Marine Ingredients’ developer for high health benefits

TENSIDYSS®

Blood pressure regulator
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PROCESS
UPGRADING BY-PRODUCTS

MACKEREL FLESH

CONTROLLED ENZYMATIC HYDROLYSIS

CENTRIFUGATION FILTRATION

CONCENTRATION

DRYING

GRINDING

TENSIDYSS®
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## COMPOSITION

**TENSIDYSS®** = hydrolysate derived from mackerel proteins

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamic acid</td>
<td>≥ 13.50%</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>≥ 9.00%</td>
</tr>
<tr>
<td>Lysine</td>
<td>≥ 8.50%</td>
</tr>
<tr>
<td>Leucine</td>
<td>≥ 8.00%</td>
</tr>
<tr>
<td>Alanine</td>
<td>≥ 7.20%</td>
</tr>
<tr>
<td>Glycine</td>
<td>≥ 7.00%</td>
</tr>
<tr>
<td>Proline</td>
<td>≥ 5.50%</td>
</tr>
<tr>
<td>Valine</td>
<td>≥ 5.20%</td>
</tr>
<tr>
<td>Arginine</td>
<td>≥ 4.50%</td>
</tr>
<tr>
<td>Threonine</td>
<td>≥ 4.50%</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>≥ 4.30%</td>
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<tr>
<td>Serine</td>
<td>≥ 4.00%</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>≥ 4.00%</td>
</tr>
<tr>
<td>Histidine</td>
<td>≥ 3.00%</td>
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<tr>
<td>Tyrosine</td>
<td>≥ 3.00%</td>
</tr>
<tr>
<td>Methionine</td>
<td>≥ 2.50%</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>≥ 0.80%</td>
</tr>
<tr>
<td>Cysteine</td>
<td>≥ 0.75%</td>
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</table>

*Peptides: 250-1000 Daltons*
ASSESSMENT OF ANTIHYPERTENSIVE EFFECTS
MECHANISMS OF HYPERTENSION

Renin-angiotensin system

- Angiotensinogen
- Angiotensin I
- Angiotensin II
- Renin
- Angiotensin I Conversion Enzyme – Bradykine (ACE)
- Vasoconstriction
- Blood Pressure
REGULATION OF HYPERTENSION

Renin-angiotensin system

Angiotensinogen → Angiotensin I → Angiotensin II → Vasoconstriction → Blood Pressure

Renin

ACE +

ACE inhibitor

Bradykine (vasodilator) +
ACE inhibitors

- prevent the conversion of angiotensine I to angiotensine II.

- measure of blood pressure regulators activity = IC$_{50}$
  (concentration of ACE inhibitor required to inhibit 50% of the ACE activity – IC$_{50}$ of Captopril = 0.62 ng/ml)

Drug (chemical substance) : Captopril

Several natural substances
Natural products present in foods with an blood pression regulator activity

Peptides from:
- fishes (sardines, bonites, hake) by Matsui et al., Jujita et al., Byun et al.
- milk by IMPLVO et al.
- maize by Suh & Whang
Peptides with a blood pressure regulator activity

<table>
<thead>
<tr>
<th>Peptidic sequence</th>
<th>Source</th>
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<tbody>
<tr>
<td>LKP</td>
<td>Dried bonito</td>
</tr>
<tr>
<td>IKP</td>
<td>Dried bonito</td>
</tr>
<tr>
<td>IY</td>
<td>Dried bonito</td>
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<tr>
<td>LKPMN</td>
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<td>IWH</td>
<td>Dried bonito</td>
</tr>
<tr>
<td>IWHHT</td>
<td>Dried bonito</td>
</tr>
<tr>
<td>GHF</td>
<td>Bonito bowels</td>
</tr>
<tr>
<td>GYALPHA</td>
<td>Bonito bowels</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peptidic sequence</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>KVLAGM</td>
<td>Sardine</td>
</tr>
<tr>
<td>HQAAGW</td>
<td>Sardine</td>
</tr>
<tr>
<td>VKAGF</td>
<td>Sardine</td>
</tr>
<tr>
<td>LKVGVKQY</td>
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<tr>
<td>LKL</td>
<td>Sardine</td>
</tr>
<tr>
<td>YALPHA</td>
<td>Squid</td>
</tr>
</tbody>
</table>

Experience: comparison of Kasuobushi Oligopeptide (KO or LKPNM) antihypertensive activity with Captopril.

Protocol:
- 30 Spontaneously Hypertensive Rats= SHR (Systolic blood pressure > 130 mm Hg and diastolic blood pressure> 85 mm Hg)
- Oral administration of 2 different substances at different dosages (4, 8 and 15mg/kg for the peptide and 1.25, 2.5 and 5mg/kg for Captopril)
- Measurement of rats blood pressure (mmHg)
Results:

- Anti hypertensive effect of both substances in quasi simultaneous time : 2 h after administration and for 5h.

→ LKPNM has practically the same activity than Captopril.

Remark : Captopril (chemical substance) is efficient at low doses, unlike natural substances.

Experience: comparison of yellofih sole peptide (YFP) antihypertensive effect with Captopril

Protocol:
- Study on spontaneously hypertensive rats
- Oral administration of 2 hypotensive substances
- Measurement of blood pressure (mmHg)
Results:

- The activity is maintained during 9h after ingestion.
- Peptide effect very similar to Captopril.

Fig. 4. Change in systolic blood pressure of SHR by administering ACE inhibitory peptide. Single oral administration was performed with the dose of 10 mg kg\(^{-1}\) body weight, and SBP was measured 0, 1, 2, 3, 6 and 9 h after the administration. Different from control at \(*P < 0.05, **P < 0.01.\)

Protocol:
- VY Group receives sardine peptide.
- Placebo Group.
- Study during 11 weeks (measurement of blood pressure every week).
Results:

Measurement of systolic (SBP) and diastolic (DBP) blood pressure in both groups.

- Placebo Group : constant pressures
- VY Group : significant lowering just after sardine peptide ingestion
- No side effects
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SAFETY EVALUATION
Study N°4 Kawasaki et al. Antihypertensive effect and safety evaluation of vegetable drink derived from sardine protein hydrolysates on mildly hypertensive, high-normal and normal blood pressure subjects. Journal of Human Hypertension (2000) 14, 519-523

Test N°1: Peptides effects on normotensive subjects

Protocol:
- 63 subjects (mildly hypertensive and normotensive subjects) separated in 2 groups: the test group and the control group
- Test group: 195g of vegetable drink with 0.5g of sardine peptides once a day for 13 weeks.
- Control group: same protocol with a drink without sardine peptides.

Results:
→ No blood pressure changes in normotensive subjects, no adverse effects.
SAFETY EVALUATION

Test N°2: Effect of an excessive ingestion of peptides on hypertensive, mildly hypertensive and normotensive subjects. 

Protocol:

- 25 subjects separated in 2 groups: the test group and the control group
- Test group: receives 3 times the amount of test N°1, that is to say 585g of vegetable drink (1.5g of sardine peptides) once a day for 14 days.
- Control group: same protocol with a drink without sardine peptides.

Results:

- We notice a normal lowering of blood pressure in subjects with hypertension and with mild hypertension, without adverse effects.
- No lowering is noticed in normotensive subjects.
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TENSIDYSS®
CHARACTERISTICS
TENSIDYSS® CHARACTERISTICS

ACE inhibition curve of TENSIDYSS®

IC$_{50}$ = 0.25 (+/- 0.06) mg / mL

Gel filtration profile of TENSIDYSS® and ACE inhibition activity of the different fractions

MW = 1652 g/mol
MW = 378 g/mol
MW = 268 g/mol
MW = 119 g/mol

Results from an internal study - 2007
Our study permitted to develop an internal analysis method (which was the subject of a scientific publication), to verify that each batch have this activity of inhibition of angiotensin conversion enzyme.

Our study also demonstrate that several peptides have this activity in our hydrolysate (peptides of 2 to 5 amino acids = low molecular weight)

Publication:
Véronique Lahogue et al. (2009) A HPLC-UV method for the determination of angiotensin I-converting enzyme (ACE) inhibitory activity. Food Chemistry 118 (2010); 870-875
TENSIDYSS® Characteristics

• TENSIDYSS®, like other fish peptides studied before (Sardine, tuna, hake), presents also an activity of blood pressure regulation

• TENSIDYSS®, controlled at each batch guarantees this ACE activity thanks to our analysis method

-TENSIDYSS®, like for all peptides which have this activity, we can notice:

-A normal lowering of blood pressure in subjects with hypertension and with mild hypertension, without adverse effects.

-No lowering is noticed in normotensive subjects.
TENSIDYSS® can be formulated with:

**Fish oils** (modest reduction in systolic and diastolic pressures - Protective effect on the cardiovascular system.)

**Co-Q10** (antioxidant lowering blood pressure)

**Calcium from food source** could help maintain normal blood pressure and protect the cardiovascular system (link scientifically demonstrated between hypertension and poor calcium metabolism)

**Plant extracts:** Horsetail, olive leaves, hawthorn, etc…
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CONCLUSION
CONCLUSION

- NATURAL PRODUCT

- BLOOD PRESSION REGULATOR ACTIVITY WITHOUT ADVERSE EFFECTS

- DAILY RECOMMENDED DOSE: 500mg – 1g /day

Scientific bibliography available on request