006 applies, requiring an article 14 authorization procedure, including a thorough EFSA evaluation of the submitted scientific evidence. Today, no health claims on digestibility have been authorized yet.

Substantiation of health claims wrt digestion in EU

In its “Guidance on the scientific requirements for health claims related to gut and immune function”, the EFSA mentions outcome measures that they consider as appropriate measures of claimed effects. These measures can be taken into account when setting up clinical trials to substantiate the specific health claim:

 Claims on bowel function
  - Transit time
  - Frequency bowel movements
  - Stool bulk

 Claims on gastrointestinal discomfort
  - Distension/bloating, abdominal pain/cramp, rumbling
  - Symptom severity questionnaires

 Claims on gastrointestinal microbiota
  - Reduction of number of pathogenic microorganisms or their toxins in stools or other suitable samples