國立高雄科技大學水產食品科學系
108學年度第二學期專題討論

Preparation and functionality of hydrolysate from dorsal meat of milkfish.
虱目魚背肉水解液的製備及其功能性

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Outline

- Introduction
- Aim
- Materials and methods
- Results and discussion
- Conclusion

Introduction

- Milkfish is a broad-salt osteoid fish, most of which are distributed in tropical and subtropical seas, and can survive in both fresh and salt water.

（李，2013）

- Milkfish is regarded as high-quality nutrition just like milk, so it is also called milkfish.

（漁業署，2012）

- Milkfish is thin and delicious, rich in high-quality protein, vitamin B group and EPA, DHA.

（濃委會，2014）

- Milkfish is rich in protein, which can be used for more than half of the daily consumption.
- Milkfish contains 8 amino acids essential for adults: Isoleucine, Leucine, Lysine, Methionine, Phenylalanine,Threonine, Tryptophan, Valine.

（水產試驗所，2007）

- Milkfish contains polyunsaturated fatty acids EPA and DHA. EPA can reduce the formation of blood clots. DHA can help the healthy development of the fetus.

（水產試驗所，2007）

- Proteins are biopolymers constructed from 20 kinds of amino acid molecules with specific chemical structures, and have a variety of structures and functions.

- Enzymes hydrolyze proteins into smaller peptide chains containing 2-20 amino acids, called hydrolysates. Protein hydrolysates produce a source of amino acids for various physiological functions in the human body, a process that provides essential nutrients to human and animal populations.

(N.R.A.Halim et al., 2016)

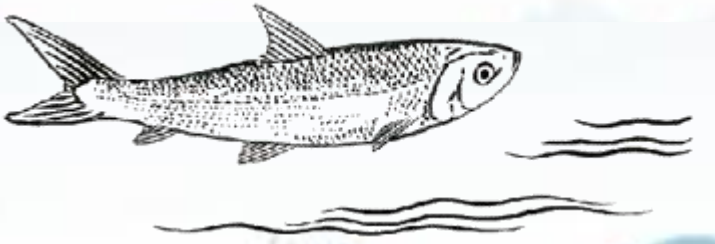
- Protein hydrolysates can be used in a variety of applications in the food industry, including replacing dairy protein supplements, stabilizers in beverages, and flavor enhancers in confectionery products.
- The benefits of hydrolyzing food proteins to make functional protein ingredients and nutritional supplements is a new technology.

- Commercially used enzymes, such as alkaline protease, pepsin, trypsin, Papain, Alaclase, flavourzyme, protamex, prolyve are often used for protein hydrolysis.

(Jianhua et al., 2019)

- Fish is rich in protein, and enzymes are used to hydrolyze fish meat. Fish protein hydrolysis (FPH) is mainly hydrolyzed from fish muscle, fish skin, head, fins, etc.

(N.R.A.Halim et al., 2016)



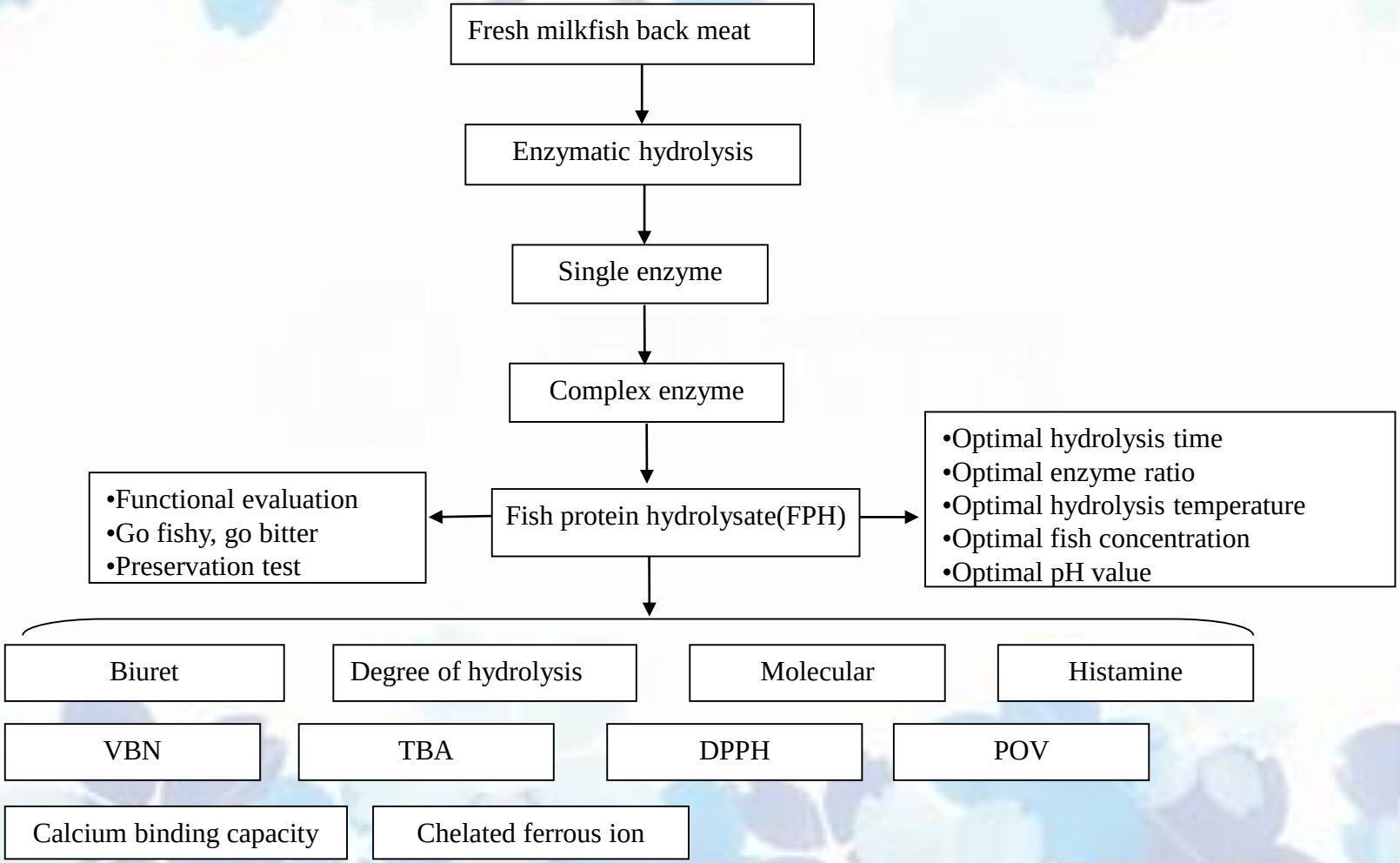
- There are three methods for protein hydrolysis:
physical methods, chemical methods (acid, alkali) and enzymatic methods, which can decompose proteins into peptides.

- Factors affecting the function of enzymes:
The type of enzyme, temperature, time, pH value, and ratio of enzyme to substrate.

Aim

- The purpose of this study was to study the effects of enzyme content, fish concentration, pH, hydrolysis temperature and incubation time on DH and yield.

Experimental architecture



Introduction

Aim

Materials and methods

Results and discussion

Conclusion

Materials and methods

Fish preparation

30 second

30min

Packing

-20℃

Preparation of protein hydrolysates

30g Fish

100mL RO water

pH adjustment with sodium carbonate

Add enzyme

10min

Cool

0.45µm filter

Biuret determination

1mL → 3mL Biuret reagent →  → 30min

→  → 540nm

OPA determination

20μL → 1mL OPA reagent →  → Dark，2min

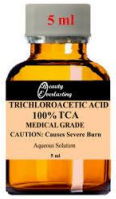
→  → 340nm

$$DH\%=(OD_{sample}-OD_{blank})/OD_{HCl}x100\%$$

VBN determination

Sample Preparation:

1g



2.2%TCA 5mL

10min

10mL

VBN:

1mL Boric acid

Inner chamber

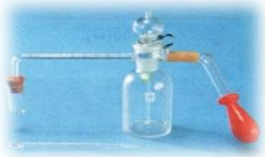
1mL Saturated potassium carbonate

Outer room

1mL sample

Outer room

37℃，90min



TBA determination

Standard preparation(1,1,3,3-Tetrathoxypropane):

319.49μg/mL

10 times dilution
31.50μg/mL

0、1、2、3、4、5mL

50mL

TBA:

3mL

3mL TBA

100℃, 30min

Cool

532nm

POV

Standard preparation:

1000ppm

Chloroform:Methanol(7:3)

Dilute 100

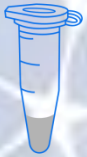
0、0.2、0.5、1、2、3、4ppm

POV:

100μL

900 μLChloroform:Methanol(7:3)

5μL $FeCl_2$



5μL KSCN

5min

500nm

DPPH determination

100μL

900μL

1mL0.2mM DPPH

Dark， 30min

517nm

$DPPH\%=(1-OD_{sample}/OD_{blank})x100\%$

Histamine determination

1mL → 200μL 2N NaOH → 300μL $NaHCO_3$ → 2mL Dansyl chloride

→  40℃，45min → 100μL NH_4OH → 30min

→  5mL Acetonitrile →  →  → 

→ 

Molecular determination

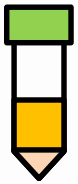
1mL →  → 

Calcium binding capacity

1mL → 5mL 15mM$CaCl_2$ → 5mL 0.06M Phosphate buffer →

→

→



24μL Supernatant →

0.488μL O-cresolphthalein → 0.488μL AMP → Dark， 15min →

570nm

Chelated ferrous ion

0.2mL → 0.74mL DDW → 20μL 2mM $FeCl_2$ → 30 sec

→ 40μL 5mM Ferro zine → 20min →

562nm

Chelating power%=(1-OD_{sample}/OD_{blank})x100%

Remove fishy smell

Activated carbon

Yeast

Introduction

Aim

Materials and methods

Results and discussion

Conclusion

Results and discussion

Table1. Yield and degree of hydrolysis (DH%) for hydrolysates from dorsal meat of milkfish treated with single enzyme.

Enzyme	Yield%	DH%
Protamex	55.44 ± 3.63^{bcd}	21.97 ± 0.38^{fg}
Flavourzyme	35.30 ± 3.64^{e}	21.97 ± 0.38^{g}
Papain	42.59 ± 8.49^{de}	15.59 ± 0.38^{fg}
Protease 826	56.94 ± 8.17^{bcd}	27.28 ± 2.63^{ef}
Alcalase	50.94 ± 2.12^{cde}	31.98 ± 1.25^{de}
Protease A Amano SD	56.52 ± 3.33^{bcd}	47.12 ± 2.38^{c}
Protease M Amano SD	49.02 ± 0.60^{cde}	35.34 ± 0.25^{de}
Protease P Amano 6SD	76.44 ± 0.60^{a}	73.69 ± 5.89^{a}
Protease N Amano	61.23 ± 6.97^{abc}	36.94 ± 1.50^{d}
Umamizyme G	70.01 ± 0.61^{ab}	58.37 ± 1.00^{b}

Table 2. Yield and degree of hydrolysis (DH%) for hydrolysates from dorsal meat of milkfish treated with two enzymes system.

Enzyme	Yield%	DH%
Protease A Amano SD +Flavourzyme	67.44±4.242	77.95±12.46
Protease A Amano SD+ Umamizyme G	80.94±0.912	91.35±0.506

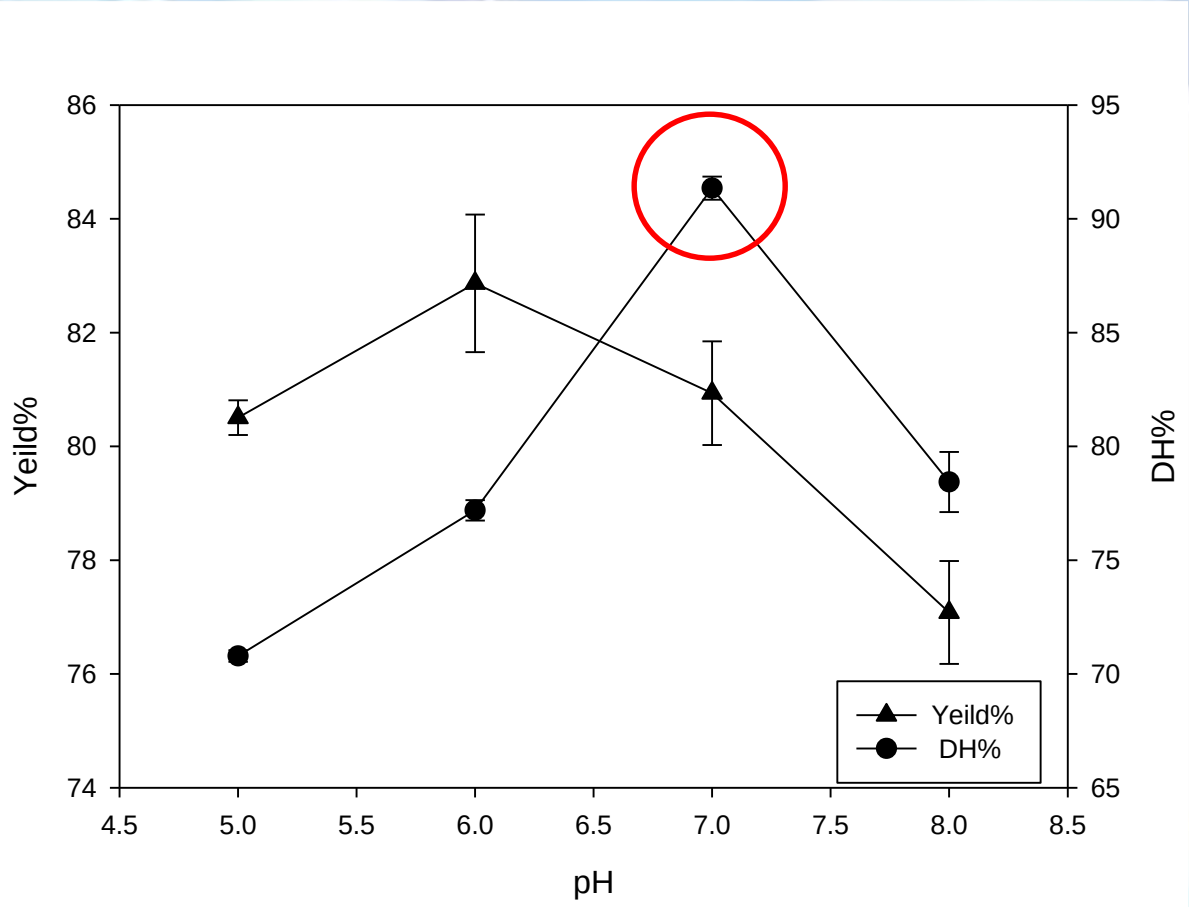


Fig1.Effect of pH on the degree of hydrolysis (DH%) and yield for hydrolysates from dorsal meat of milkfish treated with two enzymes system.

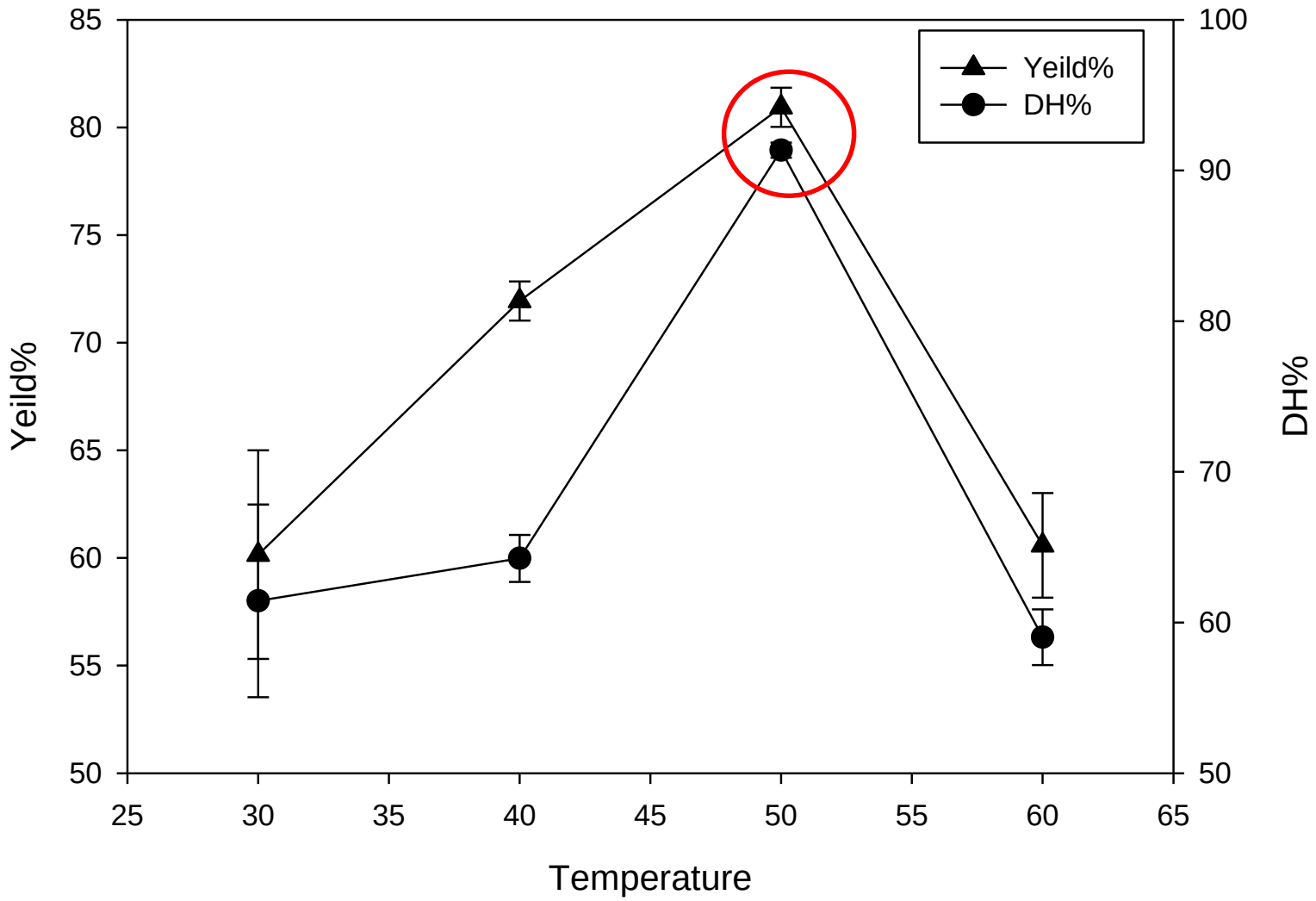


Fig2.Effects of temperature on the degree of hydrolysis (DH%) and yield for hydrolysates from dorsal meat of milkfish treated with two enzymes system.

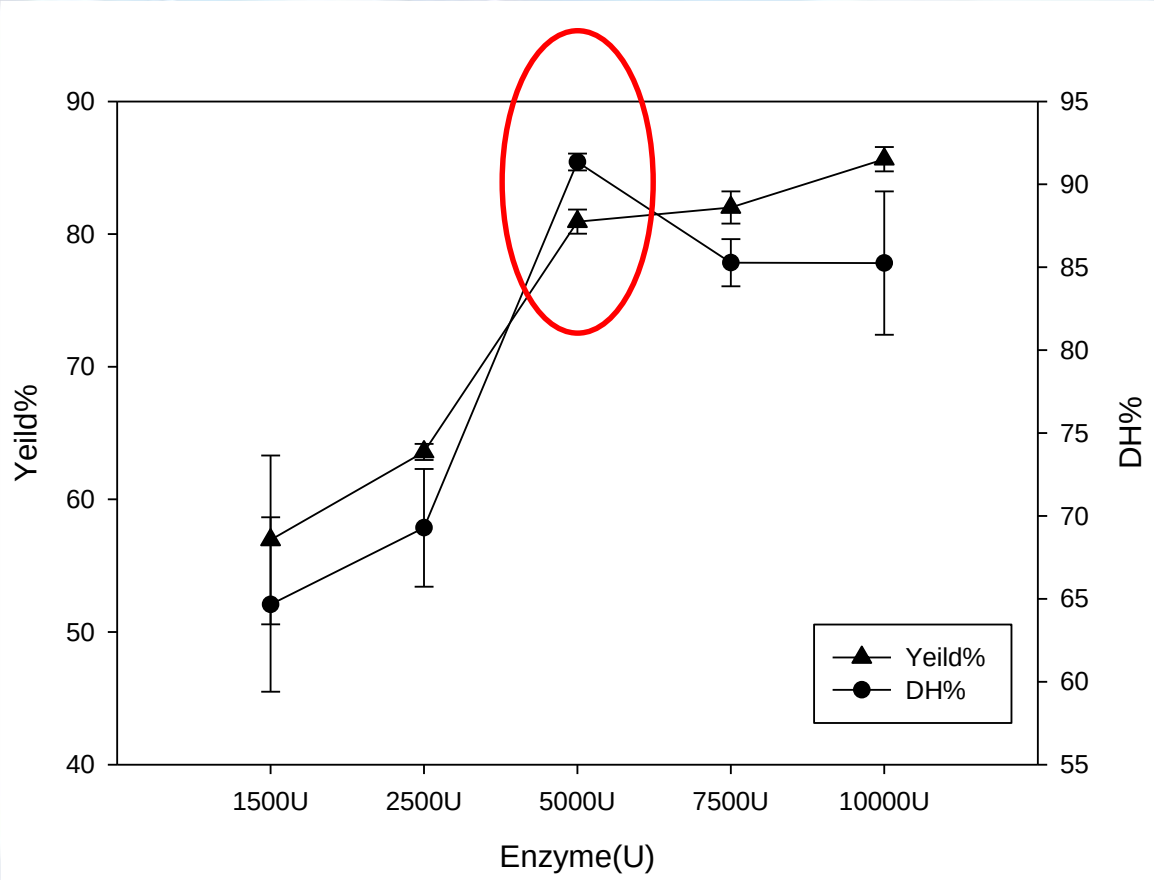


Fig3. Degree of hydrolysis (DH%) and yield for hydrolysates from dorsal meat of milkfish treated with two enzymes system at different enzyme activity. 1U=1 μg tyrosine release/ min.

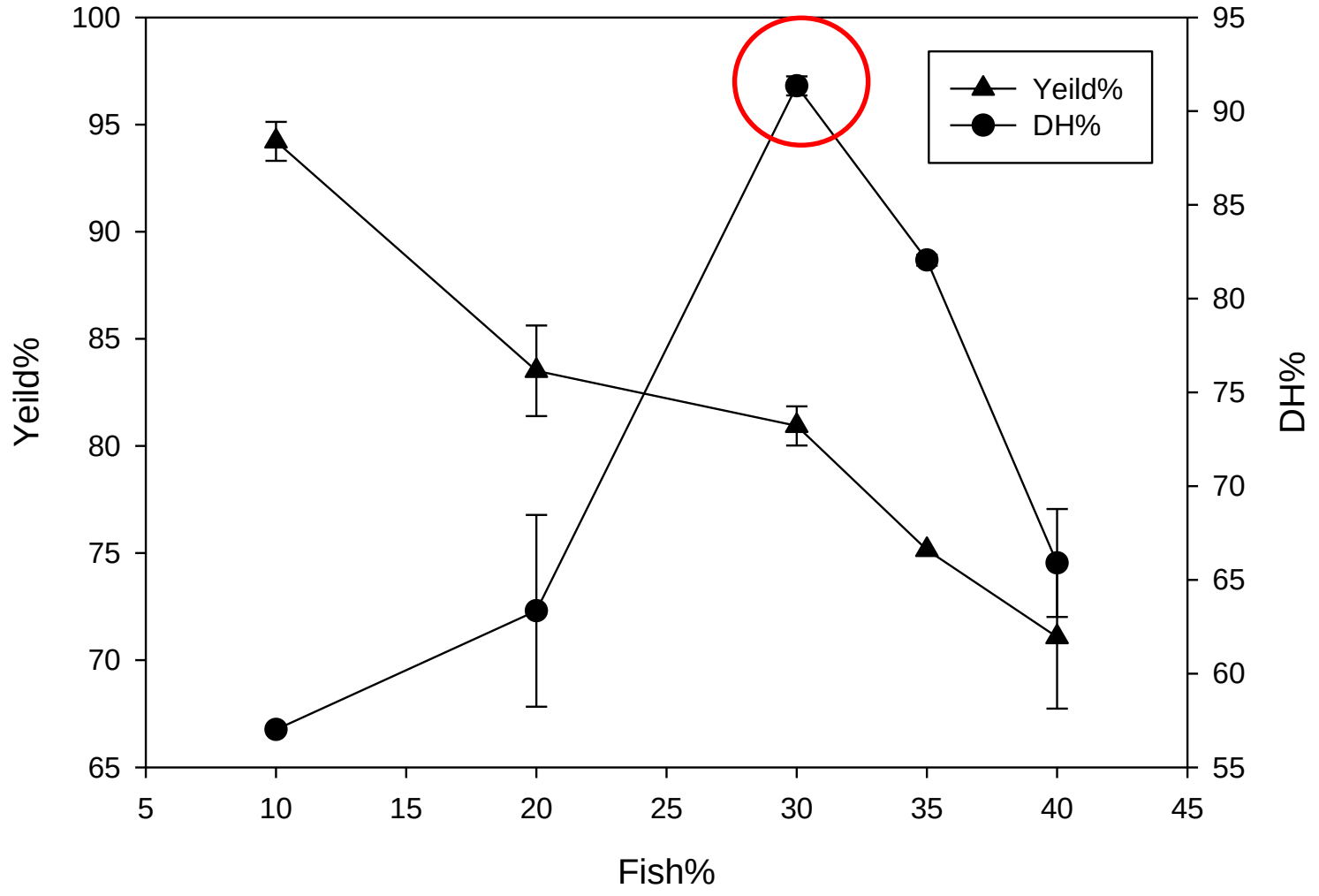


Fig4. Degree of hydrolysis (DH%) and yield for hydrolysates from dorsal meat of milkfish treated with two enzymes system at different concentration of fish meat.

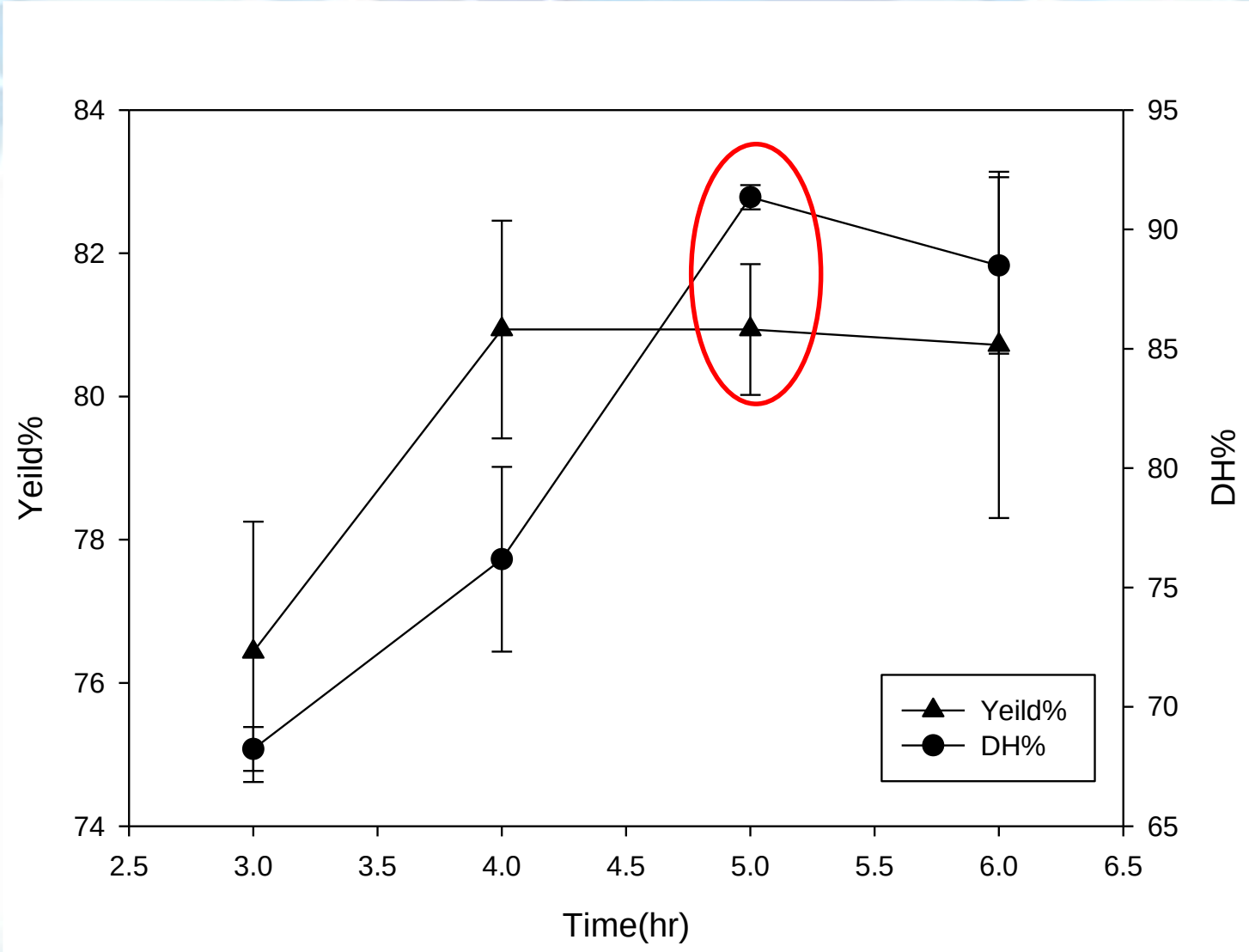


Fig5. Degree of hydrolysis (DH%) and yield for hydrolysates from dorsal meat of milkfish treated with two enzymes system at different time.

Table 3. VBN, POV, TBA and histamine of hydrolysates from dorsal meat of milkfish treated with Protease A + Umamizyme G at pH 7.0, 50 ℃ for 5 h. Concentration of fish used was 30%.

VBN(mg/100g)	POV(meq/kg)	TBA	Histamine(ppm)
2.49±0.39	0.022±0.006	0.034±0.0006	8.504

Table 4. Effect of the treatment with activated carbon and yeast on the odor of hydrolysates from dorsal meat of milkfish treated with Protease A + Umamizyme G .

	Fishy odor*	
Concentration (%)	Activated carbon	Yeast
0	+++	+++
0.3	++	-
0.5	-	-
1.0	-	-
1.5	-	-

***strong +++, common++ , weak+ , none-**

Table4. Effect of the treatment with activated carbon and yeast on the protein concentration of hydrolysates from dorsal meat of milkfish treated with Protease A + Umamizyme G .

	Protein concentration (%)*	
Concentration (%)	Activated carbon	Yeast
0	5.23 ± 0.28^{a}	5.23 ± 0.28^{a}
0.3	5.18 ± 0.42^{a}	5.54 ± 2.24^{a}
0.5	5.78 ± 0.78^{a}	5.35 ± 0.91^{a}
1.0	5.23 ± 0.14^{a}	5.25 ± 1.01^{a}
1.5	5.05 ± 0.46^{a}	5.77 ± 0.05^{a}

*Assayed with Biuret method.

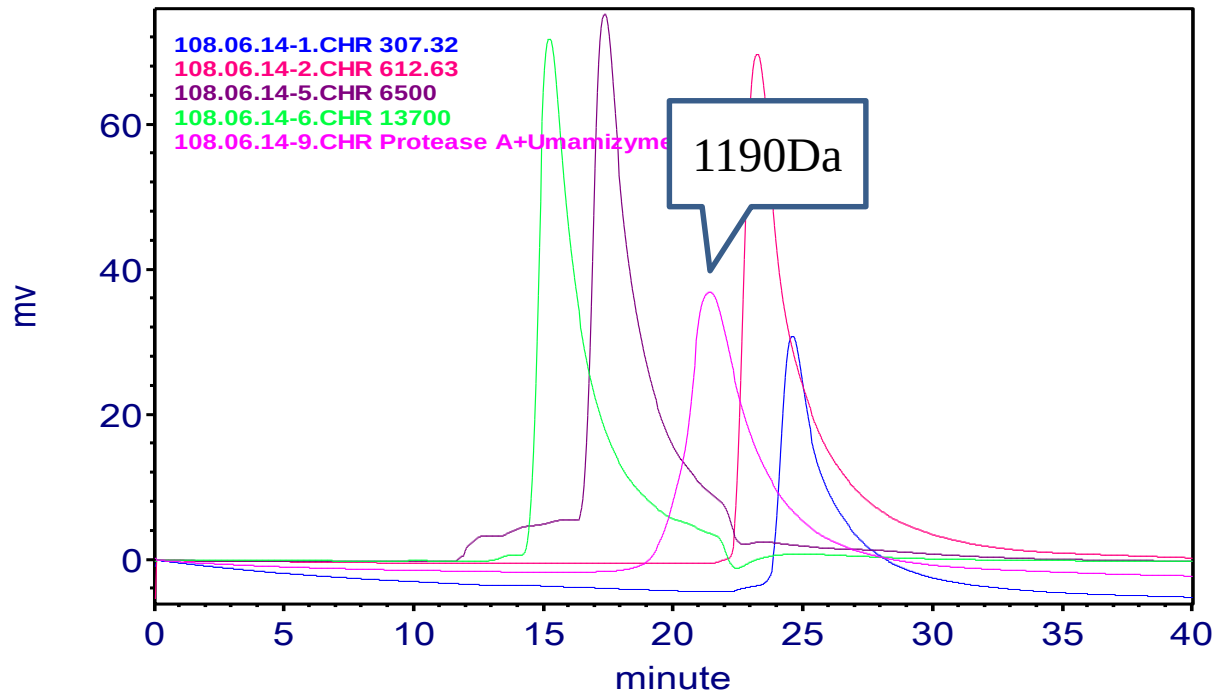
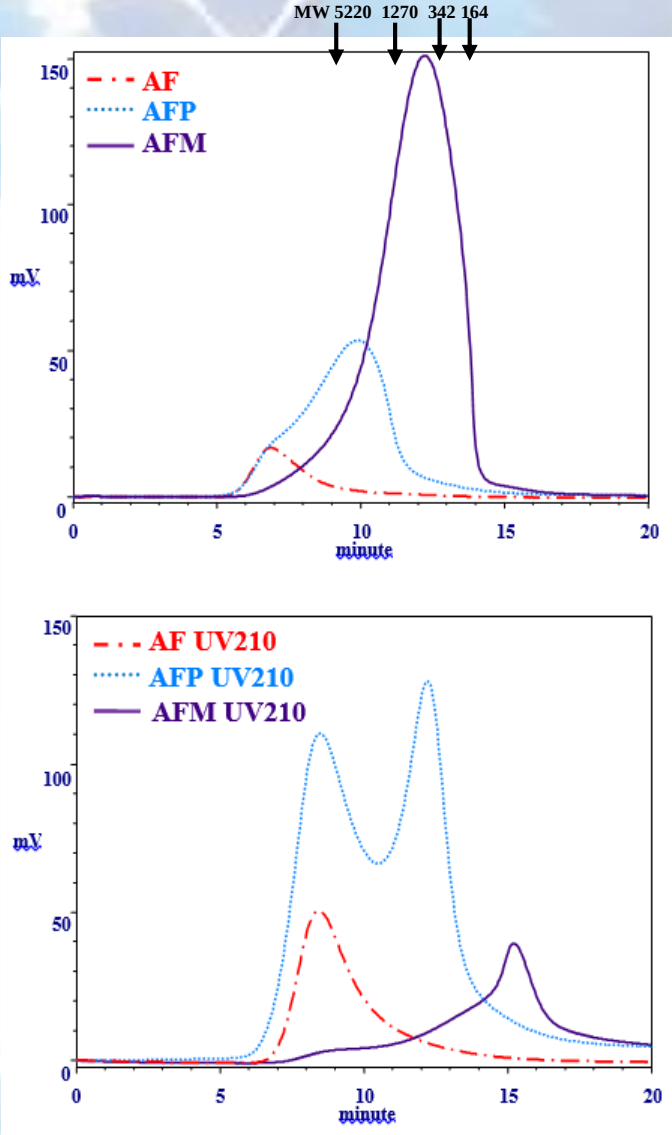


Fig6. Size exclusion HPLC chromatogram of **hydrolysates from dorsal meat of milkfish treated with Protease A + Umamizyme G .** A size exclusion column of BioSep - SEC - S2000 (Phenomenex, Torrance, CA, USA), 300 x7.8 mm(請確認) was used. The mobile phase was 45% acetonitrile (0.1% TFA) at a flow rate of 1.0 mL/min monitored at 214 nm.圖形改為標準品分子量用箭頭標示，如下頁第34張投影片


MW 5220 1270 342 164
AF
AFP
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mV
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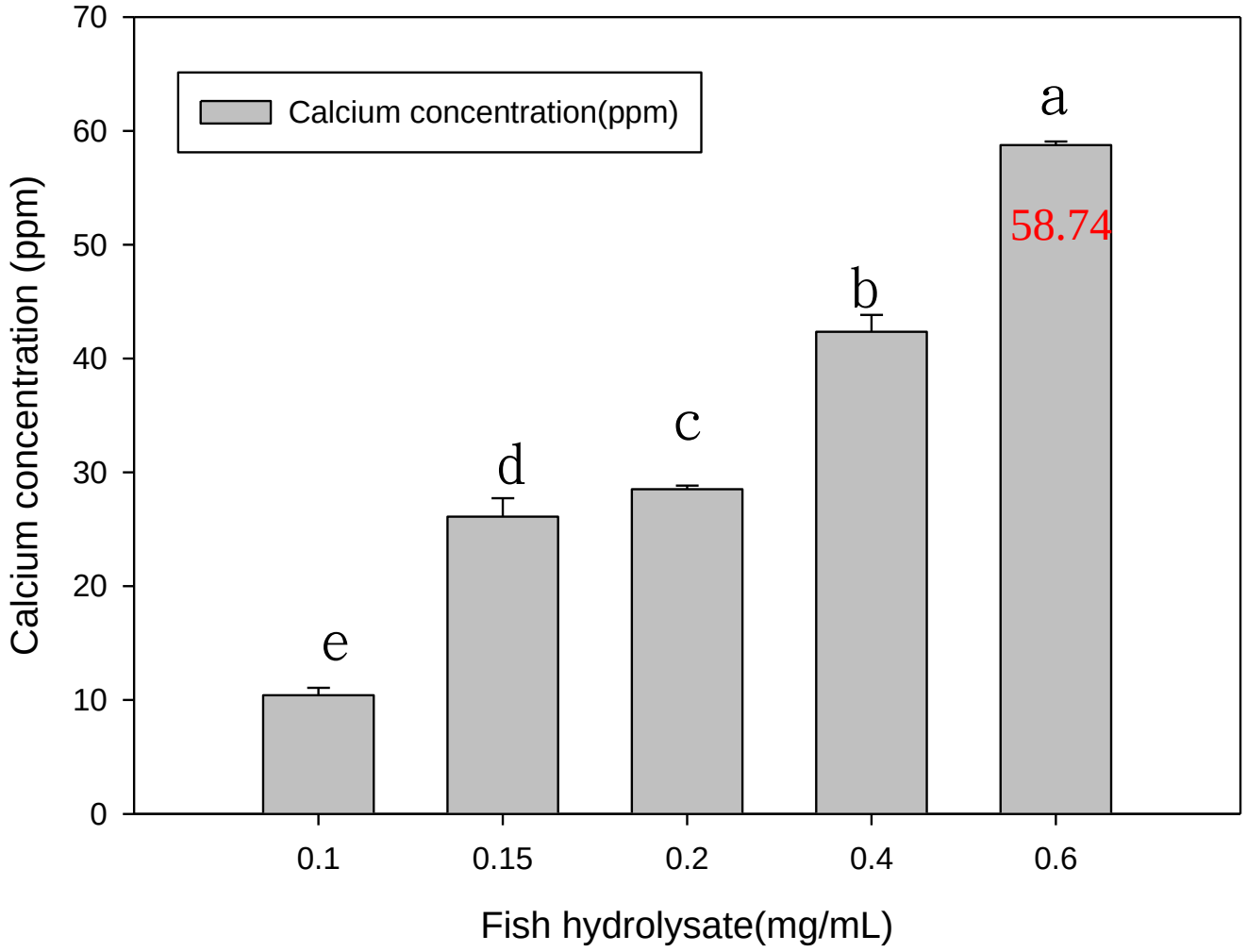

Fig7.Calcium binding activity of hydrolysates from dorsal meat of milkfish treated with Protease A + Umamizyme G .

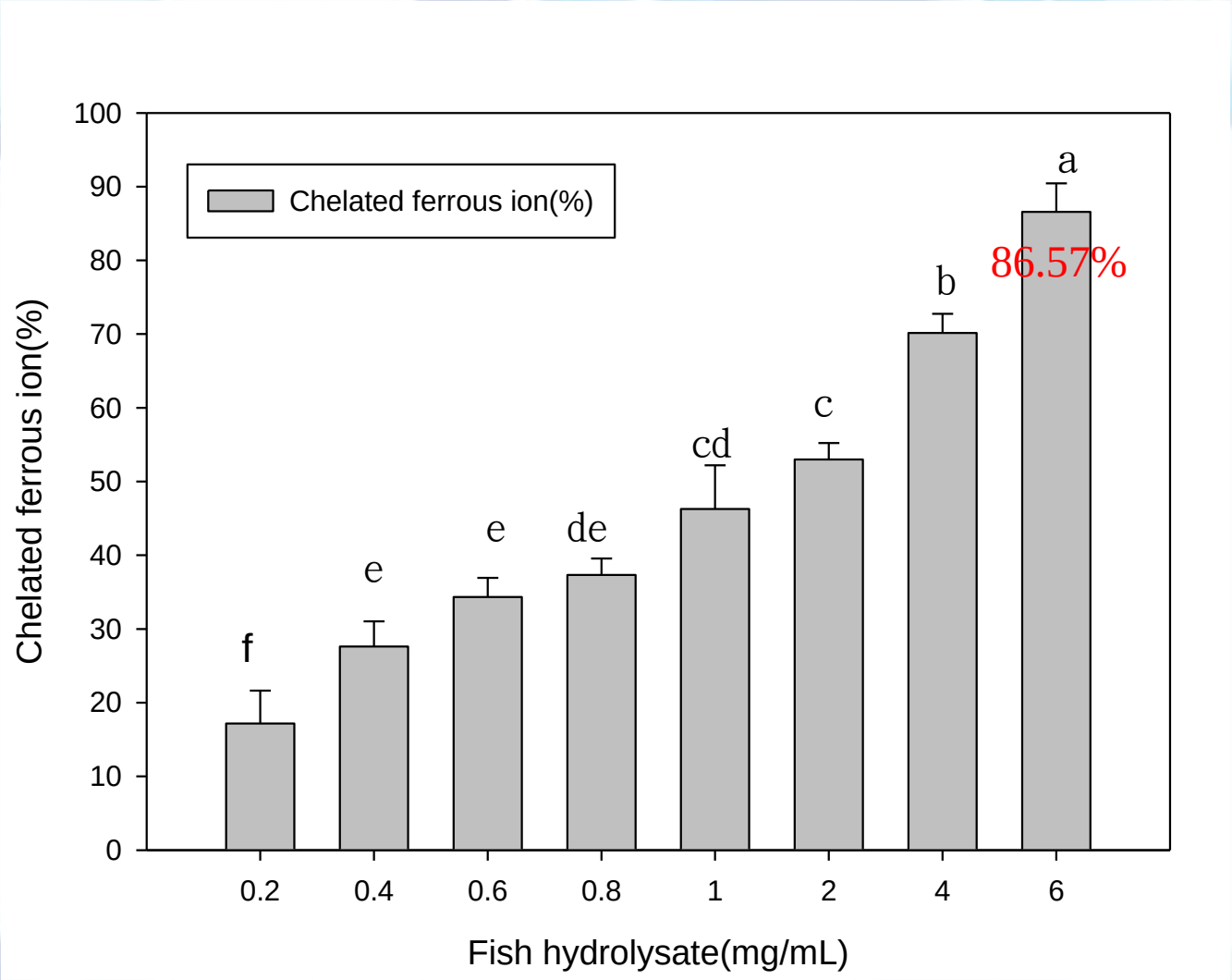

Fig8. Ferrous ion chelating activity of hydrolysates from dorsal meat of milkfish treated with Protease A + Umamizyme G .

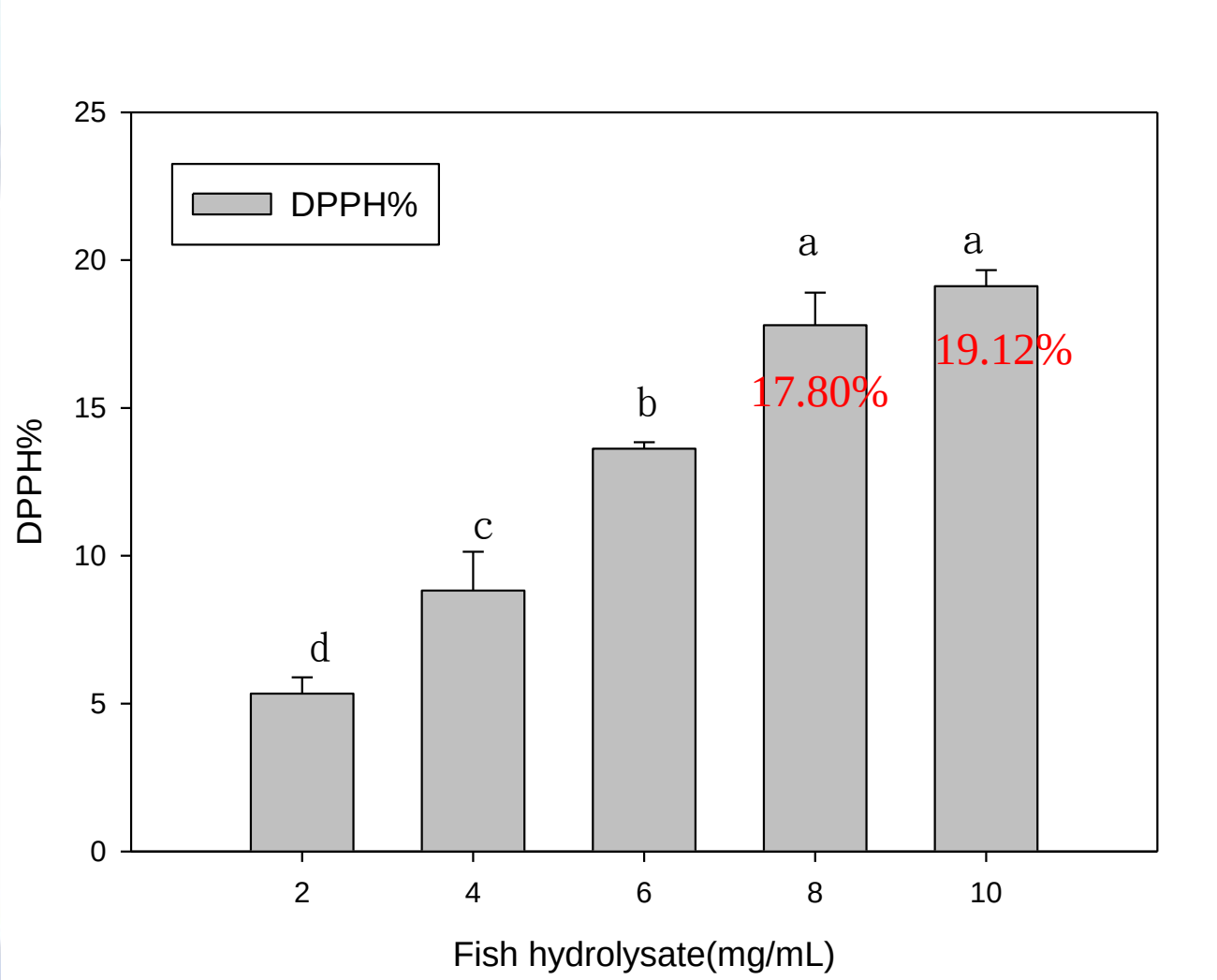

Fig9. DPPH scavenging activity of hydrolysate from dorsal meat of milkfish treated with Protease A + Umamizyme G.

Introduction

Aim

Materials and methods

Results and discussion

Conclusion

Conclusion

- Proteases of protamex, flavourzyme, papain, protease 826, alcalase, protease A, protease M, protease P, protease N and Umamizyme G were used to prepare hydrolysate from dorsal meat of milkfish . Protease P, Umamizyme G and protease A showed higher activity in degree of hydrolysis (DH%).The fish meat was then treated with two enzymes system including protease A and Umamizyme G.

- The optimal conditions for preparing hydrolysate from dorsal meat of milkfish with two enzymes system were listed as follow: temperature 50℃; pH7; reaction time 5h; enzyme activity 5000U ; fish concentration 30%. DH of 91.35% was obtained from optimal condition.

- VBN, POV, TBA and histamine of hydrolysates from dorsal meat of milkfish were 2.49±0.39 mg/100g, 0.022±0.006 meq/kg, 0.034±0.0006 and 8.504 ppm, respectively.

- None of fishy odor was obtained from hydrolysate treated with 0.5% activated carbon or 0.3% yeast.

MW of hydrolysate from dorsal meat of milkfish was 1190 Da estimated by Size exclusion HPLC.

- Hydrolysate obtained from two enzyme system showed calcium ion binding activity of 58.74 ppm at 0.6 mg/mL as well as ferrous iron chelating activity of 86.57% at 6mg/mL In addition, hydrolysate from fish meat also exhibited DPPH scavenging activity of 19.12% at 10mg/mL.


THANK
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