The Production of Bioactive Peptides from Peanuts
Composition of peanut kernel

- Fat
- Protein
- Carbohydrate
- Water
- Other
Protein Structure
Peptide Hydrolysis

The Cleavage Reaction

$$\text{H}_2\text{O} + \text{HN-} (\text{R}_A)_x \text{C} - \text{NH} - (\text{R}_B)_y \text{C} - \text{O}^-$$

$$\text{HN-} (\text{R}_A)_x \text{C} - \text{O}^- + \text{HN-} (\text{R}_B)_y \text{C} - \text{O}^-$$

Peptide 1

Peptide 2

Polypeptide

Sciissile Bond
Peptides
Amino Acid
Essential Amino Acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Peanut (mg/g)</th>
<th>Cows Milk (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Threonine</td>
<td>31</td>
<td>44</td>
</tr>
<tr>
<td>Phenylalanine and tyrosine</td>
<td>31</td>
<td>99</td>
</tr>
<tr>
<td>Methionine and cystine</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>Lysine</td>
<td>41</td>
<td>78</td>
</tr>
<tr>
<td>Leucine</td>
<td>70</td>
<td>95</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>47</td>
<td>48</td>
</tr>
<tr>
<td>Histidine</td>
<td>22</td>
<td>27</td>
</tr>
</tbody>
</table>
Peptide structure
Bioactive Peptides

• Bioactive Peptides – Those with physiological effect
• Antihypertensive (ACE inhibitor), antimicrobial, antithrombotic, antioxidant, etc.
• Various sources
  – Milk
  – Plant sources including legumes (chickpea)
• None reported from peanut
Stress
Stress
Up Regulation of BP by ACE

Blood Pressure

Angiotensin I

Angiotensin II

ACE*

* Angiotensin I Converting Enzyme
Down Regulation of BP by ACE Inhibitors

Blood Pressure

Angiotensin I  Angiotensin II

ACE*  ACE INHIBITORS

* Angiotensin I Converting Enzyme
OBJECTIVES

• To identify and isolate hypotensive peptides from proteolytic peanut digests
• To detect and evaluate bactericidal peptides from proteolytic peanut digests
Methodology

• Peanut Flour
  – Digested with Alcalase & Pepsin-Pancreatin at different times using modified pH Stat
  – Degree of hydrolysis using TNBS
• Lyophilized Digest
• ACE Activity
• Antimicrobial
  – *E. coli* & *L. monocytogenes*
• SDS PAGE
• HPLC
METHODOLOGY Con’t

• HPLC on hydrolysates with highest DH values, from both raw and roasted peanuts digested with either enzyme
  – Fractions collected and ACE inhibition determined

• Antimicrobial activity also determined using hydrolysates with the highest DH values
Isolation of Biopeptides

Crude Hydrolysate
METHODOLOGY Con’t

• Proteolytic digests tested against
  – Angiotensin Converting Enzyme (ACE) for antihypertensive activity
  – *E.coli* O157:H7 and *L. monocytogenes* for antimicrobial activity
RESULTS

• Statistical analyses reveal
  – Degree of Hydrolysis
    • Enzyme type and duration of hydrolysis significantly affect DH.
      – Alcalase recorded higher DH values
    • Peanut treatment has no significant effect on DH
RESULTS Con’t

– ACE Activity
  • Hydrolysis time, Enzyme type and Peanut treatment have a significant effect on ACE activity.
    – Raw peanuts recorded more hypotensive activity, and hydrolysates from Alcalase also recorded greater ACE inhibition.
Raw Peanut, Alcalase

![Graph showing specific ACE inhibition for unhydrolyzed and peptide fraction of Raw Peanut, Alcalase.](image-url)
Raw Peanut, Pepsin-Pancreatin

The graph shows the specific ACE inhibition for Unhydrolyzed and Peptide Fraction samples. The Peptide Fraction has significantly higher specific ACE inhibition compared to the Unhydrolyzed sample.
RESULTS Con’t

• Preliminary studies show that hydrolysates from Alcalase have more bactericidal effects on *E.coli* O157:H7 and *L. monocytogenes*
Antimicrobial Activity

- Without Peptides
- With Peptides
Eat = More peanut products

\[ E = MC^2 \]