FOOD stars — Innovative Food Product Development Cycle: Frame for Stepping Up Research Excellence of FINS

European Commission

By-product utilisation and resource utilisation in the meat processing industry: bioactive ingredients with health attributes

Dr. Maria Hayes, TEAGASC

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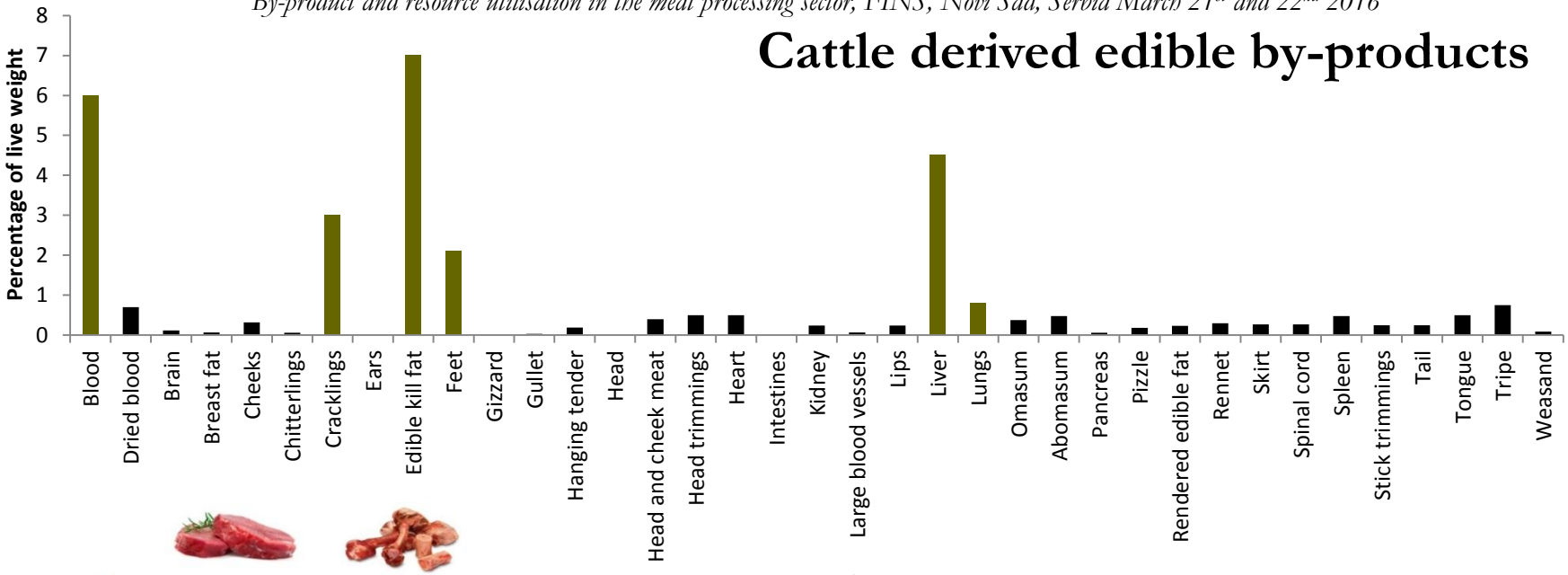
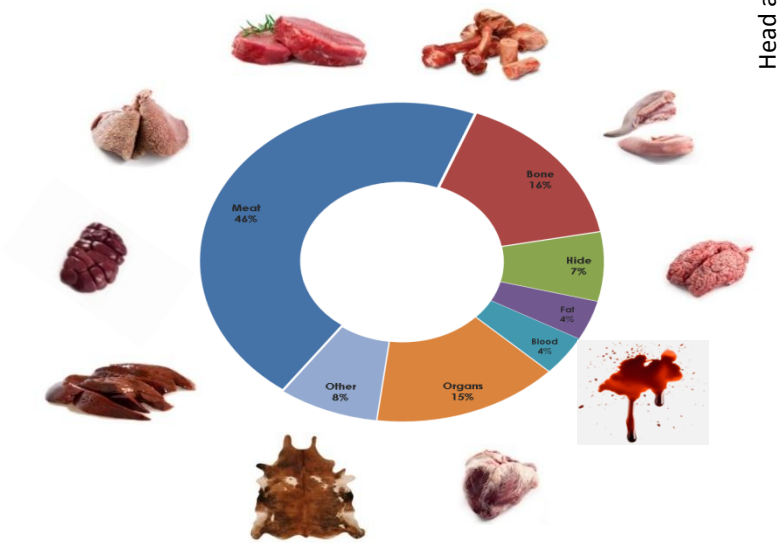
Grant Agreement number: 692276 — FOODstars | H2020-TWINN-2015

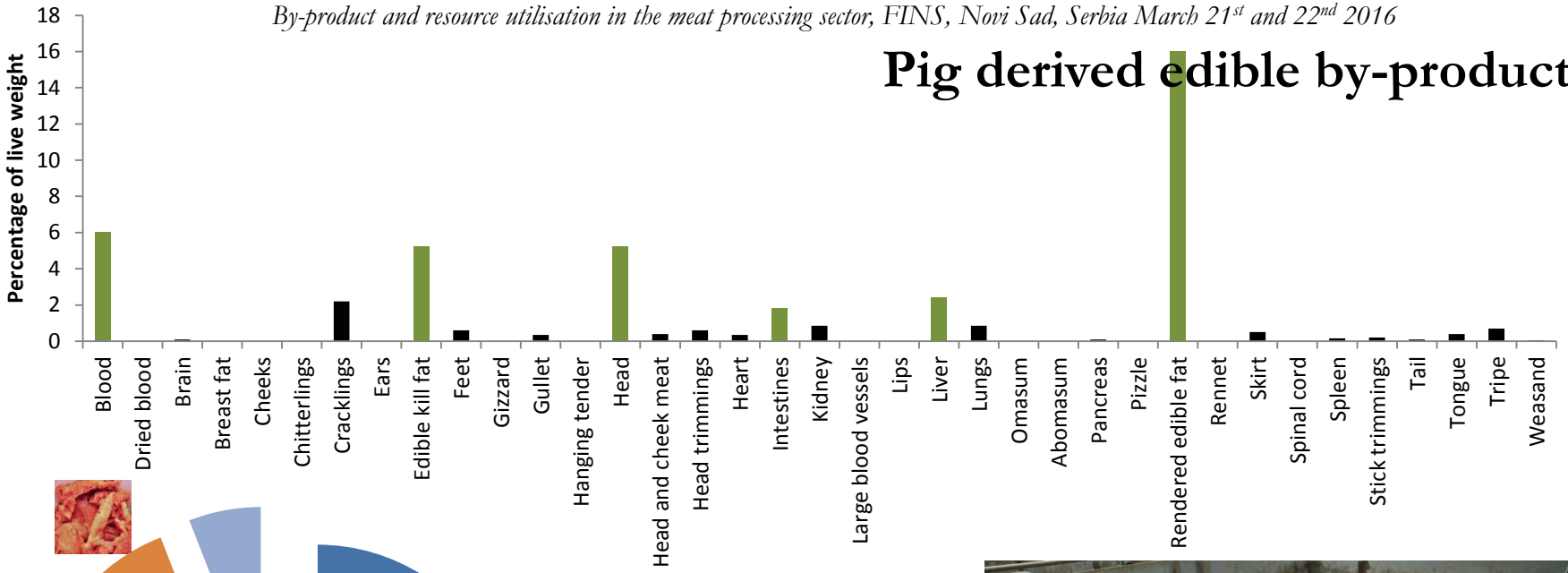
Cattle derived edible by-products





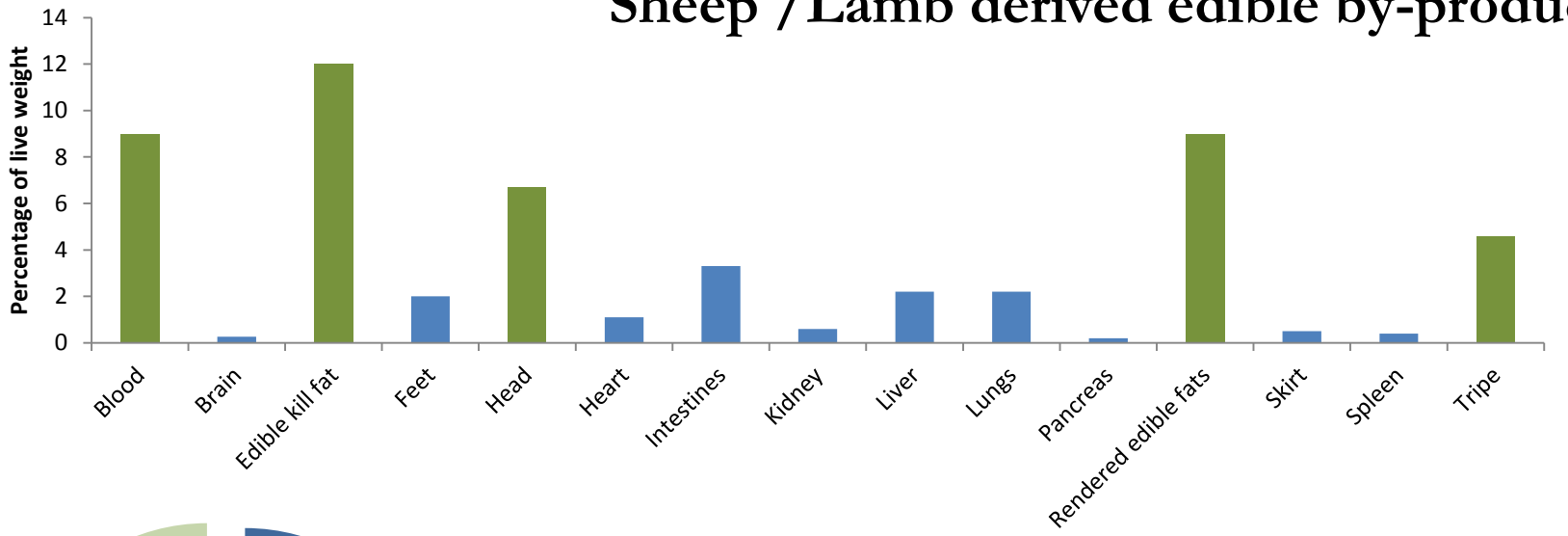
* J. Animal Pro. Adv., 2015, 5, 6, 681-696

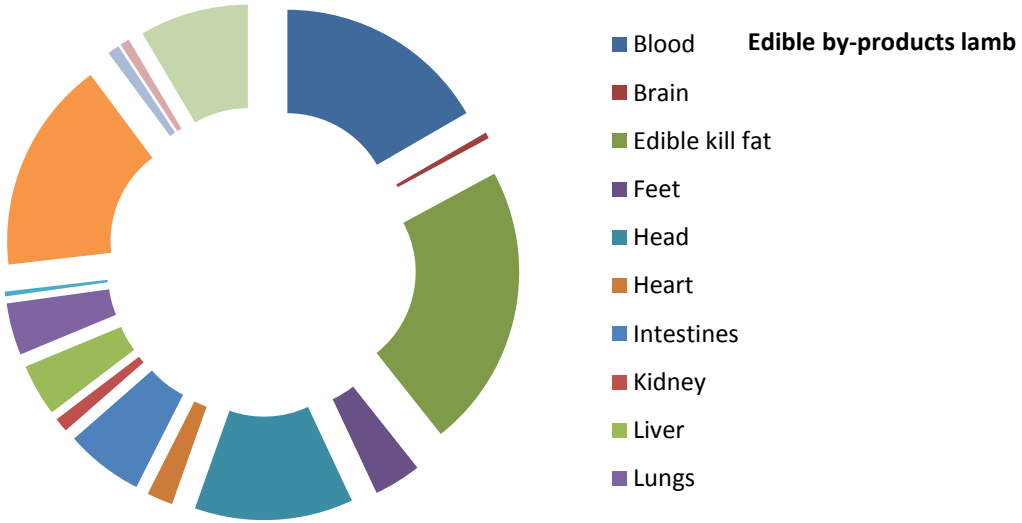
Pig derived edible by-products



* J. Animal Pro. Adv., 2015, 5, 6, 681-696

Sheep /Lamb derived edible by-products

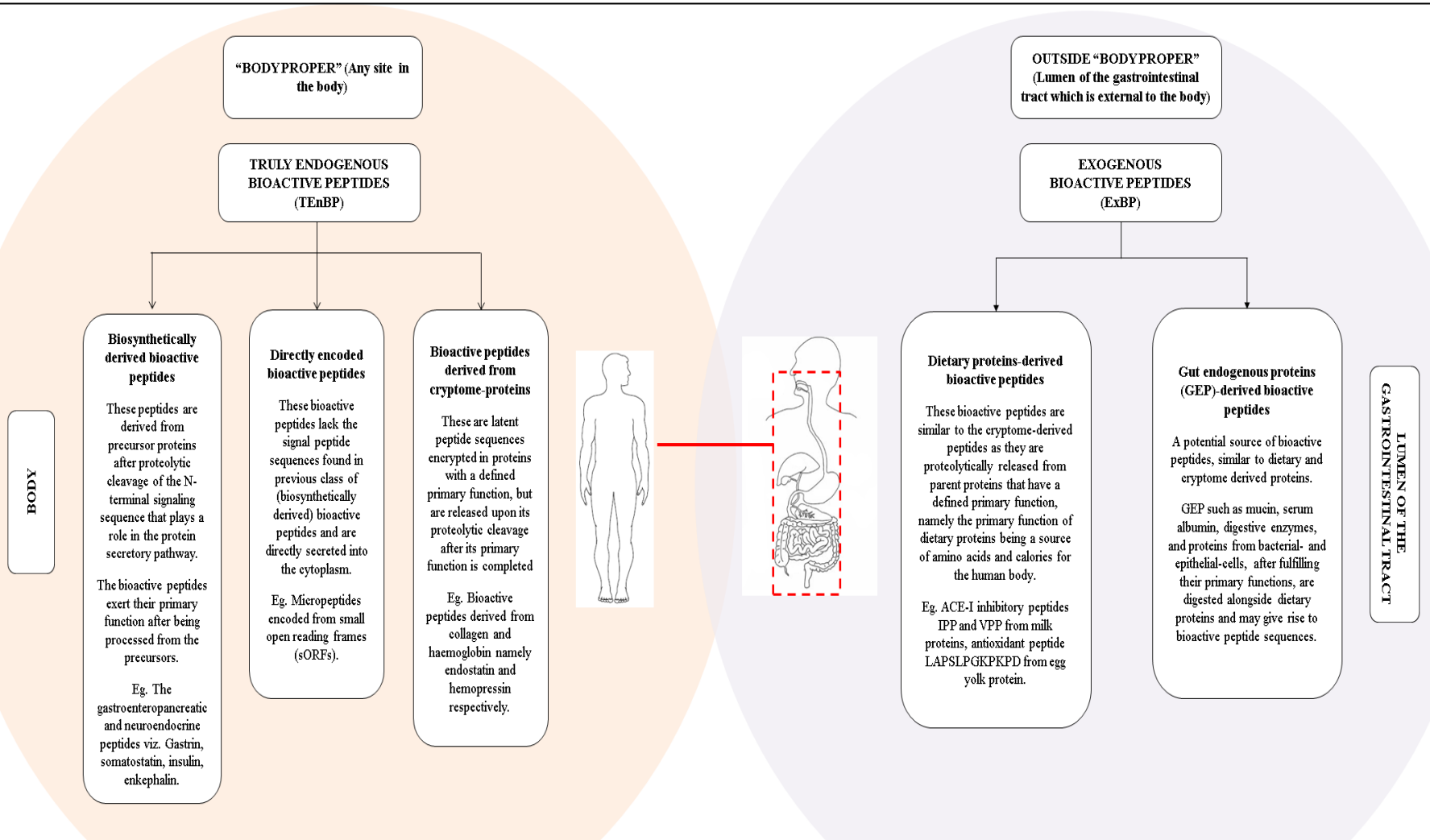




* J. Animal Pro. Adv., 2015, 5, 6, 681-696

BIOACTIVE PEPTIDE SOURCES

Schematic representation of the types of bioactive peptides that are known to date. The bioactive peptides that are derived in the body have been grouped as the truly endogenous bioactive peptides (TEnBP), while bioactive peptides that are generated in the GIT lumen are grouped as exogenous bioactive peptides (ExBP)



HUMAN GUT ENDOGENOUS PROTEINS AS A SOURCE OF BIOACTIVE PEPTIDES

- Bioactive peptides play a crucial role in regulation and modulatory functions.
- They can be broadly classified as being either truly endogenous or exogenous depending on the site in the body where they originate and act.
- Truly endogenous bioactive peptides can be defined as peptides produced from the human proteome inside the "body proper" that may either play a role in physiological regulation or exert a health benefit.
- Exogenous bioactive peptides are those that are generated outside the "body proper" such as in the lumen of the GIT, which, in anatomical terms is considered to be external to the body.
- Truly endogenous bioactive peptides can be further classified into three major types:

(1) biosynthetically-derived bioactive peptides (2) directly encoded bioactive peptides and (3) cryptome-protein derived bioactive peptides.

Reference: Dave, L.A., Hayes, M., Montoya, C. A., Rutherfurd, (2016), Human gut endogenous proteins as a potential source of angiotensin-I-converting enzyme (ACE-I), renin and antioxidant peptides, Peptides, 76, 30-44.

FOOD DERIVED BIOACTIVE PEPTIDES – EXOGENOUS BIOACTIVE PEPTIDES (EXBP)

- ExBP are bioactive peptides derived from food, such as peptides found in sour milk, or generated from the digestion of dietary or host proteins in the GIT.

- Food proteins are a well known source of ExBP and these may be generated by hydrolysis of food proteins during food processing and/or gastrointestinal digestion.

- They have a myriad of activities and health benefits including antioxidant, antimicrobial, ACE-I inhibitory, renin inhibitory opioid activities.

Reference: Dave, L.A., Hayes, M., Montoya, C. A., Rutherfurd, (2016), Human gut endogenous proteins as a potential source of angiotensin-I-converting enzyme (ACE-I), renin and antioxidant peptides, Peptides, 76, 30-44.

Sources of dietary protein and bioactive peptides










FOOD DERIVED BIOACTIVE PEPTIDES – EXOGENOUS BIOACTIVE PEPTIDES (EXBP)

- Bioactive peptides are food-derived peptides that in addition to their nutritional value exert a physiological effect in the body (*Vermeirssen *et al*. 2004).

- These bioactive peptides are inactive within the original protein but once released, function as regulatory compounds with hormone-like activity that is based on the inherent amino acid composition and sequence (*Vermeirssen *et al*. 2004).

- Digestive enzymes, naturally occurring enzymes in milk, and microbial enzymes – especially from adventitious starter LAB, generate bioactive peptides during milk fermentation and cheese manufacture, thereby enriching dairy products (*Gobbetti *et al.* 2004).

- The size of active sequences may vary from two to thirty amino acid residues, and many peptides are known to have multifunctional properties (*Meisel and FitzGerald, 2003).

- Sources include Dairy, Egg, Fish, Cereals and Meat (Myosin and Actin) (*Kitts and Weiler, 2003).

International Journal of Dairy Technology 57:173-188.

* Vermeirssen, V., Van Camp, J. and Verstraete, W. (2004). Bioavailability of angiotensin I converting enzyme inhibitory peptides. British Journal of Nutrition 92: 357-366.

*Kitts, D. D., Weiler, K. (2003) Bioactive proteins and peptides from food sources. Applications of Bioprocesses used in isolation and recovery. Current Pharmaceutical Design, 9, 1309-1323.

*Meisel, H. and FitzGerald, R. J. (2003) Biofunctional peptides from milk proteins. Mineral binding and cytomodulatory effects. Current

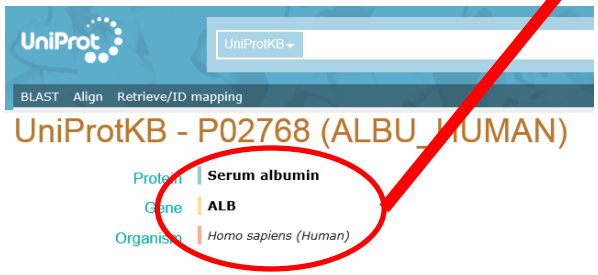
Where to start????

METHODS FOR PEPTIDE IDENTIFICATION

SCREENING FOR BIOACTIVE PEPTIDES – *IN SILICO* METHODOLOGIES

Step 1: Extraction of protein sequence

Serum albumin



MKWVTFISLLFLFSSAYSRGVFRRDAHKSEVA

HRFKDLGEENFKALVLIAFAQYLQQCPFDHVKL

VNEVTEFAKTCVADESAENCDKSLHTLFGDKL

CTVATLRETYGEMADCCAKQEPERNECFLQH

KDDNPNLPRLVRPEVDVMCTAFHDNEETFLKK

YLYEIARRHPYFYAPELLFAKRYKAAFTECCQA

ADKAACLLPKLDELRDEGKASSAKQRLKCASL

QKFGERAFKAWAVARLSQRFPKAEFAEVSKLV

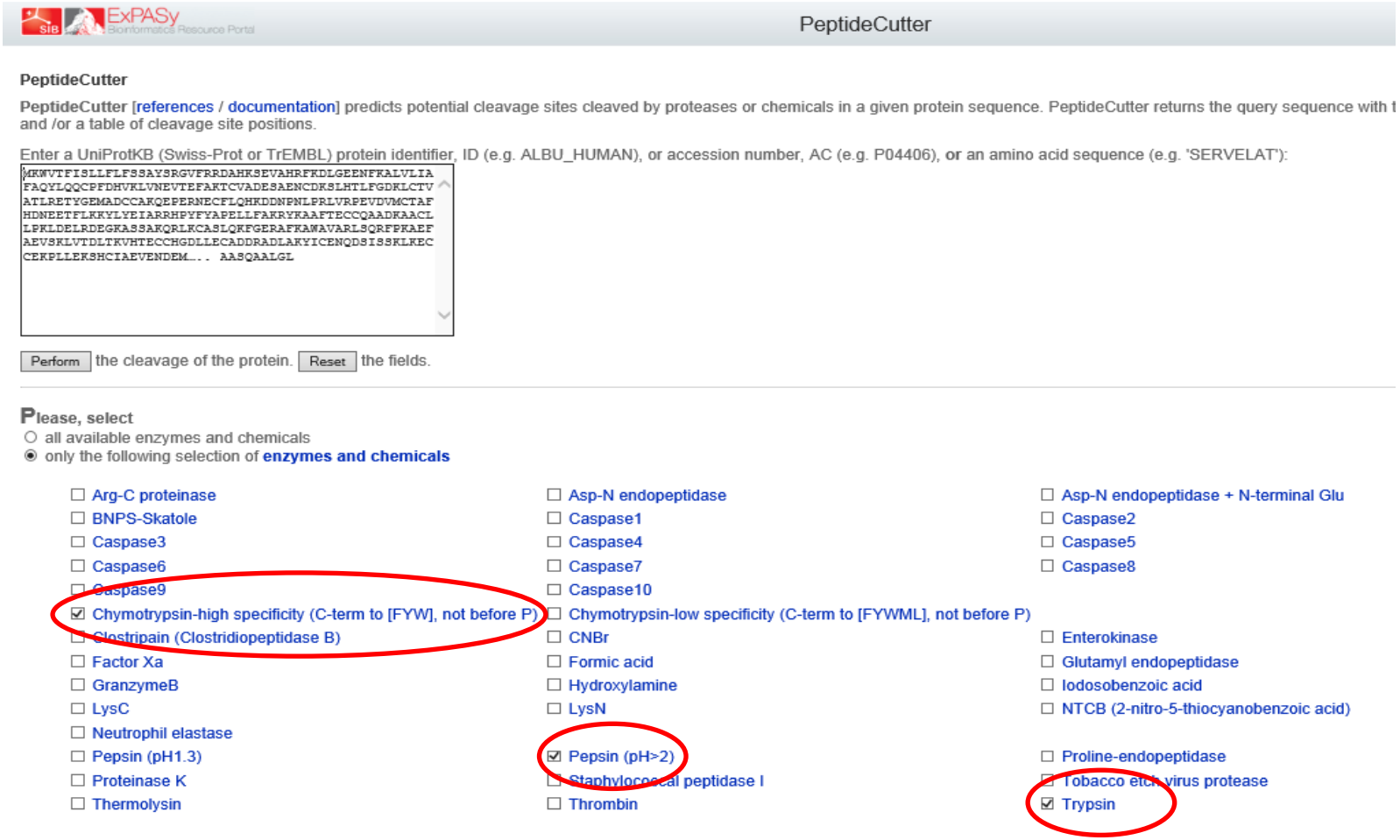
TDLTKVHTECCHGDLLECADDRADLAKYICEN

QDSISSKLKECCEKPLLEKSHCIAEVENDEM…..

AASQAALGL

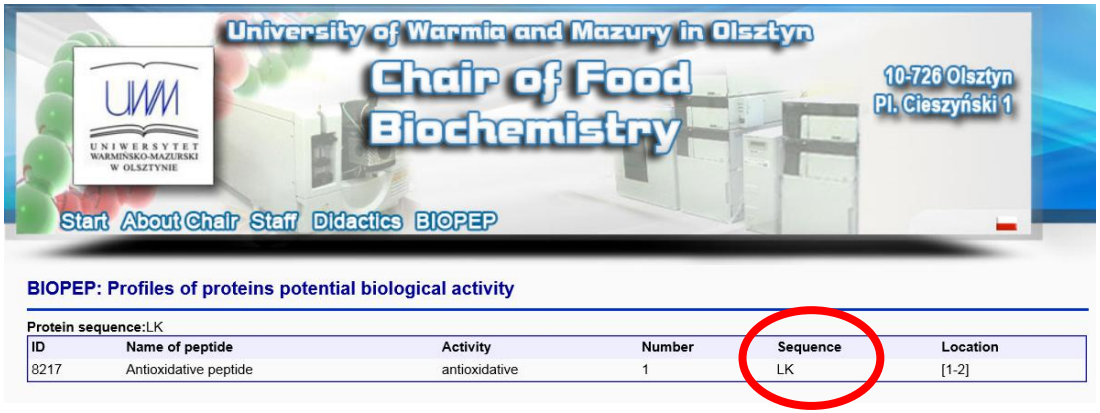
SCREENING FOR BIOACTIVE PEPTIDES – *IN SILICO* METHODOLOGIES

Step 2: Simulated *in silico* digestion



SCREENING FOR BIOACTIVE PEPTIDES – *IN SILICO* METHODOLOGIES

Step 3: Identification of known bioactive peptides



MKWV**TF**ISLLFLFSSAYSRGVFRRDAHKSEVAHRFKDLGEENF**KA**LVL**IA**FAQYL

QQCPFDHVKLVNEVTEFAKTCVADESAENCDKS**LHTLF**GDKLCTVATLRETYGE

MADCCAKQEPERNECFLQHKDDNPNL**PR**LVRPEVDVMCTAF…PELLFAKRYKA

AFTECCQAADKAACLLPKLDELRDEGKASSAKQR**LK**CASL**QK**FGERAFK**AW**AV

A**RL**SQRFPKA..*RPCF*SALEVD..KVHTECCHGDLLECADDRADLAKYICENQ

DSISSKLKECCE**KPLL**EK..*CCK*..SHCIAE**VE**NDEM..**VPK**… **AA**SQAALGL

Novel peptides predicted to be released after *in silico* digestion: Bioactive?

Screening for bioactive peptides – *in silico* methodologies

Step 4: Chemical synthesis of select peptides

Microwave-assisted solid phase peptide synthesis

Purification using reversed-phase high-pressure liquid chromatography

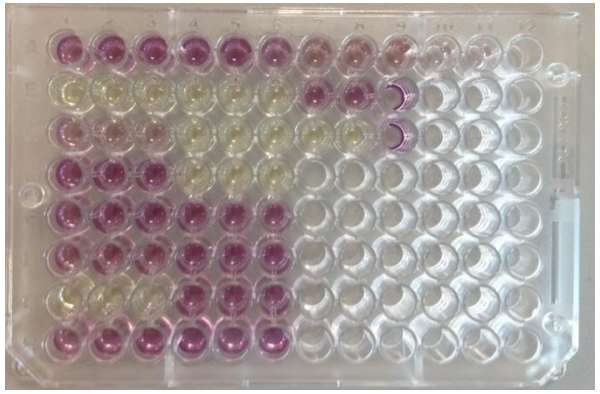
Mass spectrometry: Confirmation of the molecular weight of peptides

Synthetic peptides

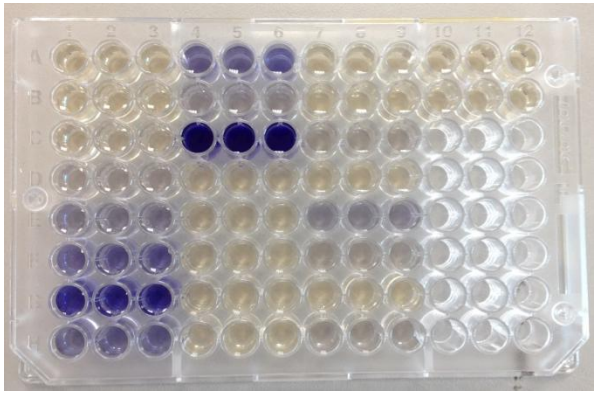
SCREENING FOR BIOACTIVE PEPTIDES – *IN SILICO* METHODOLOGIES

Step 5: in vitro screening and identification of antioxidant peptides

Synthetic peptides



DPPH inhibition



FRAP value

In silico analysis for meat derived peptide generation

I. **IDENTIFICATION OF THE AMINO ACID SEQUENCE OF SELECTED PROTEINS**

Protein databases
- **UniProt** | http://www.uniprot.org/
- **Pubmed** | http://www.ncbi.nlm.nih.gov/pubmed
- **BIOPEP** | http://www.uwm.edu.pl/biochemia/index.php/pl/biopep

II. **COMPARISON OF BOVINE AND PORCINE PROTEIN SEQUENCES**

Protein comparison
- **BLAST** | http://blast.ncbi.nlm.nih.gov/Blast.cgi
- **Expasy ProtParam Tool** | http://web.expasy.org/protparam/
- **Expasy SIM Alignment Tool** | http://web.expasy.org/sim/

III. **ENZYME SELECTION BASED ON PUBLISHED LITERATURE AND *IN SILICO* CLEAVAGE ANALYSIS**

In silico hydrolysis
- **BIOPEP** | http://www.uwm.edu.pl/biochemia/index.php/pl/biopep
- **Expasy PeptideCutter Tool** | http://web.expasy.org/peptide_cutter/

IV. **DATABASE SEARCH OF KNOWN AND UNKNOWN BIOACTIVE PEPTIDES**

Bioactive peptide databases
- **BIOPEP** | http://www.uwm.edu.pl/biochemia/index.php/pl/biopep
- **PeptideDB** | http://www.peptides.be/
- **CAMP** | http://www.camp.bicnirrh.res.in/

→ Previously described bioactive peptides

→ Inactive peptides

V. **IDENTIFICATION OF NOVEL PEPTIDES WITH POTENTIAL TO INHIBIT ACE-I, RENIN AND DPP-IV**

VI. **BIOACTIVITY PREDICTION *IN SILICO***

PeptideRanker | http://bioware.ucd.ie/~compass/biowareweb/Server_pages/peptideranker.php

VII. **TOXICITY PREDICTION AND GASTROINTESTINAL DIGESTION SIMULATION**

Toxicity prediction
- **ToxinPred** | http://www.imtech.res.in/raghava/toxinpred/

Gastrointestinal digestion simulation
- **Expasy PeptideCutter Tool** | http://web.expasy.org/peptide_cutter/

VIII. **CHEMICAL SYNTHESIS AND CONFIRMATION OF BIOACTIVITY *IN VITRO***

In vitro assays: ACE-I, DPP-IV and Renin inhibition assays

IX. **DETERMINATION OF IC50 VALUES**

Enzymatic hydrolysis using papain

↓

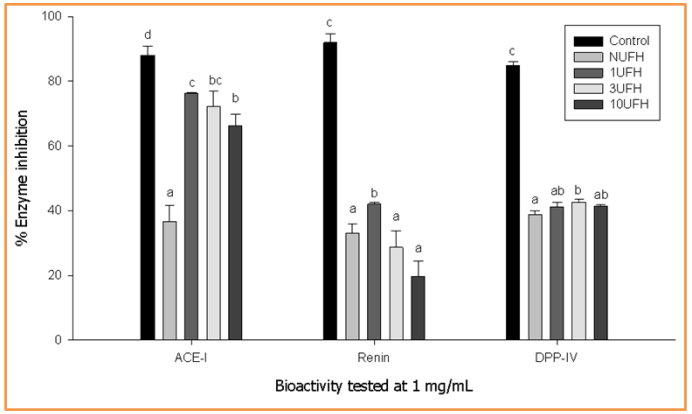
Bioactivity assessment *in vitro*

↓

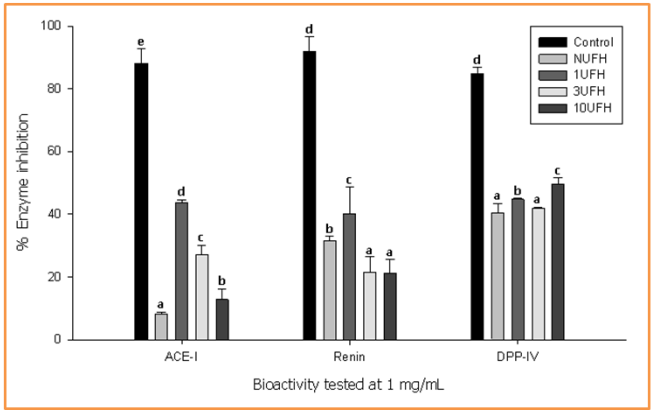
Enrichment: MWCO filtration

↓

Bioactivity assessment *in vitro*



HEMOGLOBIN



BOVINE SERUM ALBUMIN

*Lafarga, T. Hayes, M. et al., (2016) Journal of Food Biochemistry doi:10.1111/jfbc.12259

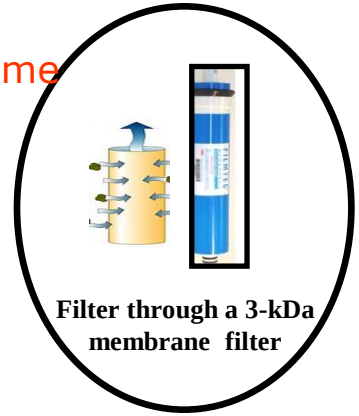
PEPTIDE GENERATION METHODS

Fermentation

Partially hydrolysed meat

Enzyme

Filtration



Stabilisation

Freeze drier

Stable powder

Analytical Scale extraction

ASE

Assays

Antioxidant

ACE-I-inhibitory

Anti-thrombotic

High through-put screening

Detection of peptides

Analytical HPLC

Separation and purification

Characterisation

Q-TOF MS

NMR

COMMON MEAT PEPTIDES

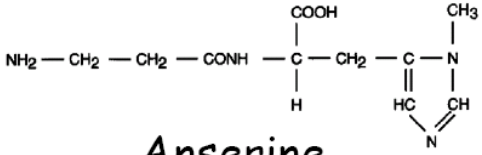
- Several endogenous antioxidant peptides are abundant in meats
- Carnosine (β-alanyl-L-histidine)
- Anserine (N-β-alanyl-1-methyl-L-histidine)
- L-Carnitine (β-hydroxy-y-trimethyl amino butyric acid)

HISTIDYL DI-PEPTIDES

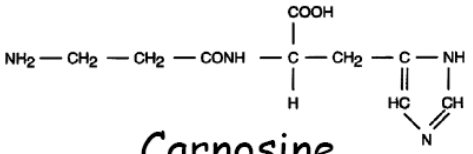
Antioxidant peptides

Histidyl di-peptides

Chicken muscle



Anserine



Carnosine

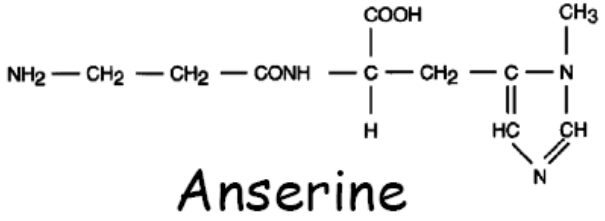
500 mg per Kg of chicken

2700 mg per Kg of pork shoulder

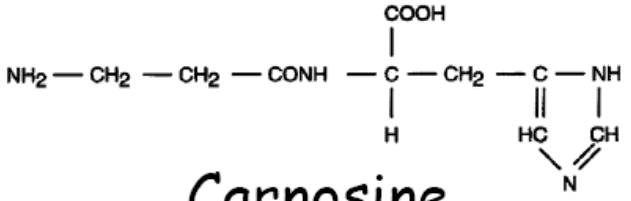
FERMENTED MEAT-PRODUCTS & PEPTIDES

BIOACTIVE PEPTIDE CONTENT????

ANTIOXIDANT ACTIVITIES OF HISTIDYL DI-PEPTIDES



Anserine



Carnosine

- Antioxidant activity results from an ability to chelate transition metals e.g., copper

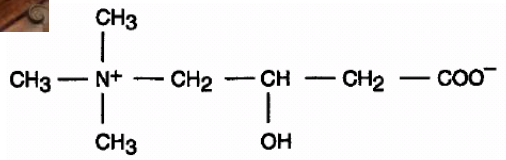
- Prevention of disease and aging related to oxidative stresses.

L-CARNITINE

Energy sports drinks

Beef

L-Carnitine



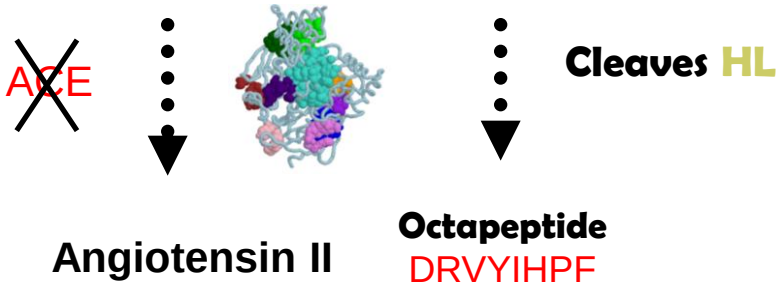
β-hydroxy-y-trimethyl amino butyric acid is biosynthesised in the human body (liver and kidneys).

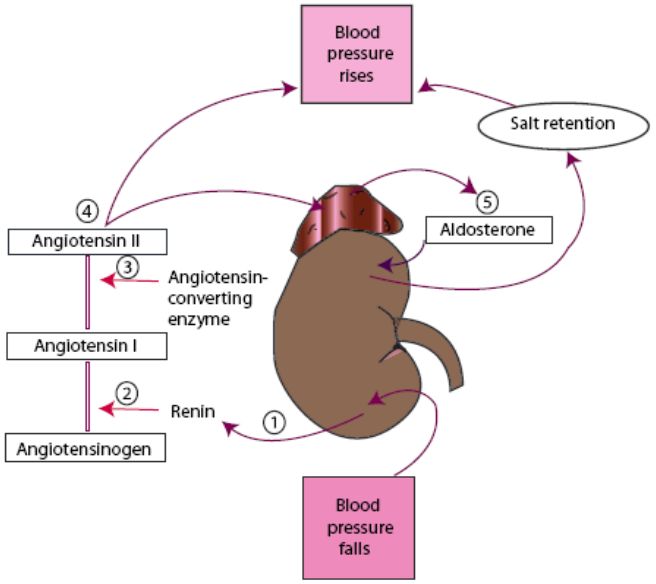
It transports long-chain fatty acids across the inner mitochondrial membranes, β-oxidised to produce energy.

Energy production in muscle – hard exercise.

Maintenance of stamina and fast recovery.

ANTIHYPERTENSIVE MEAT PEPTIDES





Meat peptide inhibitors of ACE-I enzyme

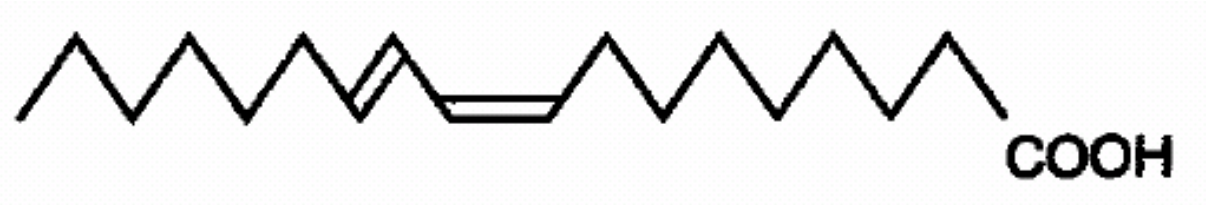
FUNCTIONAL MEAT PRODUCTS

- *Addition of proteins, fibres, antioxidants, probiotics*
- *Nine FOSHU meat products have been approved in Japan with added components.*
- *Pork sausage containing indigestible dextrin, water-soluble dietary fibre from potato starch.*
- *Low fat sausage containing soy proteins (good for cholesterol).*

Pork sausages with soy protein

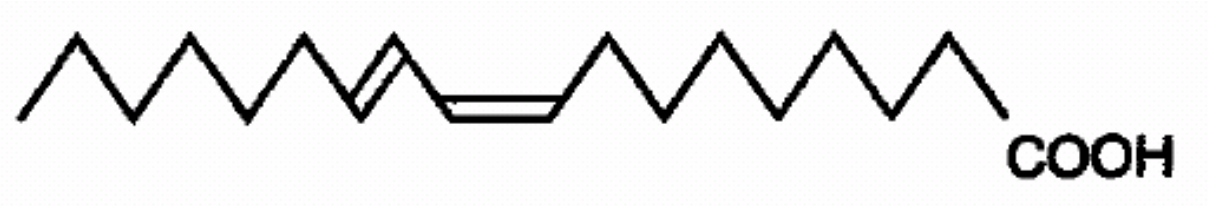
Frankfurters with dextrin

CONJUGATED LINOLEIC ACID (CLA)



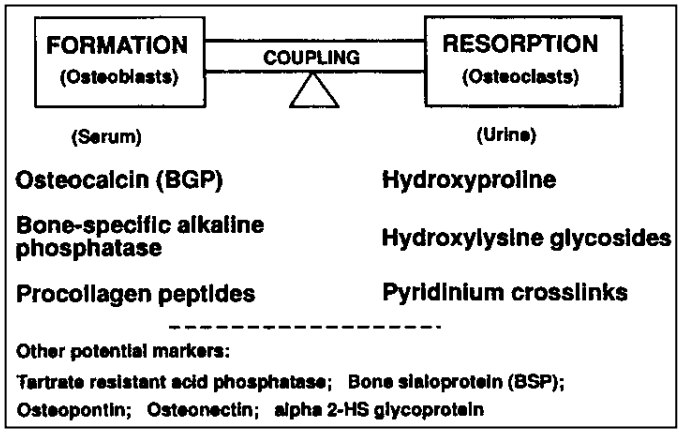
- Identified as an anti-carcinogenic compound from grilled beef.
- Geometric and positional isomers of octa-decadienoic acid.
- 3-8 mg of CLA per gram of beef fat.
- Affected by breed, age and feed composition.
- Increased by cooking, heating and processing.

CONJUGATED LINOLEIC ACID (CLA)



CLA isomer in beef is octadeca-*c*9, *t*11-dienoic acid

Diabetes



Bone metabolism

Obesity control

PROBIOTICS IN MEAT

- Salami product containing three intestinal lactic acid bacteria (Germany)
- *Lactobacillus acidophilus*
- *Lactobacillus casei*
- *Bifidobacterium* spp.
- Meat spread products (Japan)
- *Lactobacillus rhamnosus* FERM P-15120
- Fermented sausages
- *Lactobacillus paracasei*

Meat spreads

Salami

Fermented sausage

CONCLUSION

- Look at traditional
- meat products to assess
- these for bioactivities

- Develop new fermented meats rich in bioactive components

- Hurdles to development include
- Education of consumer
- Safety

Jambon

Black pudding

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ENTERPRISE IRELAND

ReValue PROTEIN

NutraMara

Marine Institute
Foras na Mara

teagasc
Agriculture and Food Development Authority

NUI Galway
OÉ Gaillimh

UCD DUBLIN

UCL

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FOOD stars
Innovative Food Product Development Cycle: Frame for Stepping Up Research Excellence of FINS

European Commission

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