Valorisation des coproduits de la pêche : la réussite d’une collaboration entre Entreprise et Université

The use of marine by-products to develop innovative ingredients: a successful collaboration between SME and University

1: ICV, Equipe ProBioGEM, Université de Lille, Villeneuve d’Ascq
2: Société COPALIS Boulogne sur Mer
2: ICV, Equipe QSA, Université d’Artois, Lens.
4: Laboratoire de Stress Périnatal, Université de Lille, Villeneuve d’Ascq

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Located in the 1\textsuperscript{st} European centre for the transformation, commercialization and distribution of seafood products

\textit{for animal nutrition}

\textit{nutraceuticals, cosmetics and food applications}
A few figures...

- Cooperative organization: 100 shareholders are Copalis raw material suppliers
- 75 employees
- Turnover: 22,000 k€
- Created in 1960 to add value to fish by-products: production of fishmeal
From fishmeal to high added-value ingredients

- Observation in the 60s: how to better use the protein resources to meet the increasing need in protein?
  - Protein solubilization to reach protein bound to non-protein substances (lipids, carbohydrates, …)

- Development of enzymatic hydrolysis process on an industrial scale: patented process
  - 1968: launch of a patented fish protein hydrolysate CPSP®
    Increased nutritional value of protein from fish by-products: +15 to 35%
A demanding raw material

✓ Filleting by-products:

✓ 40,000 T of by-products are generated in Boulogne/mer:
  ✓ Collected by Copalis dedicated service (5 trucks)
  ✓ Feed raw material in stainless steel tank
  ✓ Food raw material in refrigerated container
    (same conditions as fish fillets transportation)
General context:
- Hippocrates: Let food be thy medicine and medicine be thy food
- Molecules from natural origin
- Need to upgrade the 'waste' => by-products
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- Growing number of publications on bioactive peptides
- Isolation, characterization (Ngo et al., 2012. Int. J. Biol. Macromol.)
- Notion of cryptide (Li-Chan, 2015. Curr. Opin. Food Sci.)
- Resistance to gastro intestinal digestion (GI )=> BIOLOGICAL ACTIVITIES
Introduction – Background

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Project and network context:
- Europeen (Hydrofish, Seafood)
Clean processes and natural substances for food and nutrition

Biotechnologies and eco-design processes for clean transformation and sustainable development of agricultural resources.
● Upgrading of food proteins by enzymatic methods

Technical Platform: BIOLOGICAL ACTIVITIES STUDIED

- Antihypertensive (functional ingredients)
- Anti DPP-IV (functional ingredients: T2DM)
- Opioids (functional ingredients: anti-stress, metabolic syndrome)
- Regulation of intestinal hormones secretion (functional ingredients: obesity and associated symptoms)
- Antioxidants (functional ingredients: food conservation, healthy food)
- Anti-inflammatory
- Cytotoxicity
- ...

SEQUENCE => BIOLOGICAL ACTIVITIES => PHYSIOLOGICAL MECANISM

→ Development of new methodologies
→ Characterisation of the biological activities (identification and metabolic pathway)
→ Numerous models of cells and animals
Introduction - Background

Proteins → Hydrolysis → Peptides → Enzyme → Intermediate Peptides

Free amino acids

Sources → Hydrolysis → Biological activities → Purification and identification → Step of purification/MS → Relationship structure-function

Sequences/active Fractions and/or synergistic

Enzyme

Hydrolysis
HYDROFISH (1997 to 2000 « The search of biologically active compounds in hydrolysates of fish and crustaceans) => reproductibility of the peptidic population

=> SEAFOODPLUS Project 2004-2008 : more than 18 countries and 67 partners

=> Evidence of *in vivo* satietogen effect in rats of fish hydrolysates produce at industrial scale

- H1 : Siki (dogfish) hydrolysate (*C. squamosus*)
- H2 : Saithe fermented hydrolysate (*P. virens*)
- H3 : commercial product Nutripeptin
- **Cholecystokinin (CCK)**

Produced by I cells (duodenum) in response to lipids and **proteins**. Promotes **satiation**: increase gastric secretion, decrease gastric emptying, induces satiety feeling by vagal afferents.
Ingestion orogastric of peptide fractions

4 groups of 8 rats: control T (0.5 ml distilled water) and 3 hydrolysates H (50 mg/0.5 ml)

First step: 21 days

Measurement of the food intake and of the body weight

Second step: after 24 hrs of fasting

Measurement of the food intake and of plasmatic molecules

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H3: commercial product Nutripeptin
SeafoodPlus Project: results

Measurement of the food intake and of the body weight (3 weeks)

With H2:
- less body weight after 21 days of stuffing
- less food intake during the 3 weeks

<table>
<thead>
<tr>
<th>HYDROLYSATES</th>
<th>Body weight after 21 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>320 ± 10</td>
</tr>
<tr>
<td>H1</td>
<td>320 ± 10</td>
</tr>
<tr>
<td>H2</td>
<td>290 ± 10</td>
</tr>
<tr>
<td>H3</td>
<td>300 ± 10</td>
</tr>
</tbody>
</table>

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Measurement of the food intake and of plasmatic molecules

With H2:
- tendency to reduce food intake and glycemia
- high level of CCK
Measurement of the food intake and of the body weight

With H2:
- less body weight after 21 days of stuffing
- less food intake during the 3 weeks

Measurement of the food intake and of plasmatic molecules

Plasma glucose level after 1 h of food intake
From an academic point of view:
- 3 publications (with one book chapter)
- several communication in international congress (WEFTA 2007, MIS 2011)
- Development of innovative techniques

Effect of daily gavage with a collagen hydrolysate containing calcitonin gene-related peptide (CGRP)-like molecules on plasma CGRP-levels in rats

Oscar Martínez-Alvarez, Rozenn Ravallec, Benoit Cudennec, Laurence Guimas, Charles Delannoy, Martine Fouchereau-Péron

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Impact of ultrafiltration and nanofiltration of an industrial fish protein hydrolysate on its bioactive properties

Fish, a source of active ingredients

Whole by-product
Aquaculture
Petfood
Milk replacer
CPSP®: soluble fish protein concentrate

Skin
Collagen
Elastin

Cartilage
Fishbone
Chondroitin sulfate
Bioavailable Calcium

Fish muscle
Anti-stress peptides
GI lowering peptide
Anti-hypertensive peptides
Flavouring extract

Liver Roe
Omega 3
DNA rich extract

Enzymatic hydrolysis process

Food supplements
Nutricosmetics
Topical cosmetics
Food supplements
Food industry
Collaborative research efforts: the basis of a successful new product launch
From fish by-products to the international market of nutricsometics
- Increasingly educated consumers

- Growing competition with actives of different origin

- Increasingly demanding regulation

**New research program PepSeaNov:**
Characterisation of active peptides from fish by-products and development of new ingredients for human and animal nutrition based on innovative technics

A project led by Copalis and including 5 partners including Institut Charles Viollette

Overall budget: 1 944 117,09 €
Duration: 36 months

**ICV**  Anti stress activity and peptide identification
**In vitro digestion**

- Mouth: t = 5 min, pH = 6.5
- Stomach: t = 30 min, pH = 2-3
- Small intestine: t = 15-60 min, pH = 6.5-6.8
- Stop digestion (100°C, 15 min)

**Resistance and identification of active sequences**

- Sample collection
- Hydrolysis stop (100°C centrifugation 9500 g)

**Highlighting the physiological mechanism**

- Apical
- Basolateral
- Caco-2 cell monolayer

**Peptides**

- Opioid receptor
- Opioid like
- Antagoniste

**MS/MS**

- MS
Success story of 20 years of collaboration

- Market need
- Regulatory developments
- Industrial expertise

- Scientist expertise
- Innovative techniques

Trust and openness

Successful collaborative applied research programs between the industry and academic research lab
Institut Charles VIOLLETTE

Université de Lille 1
Sciences et Technologies
Université d'Artois

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Merci de votre attention !