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

Dr. Maria Hayes, TEAGASC
‘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.
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
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.
FERMENTED PRODUCTS AND CORRESPONDING MICROBES

<table>
<thead>
<tr>
<th>Fermented Product</th>
<th>Fermentation type</th>
<th>Reaction products</th>
<th>Principal starter/non-starter microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kefir</td>
<td>Heterofermentative lactococci, and homofermentative lactobacilli</td>
<td>Lactic acid, carbon dioxide, ethanol, and acetate, mannitol</td>
<td>Leuconostoc mesenteroides, Lactobacillus sake, Lactococcus brevis, Streptococcus spp., Pediococcus spp., Weissella spp., Leuconostoc cremoris and Lactobacillus plantarum</td>
</tr>
<tr>
<td>Beer</td>
<td>Alcoholic fermentation</td>
<td>Carbon dioxide, ethanol, dextrin</td>
<td>Saccharomyces cerevisiae, S. bayanus, S. pastorianus, S. paradoxus, Candida tropicalis</td>
</tr>
<tr>
<td>Wine</td>
<td>Alcoholic, malolactic</td>
<td>Ethanol, malic acid</td>
<td>Oenococcus oeni, Lactobacillus plantarum</td>
</tr>
<tr>
<td>Sourdough</td>
<td>Alcoholic, Heterofermentation</td>
<td>Ethanol and ethyl acetate, 2-methylpropanol and 2/3-methyl-1-butanol, dl-lactic and acetic acids</td>
<td>Bifidobacterium pseudocatenulatum, Lactobacillus sanfrancisco, Saccharomyces cerevisiae, Lactobacillus plantarum, Lactobacillus paralimentarius, Lactobacillus rossiae, Lactobacillus sanfranciscensis, Lactobacillus amylovorus, Lactobacillus fermentum, Lactobacillus reuteri</td>
</tr>
<tr>
<td>Cheese</td>
<td>Homolactic - Heterolactic</td>
<td>Lactic acid, propionic acid, acetic acid</td>
<td>Lactococcus lactis, Streptococcus thermophilus, Debrypnococcus hansenii, Geotrichum candidum Penicillium camemberti, Akkermansia muciniphila, Brevibacterium aurantiacum, Brevibacterium linens, Corynebacterium casei, Holomonas spp., Hafnia alvei, Leuconostoc spp., Psychrobacter spp., Klebsiella oxytoca</td>
</tr>
<tr>
<td>Kefir</td>
<td>Heterolactic</td>
<td>Alcohol, lactic acid</td>
<td>Lactobacillus kefir, Lactobacillus parahaemolyticus, Lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus rhamnosus, Lactobacillus delbrueckii, Lactobacillus paracasei</td>
</tr>
<tr>
<td>Sauerkraut</td>
<td>Heterolactic</td>
<td>Acetic acid, Lactic acid</td>
<td>Leuconostoc mesenteroides, Lactobacillus plantarum, Pediococcus acidilactici</td>
</tr>
<tr>
<td>Fermented</td>
<td>Lactic acid</td>
<td>Enterococcus faecium, Lactobacillus</td>
<td></td>
</tr>
</tbody>
</table>

Fermented foods, FINS, Novi Sad, Serbia March 21st and 22nd 2016
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

<table>
<thead>
<tr>
<th>Product name</th>
<th>Country</th>
<th>Major ingredients</th>
<th>Microorganisms</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sauerkraut</td>
<td>Germany</td>
<td>Cabbage, salt</td>
<td><em>Leuconostoc mesenteroides</em>, <em>Lactobacillus brevis</em>, <em>Lactobacillus plantarum</em></td>
<td>Salad, side dish</td>
</tr>
<tr>
<td>Kimchi</td>
<td>Korea</td>
<td>Korean cabbage, radish, various vegetables, salt</td>
<td><em>L. mesenteroides</em>, <em>Lb. brevis</em>, <em>Lb. plantarum</em></td>
<td>Salad, side dish</td>
</tr>
<tr>
<td>Dhamuoi</td>
<td>Vietnam</td>
<td>Cabbage, various vegetables</td>
<td><em>L. mesenteroides</em>, <em>Lb. plantarum</em></td>
<td>Salad, side dish</td>
</tr>
<tr>
<td>Dakguadong</td>
<td>Thailand</td>
<td>Mustard leaf, salt</td>
<td><em>Lb. plantarum</em></td>
<td>Salad, side dish</td>
</tr>
<tr>
<td>Burong mustasa</td>
<td>Philippines</td>
<td>Mustard</td>
<td><em>Lb. brevis, Pediococcus cerevisiae</em></td>
<td>Salad, side dish</td>
</tr>
</tbody>
</table>
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 regulation 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.
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.
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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

<table>
<thead>
<tr>
<th>Hydrolysed or fermented product</th>
<th>Observed bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sour milk</td>
<td>Phosphopeptides</td>
</tr>
<tr>
<td>Yoghurt</td>
<td>Anti hypertensive properties</td>
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<tr>
<td>Yoghurt</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Yoghurt</td>
<td>Immunomodulatory</td>
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<tr>
<td>Yoghurt</td>
<td>Antihypertensive properties</td>
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<tr>
<td>Yoghurt</td>
<td>Antiinflammatory</td>
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<tr>
<td>Yoghurt</td>
<td>Microbiocidal</td>
</tr>
<tr>
<td>Dahi</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Parmesan, Reggiana cheeses</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Comte cheese</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Cheddar cheese</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Mozzarella, Italcio cheeses</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Crescenza, Gorgonzola cheeses</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Edam, Emmental, 'Festivo' cheeses</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Feta, Swiss, Cheddar, Edam, Camembert cheeses</td>
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<tr>
<td>Gouda cheese, Havarti cheese</td>
<td>ACE-inhibitory activity</td>
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<tr>
<td>Calpis® sour milk, Calpis Co. Japan</td>
<td>ACE-inhibitory activity</td>
</tr>
<tr>
<td>Evolus® sour milk, Valio, Finland</td>
<td>ACE-inhibitory activity</td>
</tr>
<tr>
<td>Whey Protein Hydrolysate (Biozate 1), Davisco, USA.</td>
<td>ACE-inhibitory activity, anti hypertensive</td>
</tr>
<tr>
<td>Casein hydrolysate containing the C12 peptide DMV, Holland</td>
<td>ACE-inhibitory activity, anti hypertensive</td>
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<td>Antiinflammatory</td>
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<td>Antiinflammatory</td>
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</table>
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)
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-Hydroxybutyryl-Gly-Gly-Gly (3HB-GGG). 96 well format, it is possible to test multiple samples at one time.

- Spectrophotometric method (Abs 450 nm)

- % inhibition = \( \frac{(\text{Abs. Blank 1} - \text{Abs. Blank2})}{(\text{Abs. Blank 1} - \text{Abs. sample})} \times 100 \)
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 β-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

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

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**Palmaria palmata** (Linneaus)
Weber & Mohr
Renin inhibitory hydrolysate and peptide: in vivo

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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