Summary

- Literature review on digestibility and absorption of Hydrolyzed Collagen
Digestibility and absorption of hydrolyzed collagen

- Literature review:
  - Several studies have demonstrated that hydrolyzed collagen is highly digestible. If native collagen is very resistant and regarded as indigestible, denatured collagen can be easily attacked by proteolytic enzymes.

- More than 90% of the hydrolysates are digested and quickly absorbed after oral ingestion.

  - This has been known for a long time and has been especially mentioned by Asghar and Henrickson in 1982 in the article « Chemical, biochemical, functional characteristics of collagen in food system » Advanced in Food research, vol. 28.
  - More recently different studies have give more details on absorption: Oesser in 1999; Sato and al. in 2005, 2007.
Digestibility and absorption of hydrolyzed collagen

Oesser and al. in 1999 demonstrated on mice that 90% of the hydrolysate are absorbed in 6 hours after an oral ingestion:

Test on mice – absorption of $^{14}$C labeled gelatin hydrolysate compared with $^{14}$C proline + gelatin hydrolysate

A peak of radioactivity is measured after +/- 6 hours in both cases

*Oesser and al (1999) – Oral administration of $^{14}$C labeled gelatin hydrolysate leads to an accumulation of radioactivity in cartilage of mice (C57/BL). Nutrient metabolism, 0022-3166/99*
Radioactivity measured in cartilage after ingestion of:

■ $^{14}$C gelatin hydrolysate vs

▲ $^{14}$C proline + gelatin hydrolysate

Radioactivity is much more concentrated in cartilage with $^{14}$C gelatin hydrolysate than with $^{14}$C proline

That means that hydrolyzed collagen is well metabolized in the target tissues such as cartilage

Oesser and al (1999)
Digestibility and absorption of hydrolyzed collagen

- Iwai in 2005 measured the molecular weight of peptides in the blood of subjects before and after ingestion of hydrolysate.
  

![Graphs showing molecular weights of porcine hydrolysate and amino acid content](image)

**Conclusions of the measures:**

- High molecular weight peptides measured in the blood do not contain collagen sequences.
- Peaks of free hydroxyproline and hyp-containing peptides were detected in the blood 60 mn after ingestion.

**Collagen is well absorbed in small peptides in the blood.**
Digestibility and absorption of hydrolyzed collagen

- Ohara in 2007 in the same team confirmed those measures.
- Peptides with hydroxyproline (Pro/Hyp = 25% of AA) were measured in the blood before and after the oral ingestion of hydrolysates.

Ohara and al (2007) – Comparison of quantity and structures of hydroxyproline – containing peptides in human blood after oral ingestion of gelatin hydrolyzates from different sources – 55, 1532-1535

- Porcine skin collagen △
- Fish scale collagen ■
- Fish skin collagen □

Dosage = 0.385 g/kg
# 27 g / 70 kg

Peptides with hydroxyproline were present in negligible amounts before ingestion in plasma but increased significantly after ingestion.

Figure 1. Amount of free form Hyp and Hyp-containing peptide in plasma after oral ingestion of fish scale (■), fish skin (□), and porcine skin (△) gelatin hydrolysates. (A) Amount of free form Hyp in plasma (nmol/mL). (B) Amount of Hyp-containing peptide in plasma (nmol/mL). Data are
Digestibility and absorption of hydrolyzed collagen

- In both studies (Ohara, Iwai) peptides coming from hydrolyzed collagen have been characterized in the blood of the subjects.

- The main peptides containing hydroxyproline found in the blood:
  Pro-Hyp 95% in porcine hydrolyzate
  Ile-Hyp, Leu-Hyp and Phe-Hyp also detected but < 5%.

- The content of amino acids may be different depending on the origin of the hydrolysate, fish or porcine (Ohara).
NeoCell Results

- NeoCell has carried out *in vitro* digestibility measurements to confirm that our collagen has the same high digestible properties as those described in literature.

- The test has been based on the Glahn’s method and consists in the following successive steps which mimic the real digestion:
  - Oral step = mechanical food breaking and salivary enzyme action
  - Gastric step = acidic pH and addition of gastric enzymes
  - Intestinal step = pH near from the neutrality, addition of pancreatic enzymes and biliary salt actions
  - At the end of the digestion, the samples are analysed by high performance size exclusion chromatography (HPSEC) i.e. particles are separated depending on their sizes
NeoCell Results

- **In vitro vs in vivo digestion**
  - Description of the system

- Oral step = mechanical food breaking and salivary enzyme action

- Gastric step = acidic pH, Gastric enzyme action

- Intestinal step = pH near from the neutrality, pancreatic enzyme and biliary salt actions.
NeoCell Results

- After the *in vitro* digestion process, around 90% of the NeoCell collagen has been digested in peptides whose molecular weight is below 2000 dalton and which can be absorbed by the intestine.

There is no significant difference between Bovine or Porcine or Fish.

- There is a huge decrease of the molecular weight after digestion of the 3 Hydrolyzed Collagens which permits a good intestine absorption.

<table>
<thead>
<tr>
<th></th>
<th>Bovine</th>
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<th>Porcine</th>
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<th>Fish</th>
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</thead>
<tbody>
<tr>
<td>Proteins + peptides MM &gt; 2000</td>
<td>before</td>
<td>83%</td>
<td>after</td>
<td>10%</td>
<td>before</td>
<td>87%</td>
</tr>
<tr>
<td>Oligopeptides + amino acids MM ≤ 2000</td>
<td>before</td>
<td>17%</td>
<td>after</td>
<td>90%</td>
<td>before</td>
<td>13%</td>
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</table>
NeoCell Results

- The 3 figures show the molecular weight distributions of the samples before and after digestion of the samples of Peptan B, P and F.

- Those profiles confirm that the molecular weight decreased after digestion. 90% of the NeoCell collagen derived peptides are below 2000 Dalton.

- Those results are fully in line with literature.
Digestibility and absorption of hydrolyzed collagen

Conclusions

- Literature has shown from recent studies that:
  - Hydrolyzed Collagen is fully digested by the intestinal track in small peptides.
  - Those peptides are then well absorbed and transported by the blood and can be used by the target tissues such as cartilage but also bones or skins for the production of endogenous collagen.

- NeoCell results
  - NeoCell has demonstrated through *in vitro* studies that our collagen is fully digested by the intestine and can be well absorbed.