

Tasty and healthy peptides from producers to consumers

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Overview

- **Background**
- **Aims**
- **What was done ?**
- **What has been achieved ?**
- **Conclusions**
- **Future tasks**

Background of PROPEPHEALTH

Huge global issues in nutrition*



10 million deaths due to under-nutrition

15 million deaths due to over-nutrition

Data on 2000 from WHO (2002)



4bn people affected by malnutrition deserve the chance to develop physically & mentally to get more out of life.



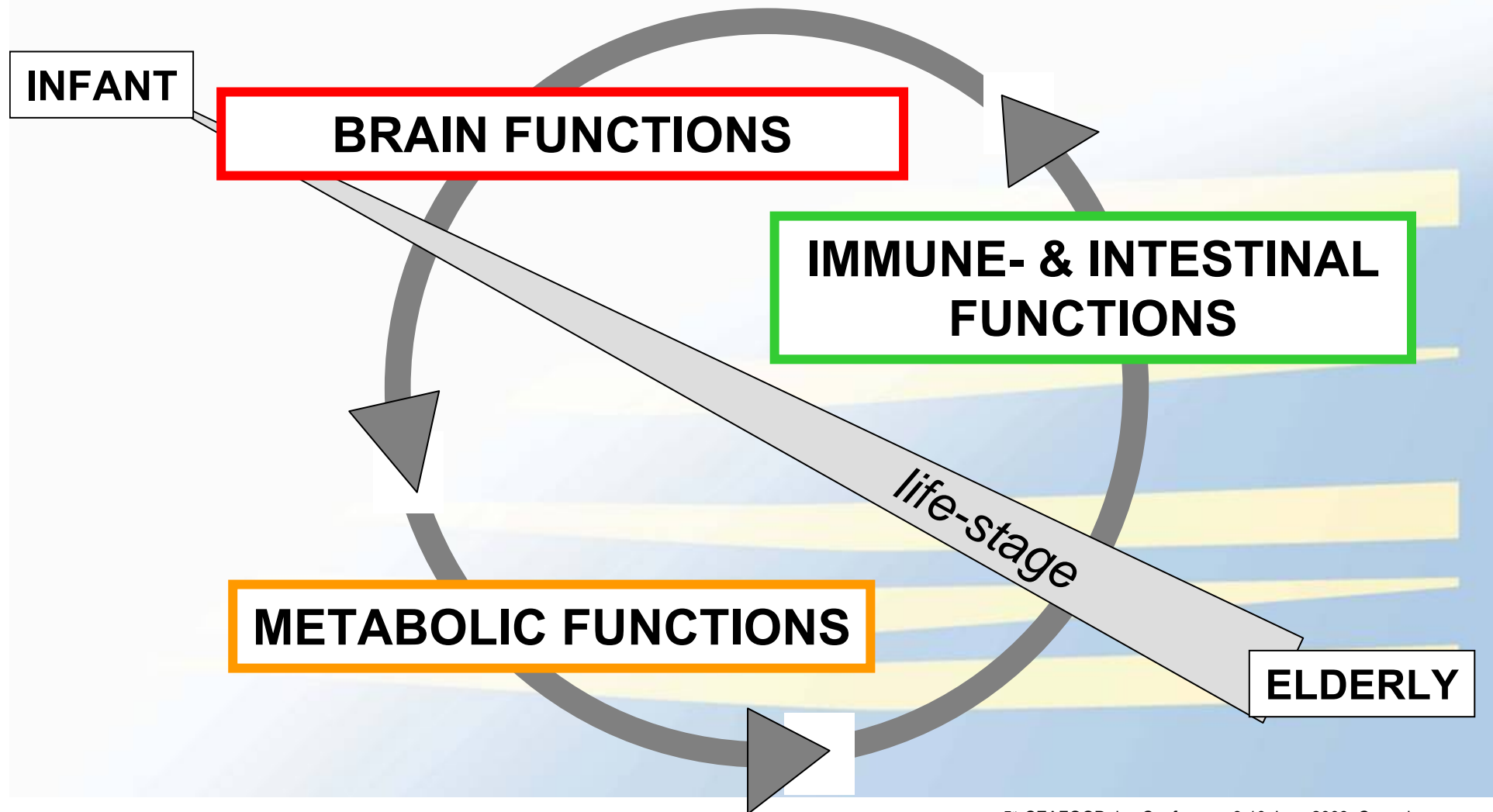
50% of world's population have blood cholesterol that's too high.



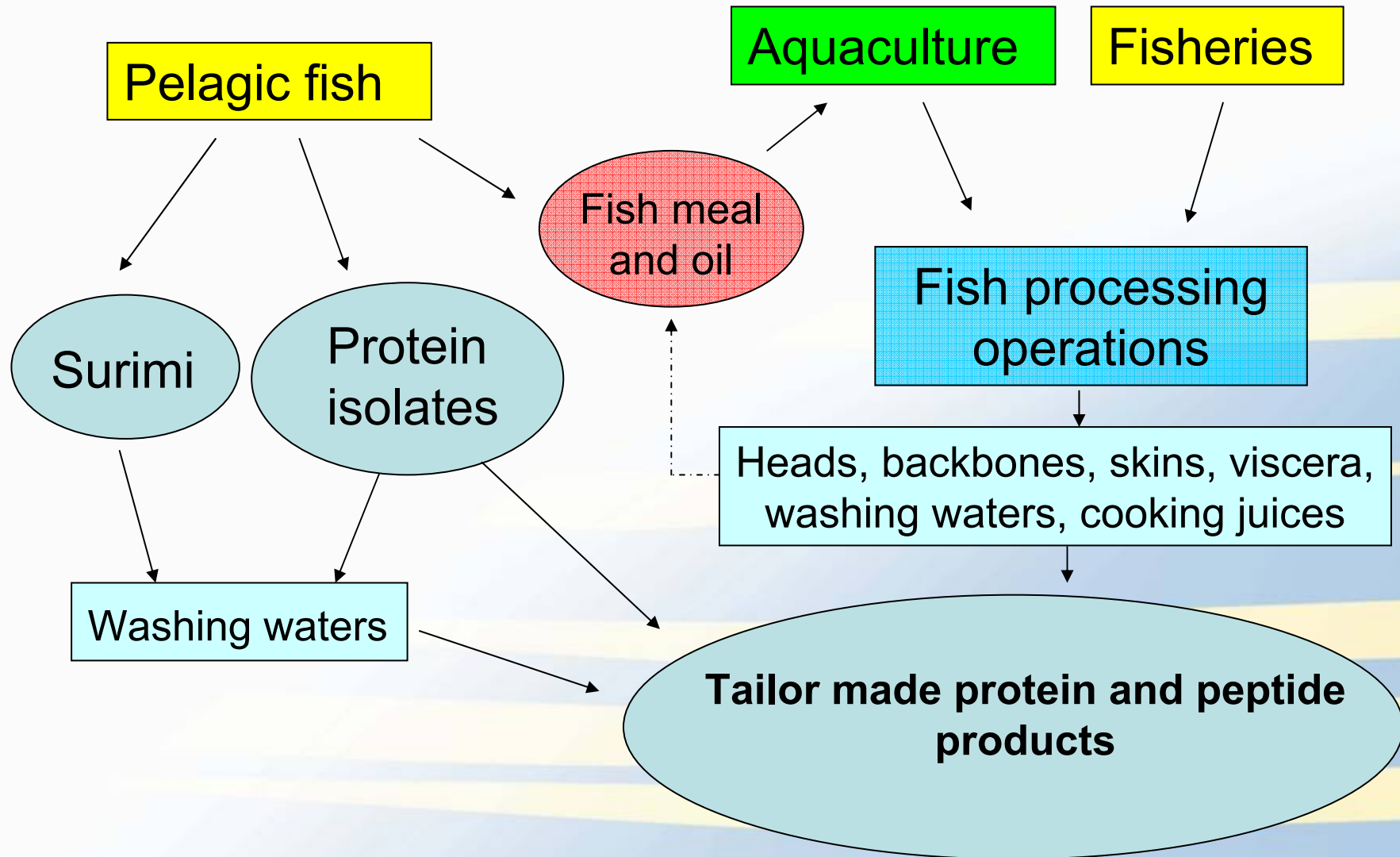
30% of world's population have blood pressure that's too high.

* From Asp

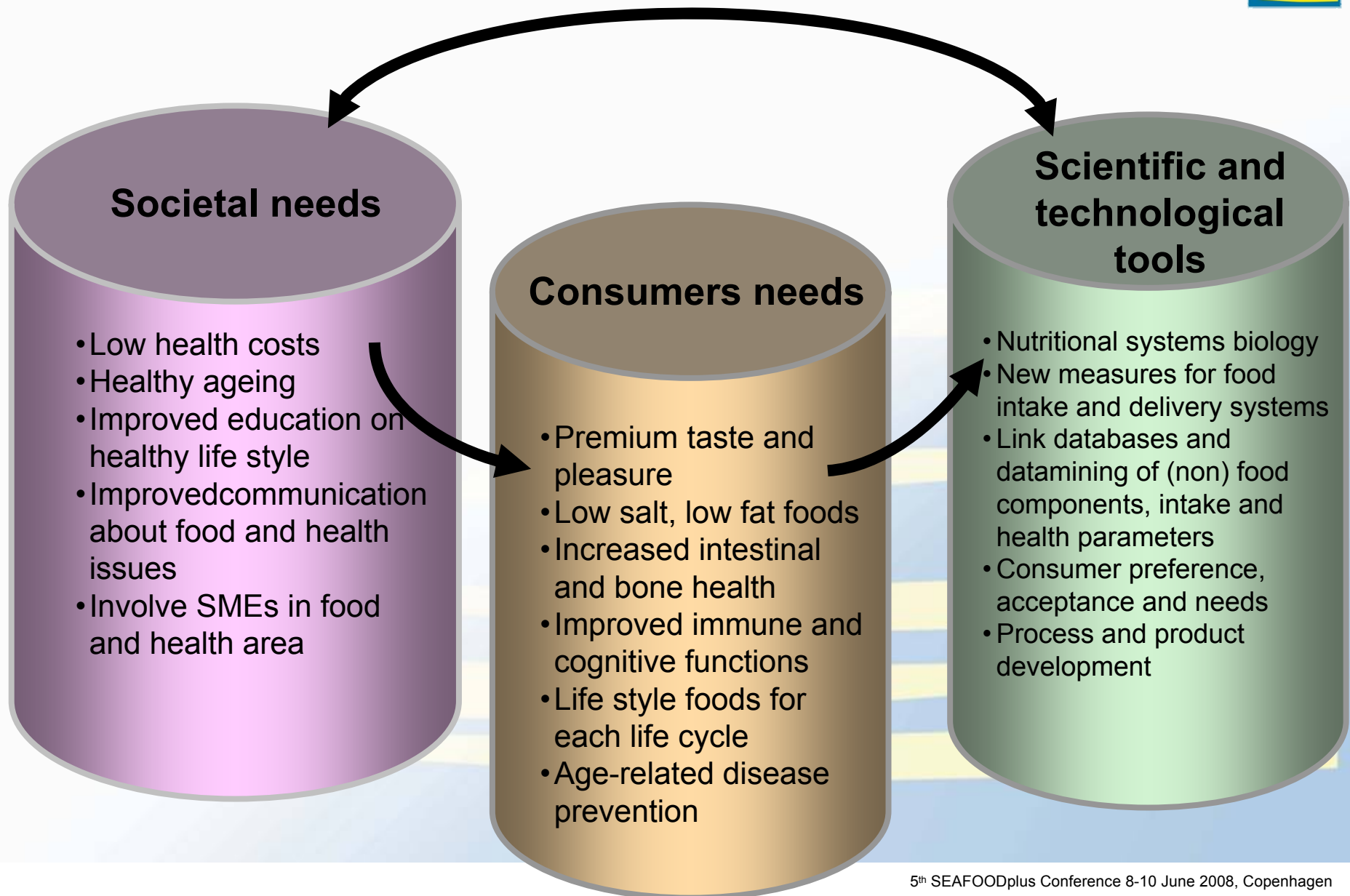
Food & Health: priority research areas



Challenges in upgrading of co-products and underutilized fish



The integrated picture

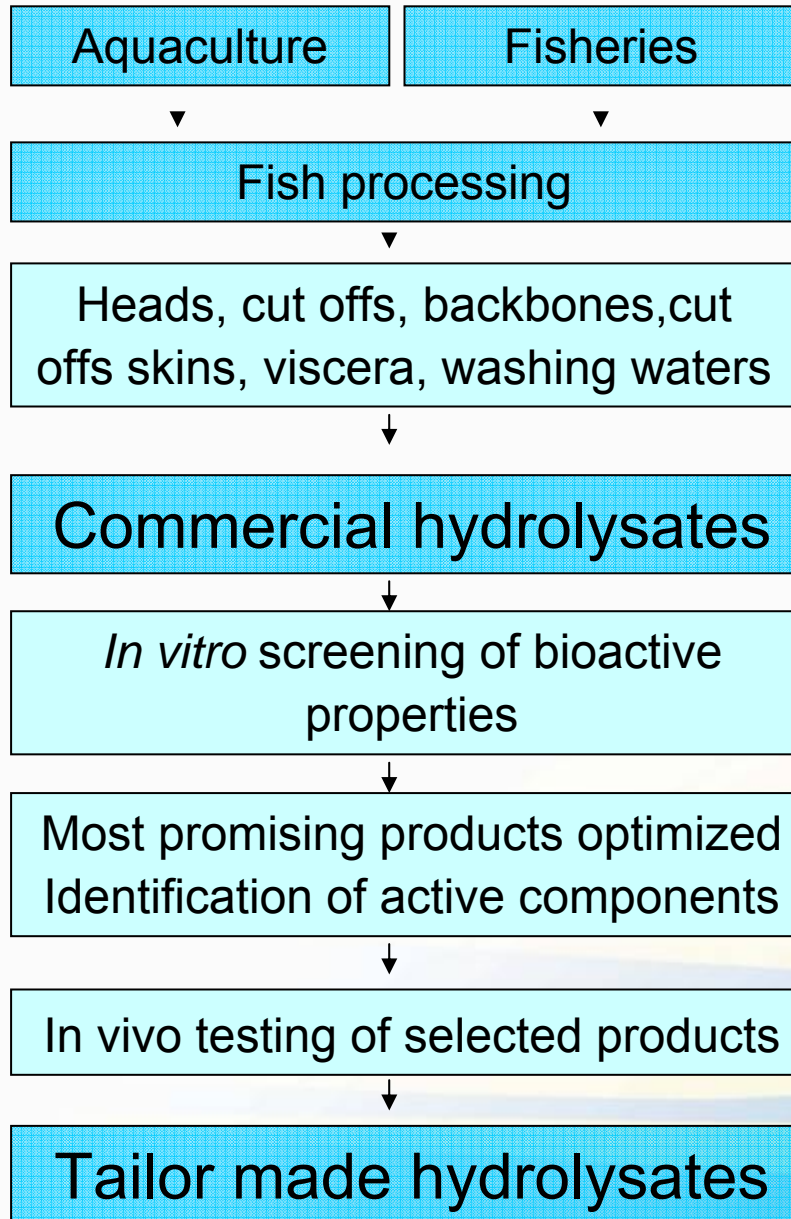


The aims of PROPEPHEALTH

The main objectives of the PROPEPHEALTH project are

- to screen and recover new health beneficial peptides from marine resources**
- document their health effects**
- to use them in tailor made products accepted by consumers**

What was done in PROPEPHEALTH



Diverse and sustainable



Same hygienic and quality criteria as other products

Mostly seafood flavours from 3 SME's

ACE-inhibition, antioxidative, anticancer, antimicrobial and hormone like activities

Degree of hydrolysis, type of enzyme, membrane filtration, fermentation and Maillard browning.

Scientific documentation of properties

Products on the market

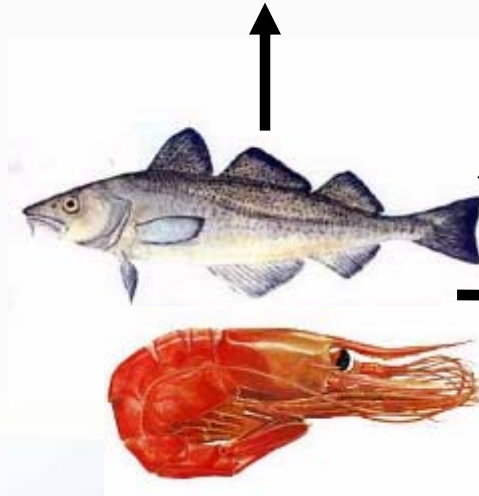
In vitro screening tests of bioactive properties carried out in PROPEPHEALTH



ANTICANCER

ANTIBACTERIAL

CALCIOTROPIC
Calcitonin- like peptides



ANTI - ACE (hypotensive)

SECRETAGOGUES
Gastrin and CCK-like peptides

ANTIOXIDANTS

**OBESITY, DIABETES
CARRIES, MIGRAINE
(CGRP-like peptides),
OTHERS**

FPH as ACE-inhibitors

- ***In vitro* and *in vivo* ACE inhibition of peptides from hydrolysates from many protein sources fairly well documented**
- **Bonito hydrolysate and sardine hydrolysate with documented ACE-inhibition have been on the market for many years**

ACE inhibitory effects of commercial fish protein hydrolysates in PPH


• Sample	• ACE IC50 ($\mu\text{g. mL}^{-1}$)*
– Captopril	– 4.78.10 ⁻³
– Cod	– 75
– Plaice	– 4
– Saithe	– 200
– Salmon	– 220
– Portuguese dogfish	– 260

* IC50 corresponds to the hydrolysate concentration ($\mu\text{g. mL}^{-1}$) inhibiting 50% of ACE activity

Antioxidative properties

Antioxidative properties of commercial FPH in the PROPEPHEALTH project



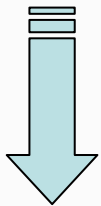
Samples	β -carotene linoleate model assay IC_{50}	DPPH scavenging assay IC_{50}	Chelating activity IC_{50}	Reducing power at OD=0,5 (mg/mL)
	0,17 – 1,8	10-36	0,3 – 7,7	4,1-18,5
Ascorbic acid	-	-	-	0,043
BHA	0,0049	-	-	0,057
Trolox	-	0.056	-	0,084
EDTA	-	-	0.06	-

THE CONTEXT...

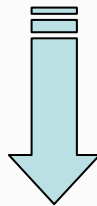
Commercial Hydrolysate



Evaluation of AAO
Peptidic profile



Low AAO



If 2nd hydrolysis
=> AAO higher



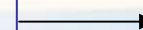
NEW EXPERIMENTS...



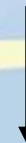
Screening of 7 proteases



Hydrolysis pH-stat
Evaluation of AAO
Peptidic profile



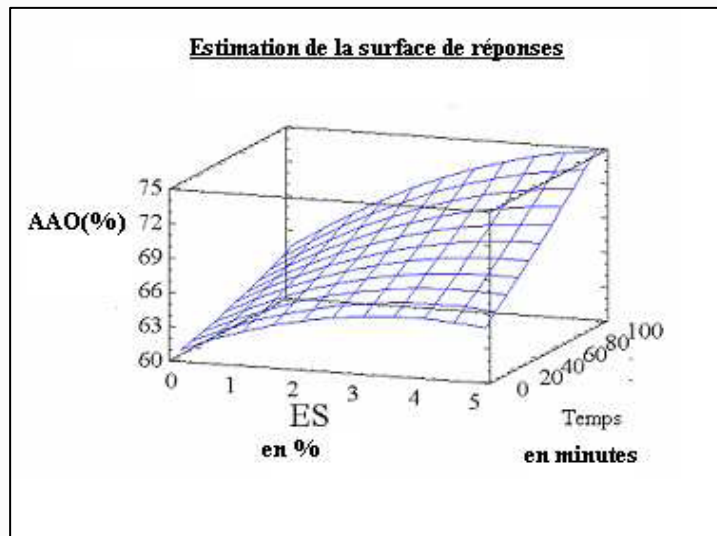
Selection of 2
proteases



Optimisation of enzymatic hydrolysis using
central composite design (2 factors)



Optimisation of antioxidative properties



Mathematical model obtained for enzyme1

$$DH = 0.12 + 4.8 E/S + 0.04\text{Time} - 0.6 (E/S)^2 + 0.006 E/S * \text{Time}$$

$$R^2 = 99.5\% ; p = 0.05$$

$$AAO = 56.5 + 3.8 E/S + 0.11 \text{Time}$$

$$R^2 = 94.1\% ; p = 0.05$$

Mathematical model obtained for enzyme2

$$DH = -0.41 + 1.31 * ES + 0.05 * \text{time} - 0.15 * (E/S)^2 - 2.10 * 10^{-4} \text{Time}^2$$

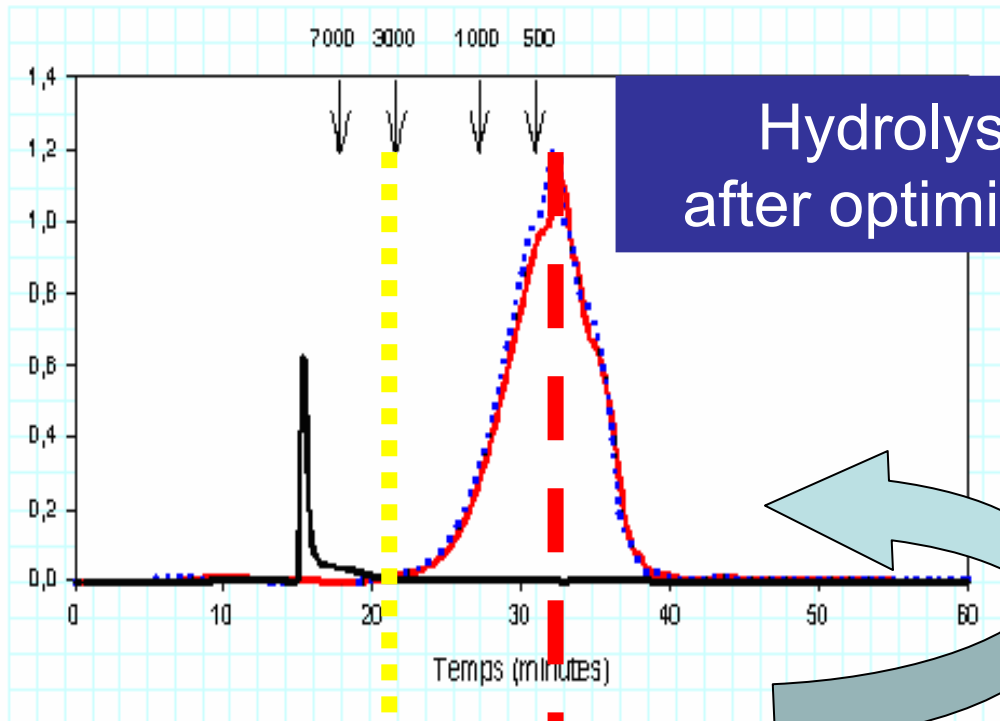
$$R^2 : 99.2 \%$$

$$p = 0.05$$

$$AAO = 60.9 + 2.2 ES + 0.03 \text{Time}$$

$$R^2 : 99.6 \%$$

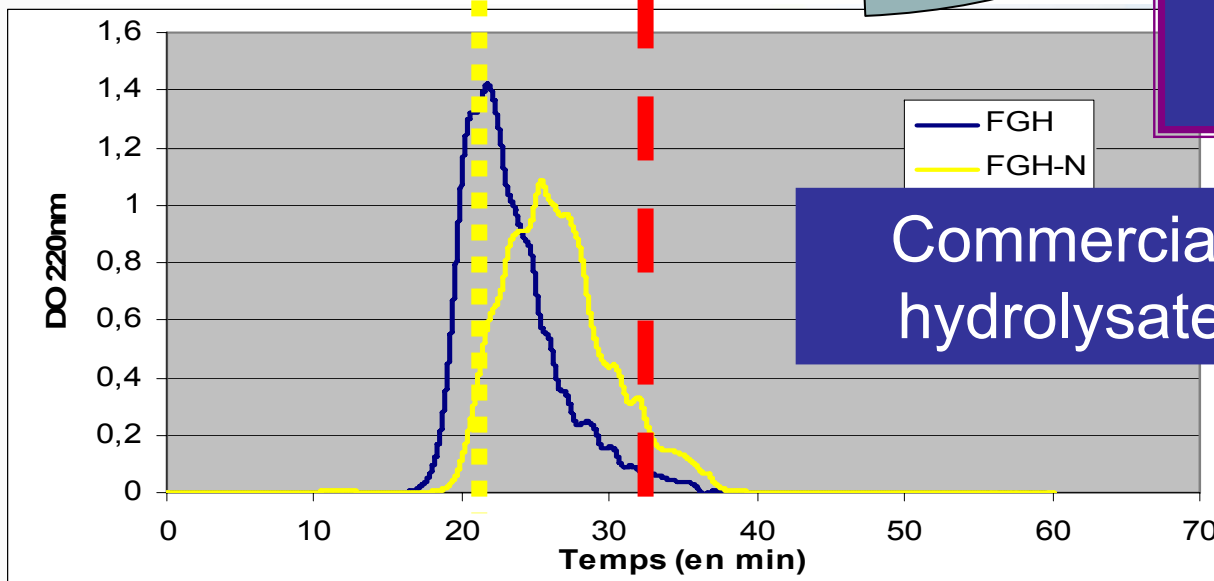
$$p = 0.05$$



Hydrolysate
after optimisation

CONCLUSION

Possibility to adapt the
industrial process on
the basis of the
chromatographic
pattern



Commercial
hydrolysate

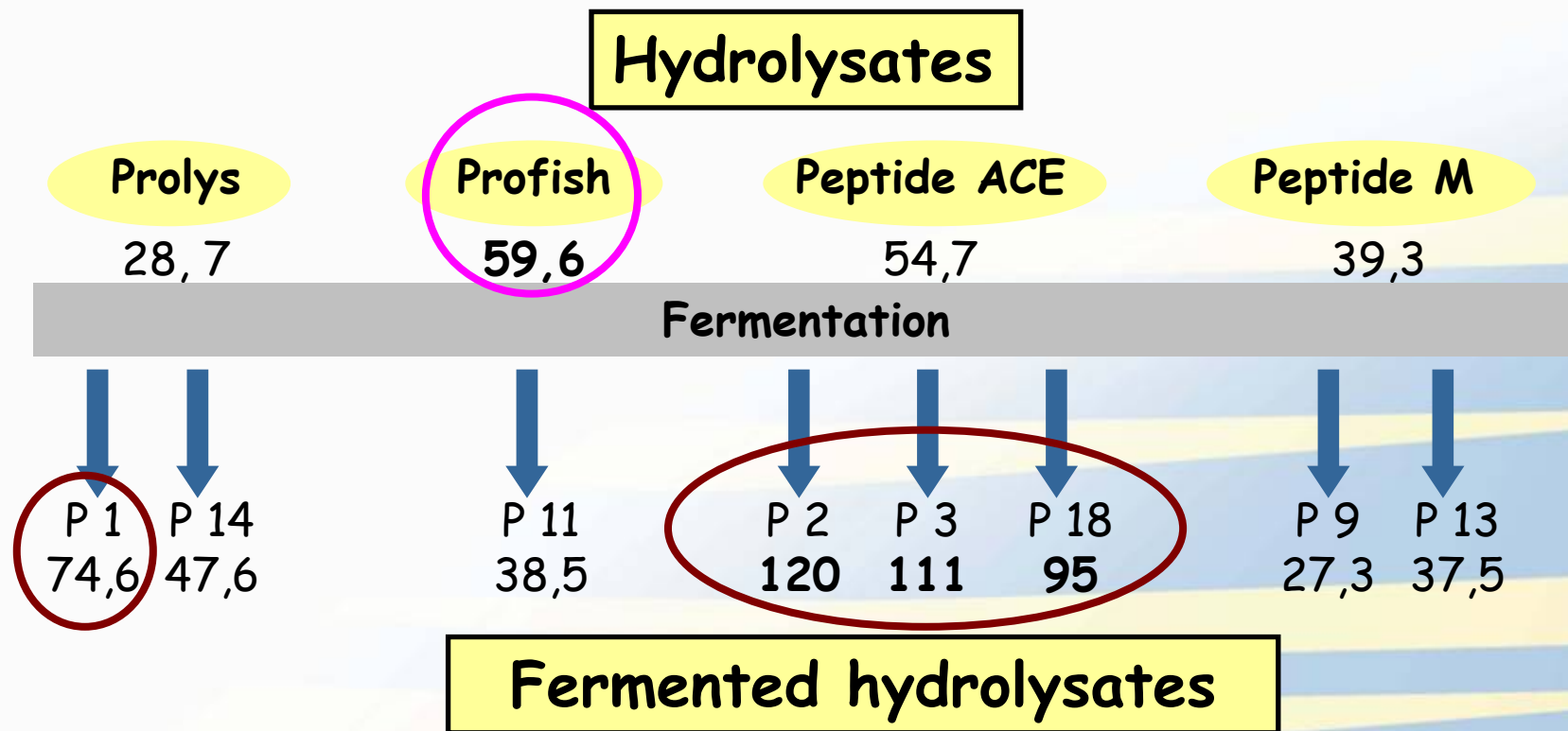
Hormone like activities

Hormone like activities in commercial fish protein hydrolysates



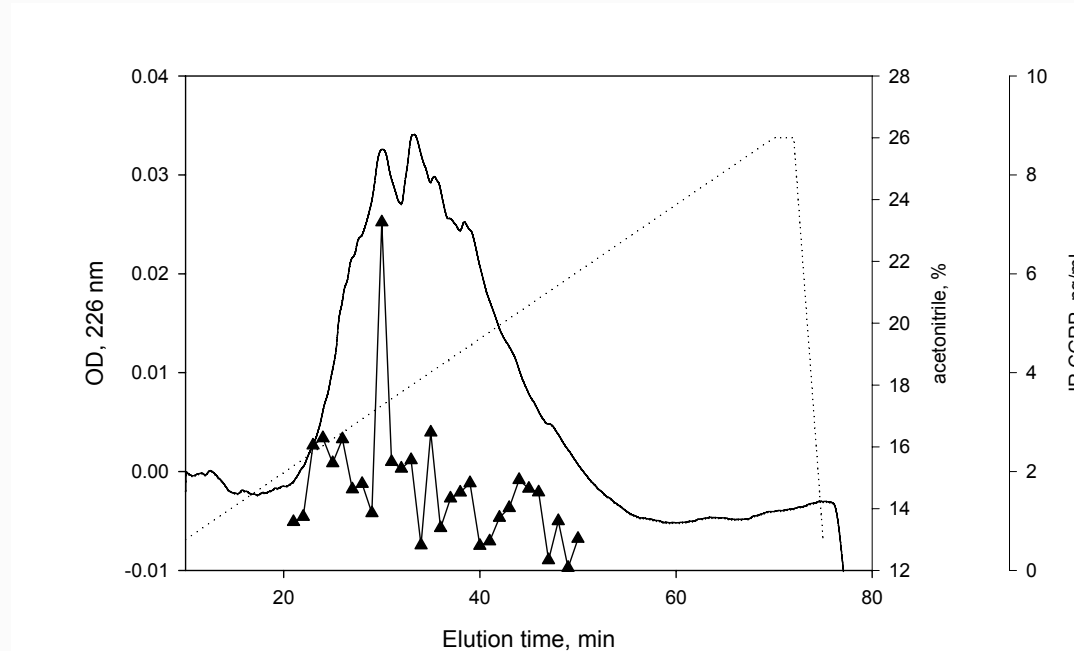
SME'S	Samples	CGRP-L	CT-L	Gastrin-L	Number of samples	Reproducibility
	Dogfish	++	++	++	4	OK
COPALIS	Saithe	+	+	+	3	OK
	Beryx	+	+	+	2	OK
	Native BW protein	+	+	-	3	-
PRIMEX	Salmon	+	+	+	3	-
	Blue Whiting	+	+	+	3	-
	Cod	++	++	+	3	OK
MARINOVA	Plaice	+	+	+	3	OK
	Salmon	+	+	+	2	OK

Task 2: Fermented saithe hydrolysates. RRA CGRP (pg/mg of dry weight)



P : Peptide

SEQUENCE OF THE PURIFIED MOLECULE FROM THE HYDROLYSATE OF PORTUGUESE DOGFISH

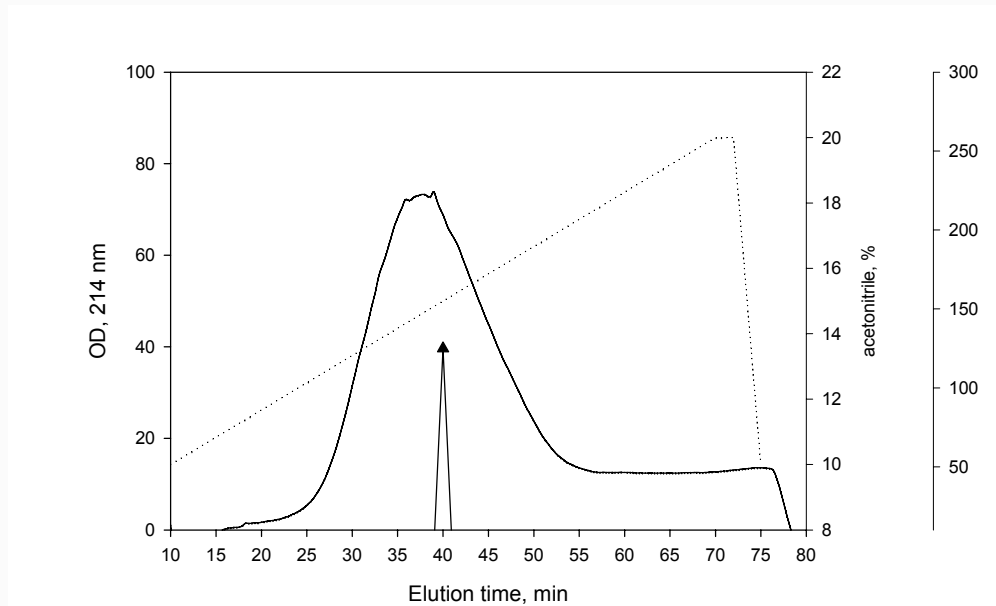


GFPGPEGL

Homology with C- terminal fragments of collagen IV and XVIII

Proteolysis of these collagens produces molecules such as tumstatin and endostatin able to inhibit the angiogenesis process

SEQUENCE OF THE PURIFIED MOLECULE FROM SAITHE



2 sequences:

VAPEEHPT: fish actin

PEDVI: no homologous sequence

Conclusions

Commercial fish protein hydrolysates can be screened and optimized for certain *in vitro* bioactive properties and the active compounds identified and the effects confirmed in *in vivo* trials

This has been, is being and will be scientifically documented

Fish protein hydrolysates with documented properties will be tried in many food applications

Application: Natural food with health claims



**Appetisers with low GI:
Nutripeptin**



**Cakes and orange juice
with Collagen HM**



**Chocolate with Protizen:
relaxing properties**



Bread with Phoscalim

Future tasks

- **Focused R/D instead of screening and mapping**
- **Focus on your fish**
- **Focus on the “function of your products”**
- **Collaborate with others on health claims**

The fish should be sustainable.

The supply should be steady and in amounts justifying the great and expensive effort needed to develop and market proteins and peptides with documented health benefits.

A better life with seafood...



www.seafoodplus.org