

## **Bioactivity of saithe (*Pollachius virens* L.) protein hydrolysates**

Patricia Y. Hamaguchi, Arnljotur B. Bergsson, Sigrun M. Halldorsdottir, Gudjon Thorkelsson, Hordur G. Kristinsson and Ragnar Johannsson

Matis, Food Research Innovation and Safety, Reykjavik, Iceland  
University of Iceland, Reykjavik, Iceland  
University of Florida, Gainesville, USA

### **Introduction**

In recent years, certain foods have been recognized as a rich source of biologically active peptides. It is well known that physiological active peptides are produced from several food proteins during gastrointestinal digestion. Bioactive peptides are specific proteins fragments that have a positive impact on body function. One common way to produce bioactive peptide is through enzymatic hydrolysis of proteins. Previous studies have shown that protein hydrolysates derived from fish proteins may have antihypertensive and antioxidative effects. In this study, Cryotin, a digestive enzyme from cod was used to produce hydrolysates with bioactive properties. The focus was to produce peptides from an underutilized fish species that may have an effect on the cardiovascular system, such as antihypertensive activity and antioxidative activity.

### **Materials and Methods**

#### **1. Materials**

Fillets of saithe (*Pollachius virens* L.) were purchased locally in Reykjavik, Iceland, and transported on ice to the laboratory. The enzyme Cryotin F was provided by North ehf., Iceland.

#### **2. Preparation of saithe proteins**

The saithe proteins were prepared in two different ways. Method A: A traditional protein isolate using the alkaline pH-shift method and Method B: Non-dewatered protein isolate. In brief, saithe muscle was minced and mixed with 6 parts of cold water. The mixed solution was homogenized and the pH adjusted to 11 using 2 N sodium hydroxide with constant stirring. The mixture was then filtrated using double-layered cheese cloth. For Method A the filtrated solution was adjusted to pH 5.5 using 2 N hydrochloric acid followed by filtration with double-layered cheese cloth. The supernatant was collected and used in the hydrolysis. For Method B the filtrate solution was adjusted to pH 8 using 2 N hydrochloric acid and used in the hydrolysis. The amount of protein in both methods were determined by Bradford.

#### **3. Enzymatic hydrolysis of saithe protein**

Enzymatic hydrolysis of saithe protein was performed using Cryotin F extracted from Atlantic cod. Previously prepared saithe proteins were adjusted to 2% w/v protein and hydrolysis was set at 45 °C, pH 8. The enzyme (2% w/v) was added and degree of hydrolysis (DH) calculated using the equation described by Raghavan and Kristinsson (2007). The mixtures were incubated for 100-120 min to achieve 11.2 % DH. The enzyme was inactivated by heating up to 90°C for 10 min followed by rapid cooling and filtrated using a cheese cloth. Hydrolysed samples were stored at -20°C until further use.

#### **4. Ultra-filtration of hydrolysate**

All the hydrolysate samples were ultra-filtrated using Millipore centrifuge tubes with different molecular weight cut offs (30 KDa, 10KDa and 5 KDa). Only the permeate samples were used.

#### **5. Lipid oxidation analysis**

Saithe protein isolates and hydrolysates were kept at 5°C for 12 days and sampled at day 1, 4, 6, 7, 11 and 12.

##### **5.1. Thiobarbituric acid reactive substance (TBARS)**

TBARS were measured by extracting the samples with trichloroacetic acid solution following the method described by Lemon (1975). The results were expressed in µmol/kg.

##### **5.2. Lipid hydroperoxides**

Samples were mixed with chloroform:methanol (1:1) with sodium chloride. The chloroform phase was removed and reacted with ammonium thiocyanate and ferrous chloride. The absorbance was measured at 500 nm.

#### **6. Bioactivity analysis**

##### **6.1. Angiotensin converting enzyme (ACE) assay**

ACE inhibitory activity was performed according to the method described by Vermeirssen *et al.* (2003) with slight modification. Concentration of selected protein hydrolysates needed to inhibit ACE by 50% (IC<sub>50</sub>) was determined by assaying various diluted hydrolysates and plotting ACE inhibition percentage as a function of protein concentration.

### 6.2. Oxygen radical absorbance capacity (ORAC) assay

ORAC values of saithe hydrolysate fractions were measured using the methods of Davalos *et al.* (2004) with slight modification. The ORAC assay monitors the decay inhibition of fluorescein in the presence of 2,2'-azobis (2-amidinopropane) dihydrochloride, a peroxy radical generator. The fluorescein decay rate was tracked by calculating the area under the decay curve. Fluorescein decay products were quantified using a standard curve of Trolox. Results were expressed as  $\mu\text{mol}$  of Trolox Equivalent per g of protein in the hydrolysate.

#### Results and Conclusions

1. Isolates and hydrolysates made with both methods had good oxidative stability (Fig. 1, 2, 3 and 4)
2. The traditional method (Method A) led to more antioxidative activity than Method B (Fig. 5)
3. Antihypertensive activity significantly increased with decreased molecular weight of peptides (Table 1 and Table 2)
4. Method B led to higher antihypertensive activity than Method A (Table 2)

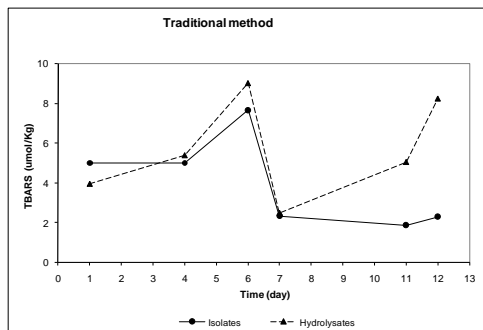


Fig. 1. TBARS ( $\mu\text{mol}/\text{Kg}$ ) of saithe protein isolates and hydrolysates made with Method A

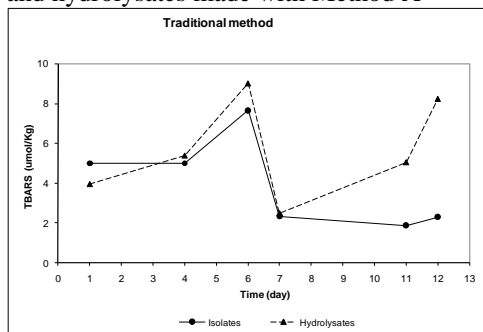


Fig. 2. TBARS ( $\mu\text{mol}/\text{Kg}$ ) of saithe protein isolates and hydrolysates made with Method B

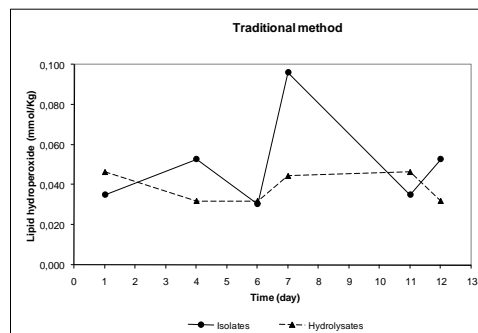


Fig. 3 Lipid hydroperoxide values (mmol/Kg) of saithe protein isolates and hydrolysates made with Method A

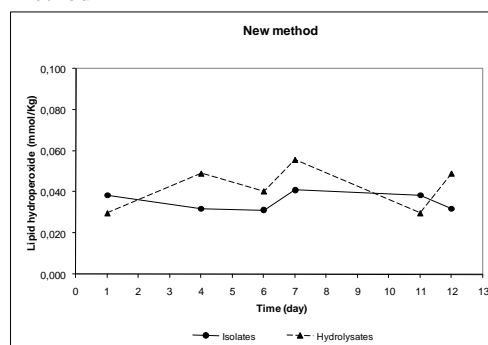


Fig. 4 Lipid hydroperoxide values (mmol/Kg) of saithe protein isolates and hydrolysates made with Method B

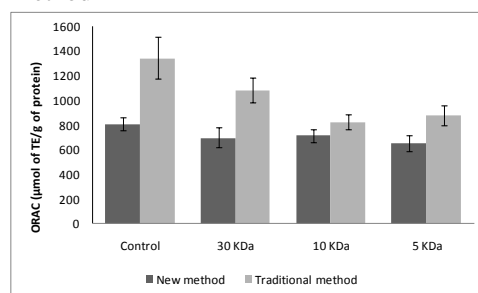


Fig. 5. Antioxidative capacity of saithe protein hydrolysates

Table 1 The protein concentration (mg/ml), ACE inhibition (%), and  $\text{IC}_{50}$  (mg/ml) of ultra-filtrated saithe protein hydrolysates (Method A)

Sample	Protein concentration (mg/ml)	ACE inhibition (%)	$\text{IC}_{50}$ (mg/ml)
Traditional method			
Control	10,2	44,2	nd
30 KDa	10,8	51,2	11,0
10 KDa	11,6	71,4	3,4
5 KDa	10,0	79,1	2,1

\*nd means not detected

Table 2 Protein concentration (mg/ml), ACE inhibition (%), and  $\text{IC}_{50}$  (mg/ml) of ultra-filtrated saithe protein hydrolysates (Method B)

Sample	Protein concentration (mg/ml)	ACE inhibition (%)	$\text{IC}_{50}$ (mg/ml)
New method			
Control	17,2	30,2	nd
30 KDa	18,7	51,2	10,9
10 KDa	14,9	86,0	1,2
5 KDa	14,6	95,2	1,1