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

European Commission

Fermented food products in traditional food use and potential for use as functional foods: overcoming challenges and bottlenecks in production

Dr. Maria Hayes, TEAGASC

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Fermented Foods

'The processes required for fermented foods were present on earth when man appeared on the scene… When we study these foods, we are in fact studying the most intimate relationships between man, microbe and foods.'

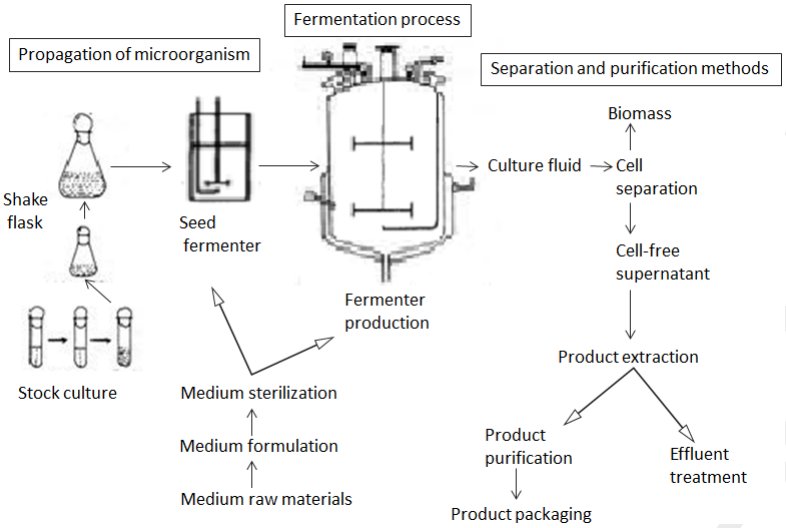
-Prof. Keith H. Steinkraus, Cornell University, 1993

- The word fermentation is derived from the Latin verb *fevere* which means “to boil” and fermentation was defined by Louis Pasteur as “La vie sans l'air” (life without air) (Bourdichon et al., 2012).

- It involves transformation of organic substances into simpler compounds by the action of microorganisms including bacteria, yeasts, fungi and moulds (Hayes et al., 2007; Subramaniyam and Vimala, 2012) often without air. Microorganisms produce enzymes that can break down organic molecules into smaller and often more bioactive molecules.

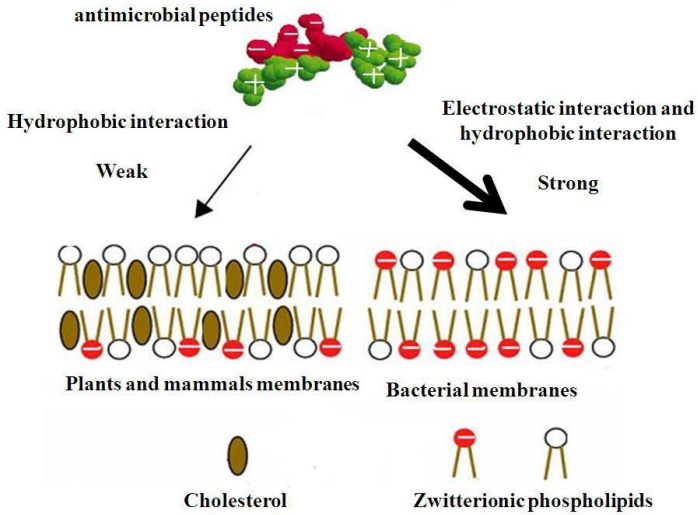
- The process can produce effervescence and heat and is the oldest form of biotechnology.

Fermentation process

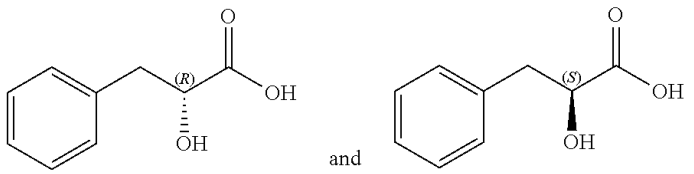


Bioactive compounds including antimicrobial compounds produced during fermentation processes as secondary metabolites include:

- hydroxy acids such as phenyl lactic acid
- Hydroxy-phenyl lactic acid, indole lactic acid
  - alcohols such as phenyl-ethyl alcohol
- antimicrobial peptides



[Chemical formula 5]

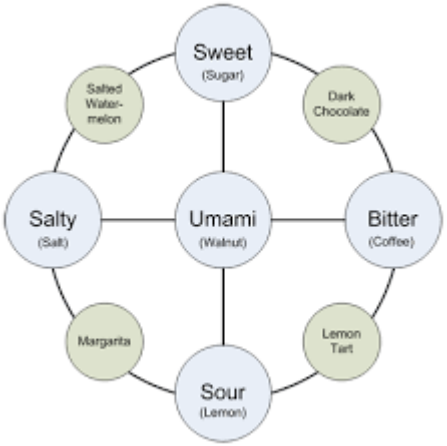


D(+)-phenyl lactic acid
CAS7326-19-4

L(-)-3-phenyl lactic acid
CAS20312-36-1

FERMENTED MARINE PRODUCTS

- Fermentation of fish was first introduced as a means of preservation, and fish sauces and pastes or condiments are staples of the diets of people from Southeast Asian, Scandinavian, and within the Innuit cultures (Fitzgerald et al., 2015).



- Rakfish – found primarily in Norway and produced from freshwater fish including trout and charr

- In general, fermented fish products are served mainly as a salty and umami condiment that assists in the consumption of large quantities of rice (Ruddle and Ishige, 2010).

FERMENTED MARINE PRODUCTS

- Hákaral – a fermented or cured shark product
- Rakfish – found primarily in Norway and produced from freshwater fish including trout and charr
- Swedish surströmming, produced from half-salted herring and traditionally consumed on thin bread known as tunnbröd
- *Lona ilish* is a salt fermented product produced from Indian shad (*Tenualosa ilisha*) a high-fat fish

FERMENTED MEAT PRODUCTS

- Meat fermentation is a low energy, acidulation method (due to lactic acid production, low water activity, salt and drying) that results in preservation and distinctive properties that include colour, microbiological safety, palatability, tenderness and other desirable attributes

- Acidulation generally results from wild microorganisms/cultures which can lower pH. These microbial strains generally belong to the LAB.
- Spontaneously fermented meat products have a long tradition of production in certain regions of the world.
- The two major European producing and consuming countries for fermented meat products include Germany and France.

FERMENTED MEAT PRODUCTS

Traditional products

- Italian salami, Spanish salchichon and chorizo, Icelandic Slátur (blood sausage), Irish pig-blood derived black pudding (blood sausage), beef sticks, pepperoni and others including Bosnian sudžuk -a dry fermented beef sausage produced in a rural household near the town of Visoko in central Bosnia and Herzegovina

Types of fermented meat products

FERMENTED PRODUCTS AND CORRESPONDING MICROBES

Fermented Product	Fermentation type	Reaction products	Principal starter/non-starter microorganisms
Kimchi	Heterofermentative lactococci, and homofermentative lactobacilli	Lactate, carbon dioxide, ethanol, and acetate, mannitol	*Leuconostoc mesenteroides, Lactobacillus sake, Lactobacillus brevis, Streptococcus* spp., *Pediococcus* spp., *Weissella* spp., *Leuconostoc citreum* and *Lactobacillus plantarum*
Beer	Alcoholic fermentation	Carbon dioxide, ethanol, dextrin	*Saccharomyces cerevisiae, S. bayanus, S. pastorianus, S. paradoxus, Candida tropicalis*
Wine	Alcoholic, malolactic	Ethanol, malic acid	*Oenococcus oeni , Lactobacillus plantarum*
Sourdough	Alcoholic, Heterofermentation	Ethanol and ethyl acetate, 2-methyl-propanol and 2/3-methyl-1-butanol, d/l-lactic and acetic acids	*Bifidobacterium pseudocatenulatum, Lactobacillus sanfrancisco, Saccharomyces cerevisiae, Lactobacillus pontis, Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus paralimentarius, Lactobacillus rossiae, Lactobacillus sanfranciscensis, Lactobacillus amylovorus, , Lactobacillus fermentum, Lactobacillus reuteri*
Cheese	Homolactic + Heterolactic	Lactic acid, propionic acid, acetic acid,	*Lactococcus lactis, Streptococcus thermophilus, Debaryomyces hansenii, Geotrichum candidum Penicillium camemberti, Arthrobacter arilaitensis, Brevibacterium aurantiacum, Brevibacterium linen, Corynebacterium casei Halomonas* spp., *Hafnia alvei, Leuconostoc* sp., *Psychrobacter sp.,Kluyveromyces marxianus*
Kefir	Heterolactic	Alcohol, lactic acid	*Lactobacillus kefiranofaciens ssp. kefirgranum, Lactobacillus parakefiri, Lactobacillus kefiri, Kluyveromyces marxianus, Kazachstania exigua, Rhodosporidium kratochvilovae, Streptococcaceae* (primarily *Lactococcus* spp.), *Gluconobacter japonicus* and *Lactobacillus uvarum, Lactobacillus helveticus, Acetobacter syzygii, Lactobacillus satsumensis*
Sauerkraut	Heterolactic + Homolactic	Acetic acid , Lactic acid	*Leuconostoc mesenteroides, Lactobacillus plantarum, Pediococcus acidilactici*
Fermented		Lactic acid	*Enterococcus faecium, Lactobacillus*

Fermented vegetables

- The primary retail fermented vegetable products produced in the United States and Europe are cucumber, pickles, olives, and sauerkraut.
- Korean-style fermented cabbage, kimchi, is thought to have originated in the primitive pottery age from the natural fermentation of withered vegetables stored in seawater.
- Kimchi

Product name	Country	Major ingredients	Microorganisms	Usage
Sauerkraut	Germany	Cabbage, salt	*Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus plantarum*	Salad, side dish
Kimchi	Korea	Korean cabbage, radish, various vegetables, salt	*L. mesenteroides*, *Lb. brevis*, *Lb. plantarum*	Salad, side dish
Dhamuoi	Vietnam	Cabbage, various vegetables	*L. mesenteroides*, *Lb. plantarum*	Salad, side dish
Dakguadong	Thailand	Mustard leaf, salt	*Lb. plantarum*	Salad, side dish
Burong mustasa	Philippines	Mustard	*Lb. brevis*, *Pediococcus cerevisiae*	Salad, side dish

BIOACTIVITIES

- Arihara first described the use of probiotic bacteria in fermented meat products.
- Probiotics including bifidobacteria and LAB can provide organoleptic and nutritional advantages as well as health benefits to the fermented meat products.
- Several meat products containing probiotics with claims for health benefits have been commercialized.
- Salami containing three intestinal LAB (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium* spp.) was produced by a German company in 1998 and a meat spread containing an intestinal LAB (*Lactobacillus rhamnosus* FERM P-15120) was produced by a Japanese company.
- Fermented sausages are suitable for the incorporation of probiotic bacteria since mild or no heat treatment is usually required by dry fermented meat products, thus providing the suitable conditions required for the survival of probiotics.

Fermented Functional Foods – Probiotics and Prebiotics

- Functional foods are foods that claim to promote human health over and above the provision of basic nutrition.
- First proposed in Japan – FOSHU**
- **Probiotics** are defined as "living microorganisms that when consumed in adequate numbers confer a health benefit to the consumer.
- There is ongoing controversy as to whether cultures must be viable for efficacy in all cases.
- **Prebiotics** are non digestible food ingredients that beneficially affect the host by stimulating growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health.
- **Synbiotic** is a term used when referring to a product that uses both prebiotics and probiotics in combination.
- Microorganisms may also indirectly impart health benefits through the production of bioactive metabolites referred to as **biogenics** in fermentation.

**** Link to later lecture regarding EFSA, FDA and reguation in China and Japan for Functional Foods and health claims**

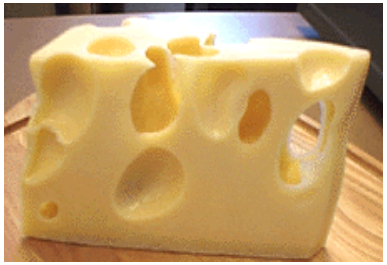
History of Functional foods

- As far back as 76 AD the Roman historian Plinio advocated the use of fermented milks for treating gastrointestinal infections.
- Tissier (1900s) proposed that Bifidobacteria could be effective in preventing infections in infants as they were predominant in breast milk.
- However, Metchnikoff, developed the probiotic concept – he observed that the consumption of fermented milks could reverse putrefactive effects of the gut microflora.

Fermented foods and live probiotic cultures

- Certain microbial culture consumption exerts health benefits in the GI tract, as well as the respiratory and urogenital tracts.
- Effective in treatment/prevention of conditions including irritable bowel syndrome, inflammatory bowel disease, depressed immune function, cancer and genitourinary tract infections.
- The effective dose is unknown but high numbers of bacteria that are viable are recommended for efficacy of probiotic foods.
- In Japan, the Fermented Milks and Lactic Acid Bacteria Beverage association demand a minimum number of 10^7 colony forming units (CFU/ml) of probiotic microorganisms at the end of shelf-life.

Italian varieties



Emmental

CHEESE

Challenges in development of fermented functional foods?

- Probiotic cultures have to be suitable for large-scale industrial production and processing
- They must maintain good viability during storage
- Problem, as probiotic cultures are from the gut originally and are sensitive to stresses including oxygen, heat and acid exposure.
- They may perform poorly in food environments
- Yoghurts and fermented milks are the most common probiotic foods available as well as cheese

Probiotic powdered ingredient development

- Dried preparation of live probiotic cultures are most convenient for long-term preservation and use in functional food applications.
- Freeze-drying is frequently used
- However, freezing and drying can lead to cell injury and decreased viability in some instances
- It is gentler than spray-drying but is more expensive and time-consuming
- Strain selection is important in terms of drying and storage and some probiotic strains exhibit better survival rates than others.

Probiotic powdered ingredient development

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Fermented foods for delivery of probiotics

- Cheese offers an attractive food-based delivery vehicle for probiotic cultures and biogenic substances such as conjugated linoleic acid (CLA) and bioactive peptides.
- Compared to many other fermented foods it has a relatively high pH and fat content, a solid consistency and a higher buffering capacity.
- Together these features probably afford improved protection of biological activity during manufacture, storage and gastrointestinal transit.

Bioactivities produced by hydrolysates and fermentates of dairy products

Hydrolysed or fermented product	Observed bioactivity
Sour milk	Phosphopeptides
Sour milk	Antihypertensive properties
Yoghurt	ACE-inhibitory activity
Yoghurt	Immunomodulatory
Yoghurt	Antihypertensive properties
Yoghurt	Antiamnesic
Yoghurt	Microbiocidal
Yoghurt	Antithrombotic
Quarg	ACE-inhibitory activity
Dahi	ACE-inhibitory activity
Parmesan, Reggiana cheeses	Opioid activity
Comte cheese	Phosphopeptides
Cheddar cheese	Phosphopeptides
Mozzarella, Italico cheeses	ACE-inhibitory activity
Crescenza, Gorgonzola cheeses	ACE-inhibitory activity
Edam, Emmental, 'Festivo' cheeses	ACE-inhibitory activity
Feta, Swiss, Cheddar, Edam, Camembert cheeses	ACE-inhibitory activity
Feta, Swiss, Cheddar, Edam, Camembert cheeses	Immunomodulatory
Feta, Swiss, Cheddar, Edam, Camembert cheeses	Antiamnesic
Feta, Swiss, Cheddar, Edam, Camembert cheeses	Opioid activity
Gouda cheese, Havarti cheese	ACE-inhibitory activity
Calpis® sour milk, Calpis Co. Japan	ACE-inhibitory activity, antihypertensive
Evolus® sour milk, Valio, Finland	ACE-inhibitory activity, antihypertensive
Whey Protein Hydrolysate (Biozate 1), Davisco, USA.	Antihypertensive properties
Casein hydrolysate containing the C12 peptide DMV , Holland	Antihypertensive properties

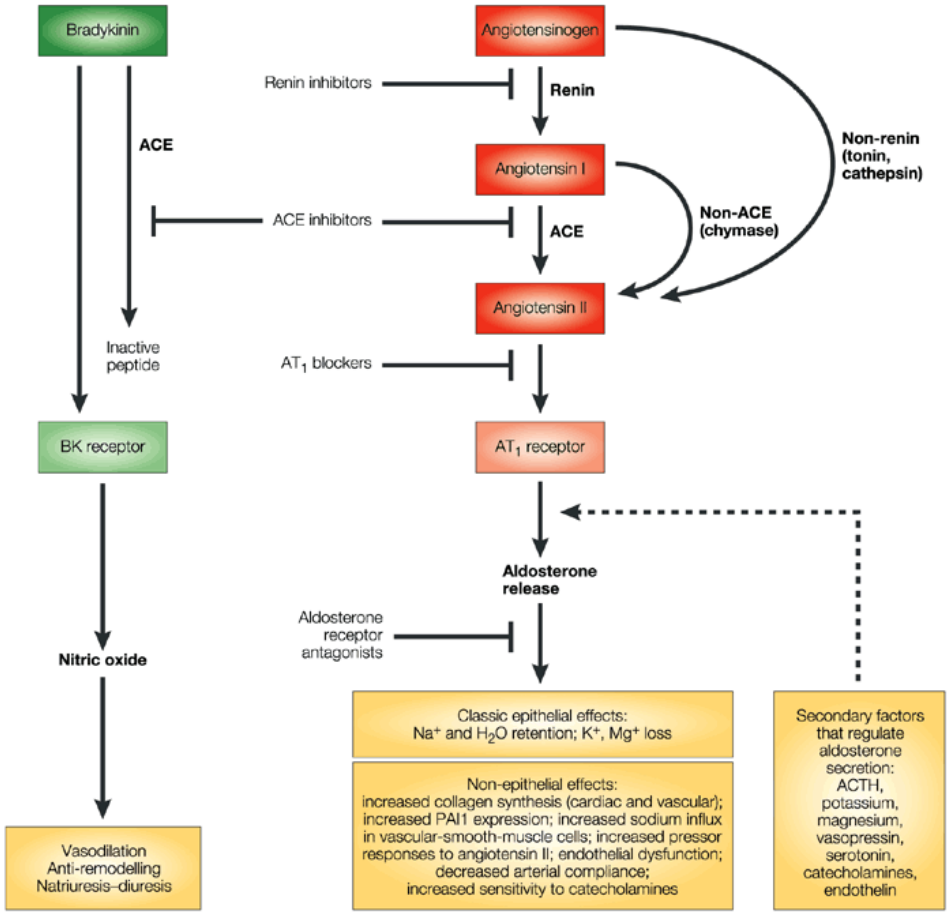
COMMON MEAT PEPTIDES

- 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)

Bioassays for heart health

ACE-I inhibition

- Angiotensin converting enzyme (ACE-1) is a metalloprotease with two isoforms.
- Its main function is to hydrolyse angiotensin I into angiotensin II and convert a vasodilator to a vasoconstrictor.
- Can inhibit ACE-I
- *In vitro* use a colorimetric assay and read absorbance at 450 nm using a positive control (Captopril)
- ACE works in the Renin-Angiotensin system, which is one of the mechanisms of blood pressure control, to convert Angiotensin I to the vasopressor Angiotensin II. This enzyme also contributes to elevated blood pressure due to its role in breaking down the antihypertensive peptide Bradykinin.



ACE-I inhibition assay

- In recent years, food and supplements containing ingredients that block ACE-I have received attention for their use in preventing high blood pressure.

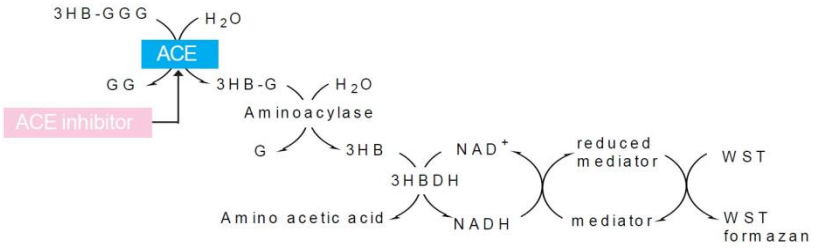
- The conventional method of measuring ACE-I inhibition employs the synthetic substrate Hippuryl-His-Leu. Hippuric acid from the synthetic substrate is extracted with ethyl acetate, condensed, re-dissolved, and then read at an absorbance of 228 nm. This method is cumbersome and measurement is subjected to error due to residual ethyl acetate.

- Kit enzymatically detects 3-Hydroxybutyric acid (3HB), which is made from 3-Hydryoxybutyryl-Gly-Gly-Gly (3HB-GGG). 96 well format, it is possible to test multiple samples at one time

- Spectrophotometric method (Abs 450 nm)

- % inhibition = (Abs. Blank 1- Abs. Blank2)/(Abs. Blank 1- Abs. sample) X 100

Principle of the assay system to determine ACE activity or inhibition activity



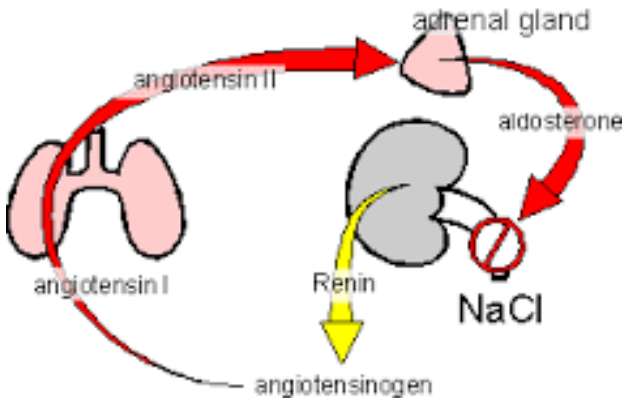
Renin inhibition assay

- Renin is an aspartyl protease of approximately 40 kDa that is released in active form from renal juxtaglomerular cells in response to sodium depletion, decreased blood volume and blood pressure, and b-adrenergic stimulation.

- Renin catalyzes the initial and rate limiting step in the renin-angiotensin system (RAS) pathway, converting angiotensinogen into angiotensin I.
- Angiotensin Converting Enzyme (ACE) subsequently converts angiotensin I to angiotensin II, which is a potent vasoconstrictor.
- A convenient assay in a 96-well format.

- The assay utilizes a renin-based synthetic peptide substrate which incorporates the fluorophore EDANS at one end and an EDAN-quenching molecule (Dabcyl) at the other end.

- After cleavage by renin, the peptide-EDANS product is released yielding bright fluorescence which can be easily analyzed using excitation wavelengths of 335-345 nm and emission wavelengths of 485-510 nm.
- Fluorometric method



Bioactive peptides from Macroalgae: Heart Health

Palmaria palmata (Linneaus) Weber & Mohr

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

REVIEW

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Heart Health Peptides from Macroalgae and Their Potential Use in Functional Foods

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[§]Department of Pharmaceutical and Biological Chemistry, School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, United Kingdom

ABSTRACT: Macroalgae have for centuries been consumed whole among the East Asian populations of China, Korea, and Japan.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Isolation and Characterization of Bioactive Pro-Peptides with *in Vitro* Renin Inhibitory Activities from the Macroalga *Palmaria palmata*

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Potential of a Renin Inhibitory Peptide from the Red Seaweed *Palmaria palmata* as a Functional Food Ingredient Following Confirmation and Characterization of a Hypotensive Effect in Spontaneously Hypertensive Rats

Ciaran Fitzgerald,*[,†] Rotimi E. Aluko,[‡] Mohammad Hossain,[†] Dilip K. Rai,[†] and Maria Hayes[†]

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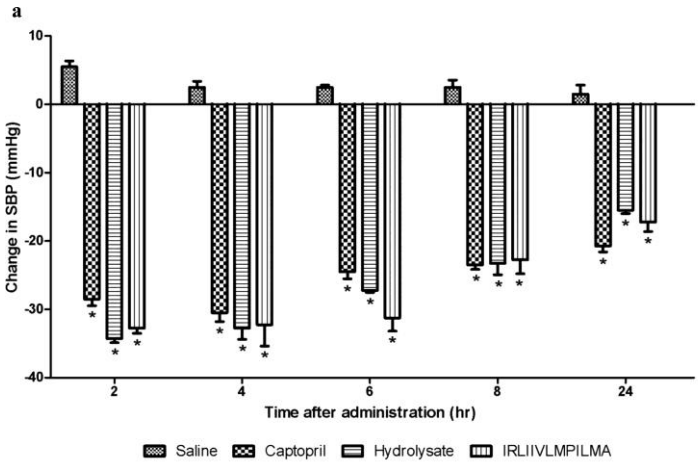
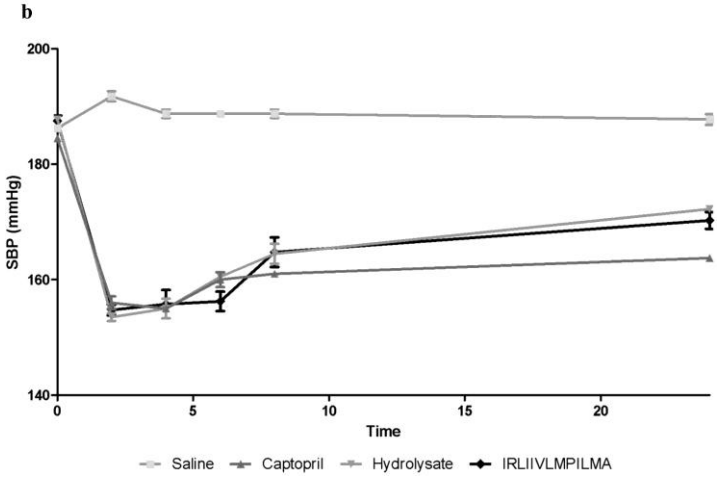
ABSTRACT: This work examined the resistance of the renin inhibitory, tridecapeptide IRLIIVLMPILMA derived previously from a *Palmaria palmata* papain hydrolysate, during gastrointestinal (GI) transit. Following simulated GI digestion, breakdown products were identified using mass spectrometry analysis and the known renin and angiotensin I converting enzyme inhibitory dipeptide IR was identified. In vivo animal studies using spontaneously hypertensive rats (SHRs) were used to confirm the antihypertensive effects of both the tridecapeptide IRLIIVLMPILMA and the seaweed protein hydrolysate from which this peptide was isolated. After 24 h, the SHR group fed the *P. palmata* protein hydrolysate recorded a drop of 34 mm Hg in systolic blood pressure (SBP) from 187 (±0.25) to 153 (± 0.64) mm Hg SBP, while the group fed the tridecapeptide IRLIIVLMPLIMA presented a drop of 33 mm Hg in blood pressure from 187 (±0.95) to 154 (±0.94) mm Hg SBP compared to the SBP recorded at time zero. The results of this study indicate that the seaweed protein derived hydrolysate has potential for use as antihypertensive agents and that the tridecapeptide is cleaved and activated to the dipeptide IR when it travels through the GI tract. Both the hydrolysate and peptide reduced SHR blood pressure when administered orally over a 24 h period.

KEYWORDS: *in vitro simulated gastrointestinal digestion, spontaneously hypertensive rats, renin inhibitory peptides, red macroalga*

Formed part of a Ph.D thesis carried out by Ciarán Fitzgerald with UCL London and Teagasc

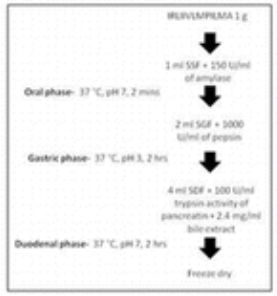
Renin inhibitory hydrolysate and peptide : *in vivo*





Palmaria palmata

Spontaneously Hypertensive Rats



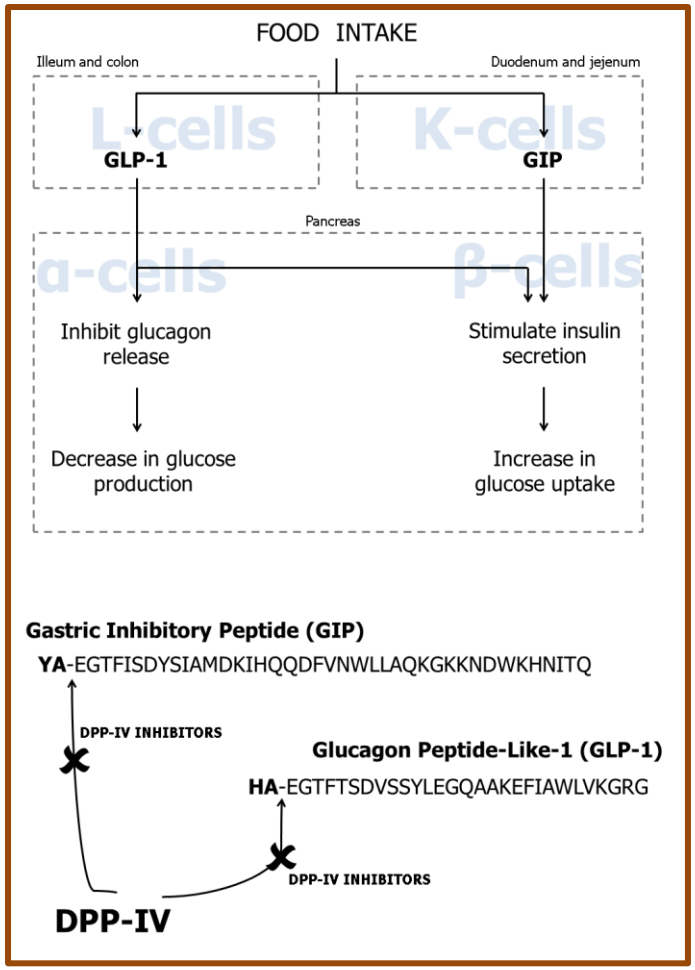
***In vitro* simulated gastric digestion**

Hypotensive effects

Fitzgerald, C., Aluko, R. E., Hossain, M., Rai, D. P., Hayes, M. (2014), Potential of a renin inhibitory peptide from the red seaweed *Palmaria palmata* as a functional food ingredient following confirmation And characterisation of a hypotensive effect in Spontaneously Hypertensive Rats. Journal of Agricultural and Food Chemistry, 62, 8352-8356.

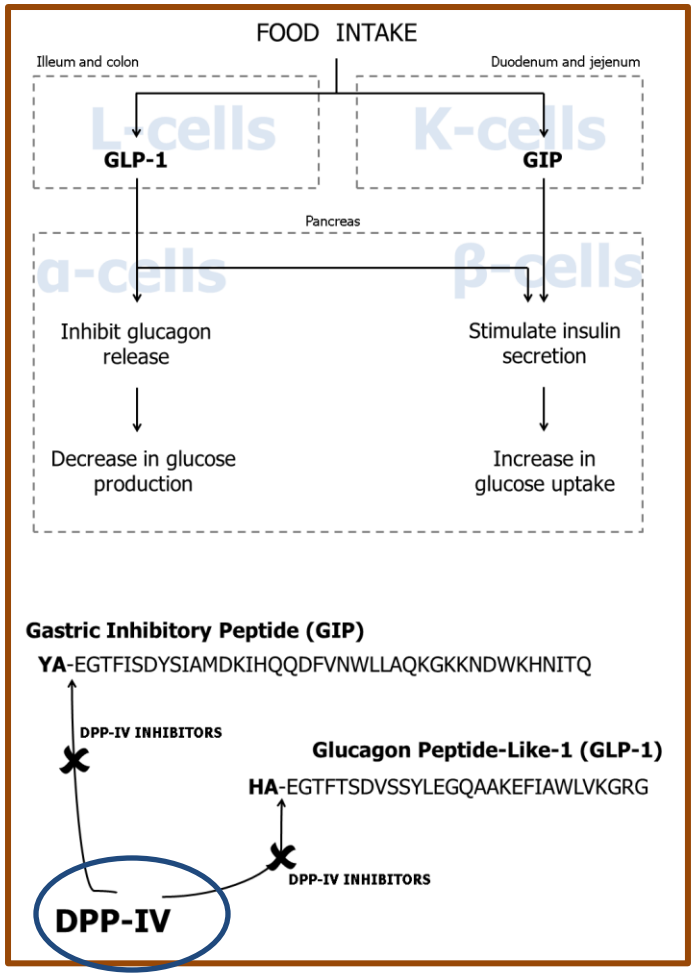
DPP-IV inhibition assay (Diabetes, hearth health)

- Dipeptidyl peptidase IV (DPP-IV) known also as CD26, adenosine deaminase complexing protein 2.
- Membrane bound glycoprotein.
- Present on the surface of most cells.
- Immune regulation, signal transduction and apoptosis.
- Soluble form found in human serum and seminal fluid
- Cleaves X-proline and X-alanine dipeptide at the N-terminus of polypeptides

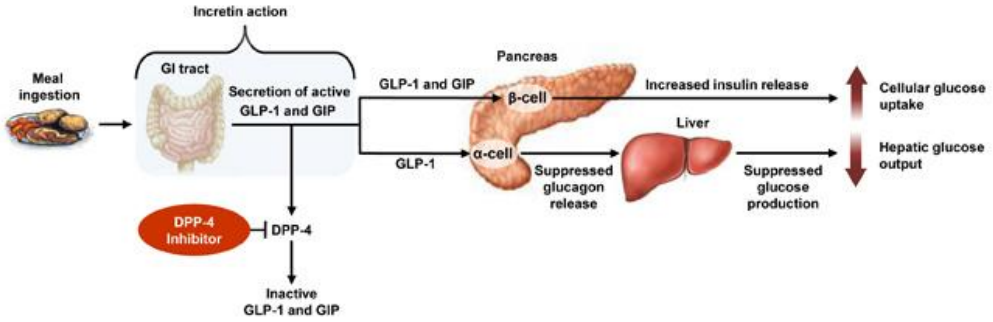


DPP-IV inhibition assay (Diabetes, hearth health)

- Inhibitors of DPP-IV considered oral anti-diabetic agents
- Promote glucose homeostasis by inhibiting DPP-IV
- DPP-IV is the enzyme responsible for degrading two key gluco-regulatory hormones GIP and GLP-1



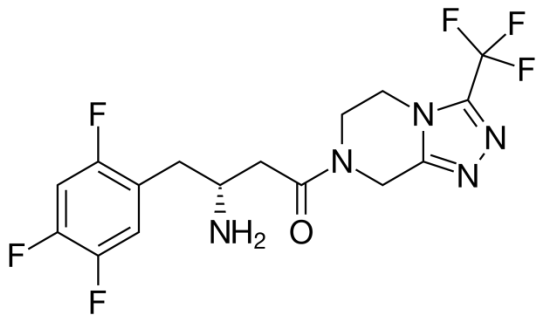
DPP-IV inhibition assay (Diabetes, hearth health)



- Glucose dependent insulinotropic polypeptide (GIP).
- Glucagon-like peptide 1 (GLP-1).
- GLP-1 extends the action of insulin while suppressing the release of glucagon.
- DPP-IV also involved in tumour biology.
- Useful marker for various cancers.
- Level increased or decrease in subjects in serum or on the cell surface.

DPP-IV inhibition assay

- Carried out using the fluorogenic substrate Gly-Pro-Aminomethylcoumarin (AMC).
- Cleavage of peptide bond by DPP releases the free AMC group.
- Fluorescence analysed at excitation wavelength 350nm-360 nm and emission wavelength of 450-465 nm.
- 96 well plate assay format can be used.
- Positive control sitagliptin.



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This presentation reflects only
the opinion of authors and not the opinion of European Commission.

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

European Commission

NAPOMENA:

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