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Seal meat enzymatic hydrolysates and its digests: A comparison on protein and minerals profiles

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ABSTRACT

Seal meat is of high nutritive value but currently a by-product and waste from seal oil processing. Seal meat hydrolysates were generated from alcalase catalysis and were subjected to a soft dynamic digestion system to study their nutritional value and the health benefits. The total amino acid content was determined as 568.54 and 384.65 mg/g for seal meat hydrolysates and its intestinal digests, respectively, using UPLC, and both of the samples were rich in essential amino acids such as Leu and Lys. There were no harmful minerals observed by ICP-MS analyses. Both the gastric and intestinal digests revealed nutritive minerals, i.e., Ca, Fe, Mg, Zn, among which the contents of Fe were 548 and 602 mg/kg, respectively, higher than common meat products. The absorbable Fe^{2+} ions content remained almost the same during the gastric and intestinal digestion, while the content of undesirable Fe^{3+} ions declined drastically in intestinal digests. The change of Fe^{2+} and Fe^{3+} ions was probably due to the antioxidant activities of seal meat hydrolysates observed via scavenging of DPPH and hydroxyl radicals. This study provides knowledge on seal meat hydrolysates as healthy food supplement.

1. Introduction

The harp seal (*Pagophilus groenlandicus*), also known as the Greenland seal, inhabits the northernmost Atlantic and Arctic Oceans. The population of Northwest Atlantic harp seal was estimated in 2016 as 7.4 million animals by Canada's Department of Fisheries and Oceans (DFO), which was 6 times higher than that in the 1970s (Lafrance, 2017). Harp seal blubber has been used for seal oil extraction for centuries, however, the seal meat is still considered as a by-product or waste from the processing of seal oil. Moreover, seal meat has an intense dark red color due to its high myoglobin content, and the dark color as well as the fishy flavor are unappealing to most consumers. In Atlantic Canada, the seal harvest generates several tons of by-products that are put to low value uses such as feed for fish and livestock. Thus, the utilization of seal meat, which is currently underutilized as human food, can be expanded to take advantage of its high nutritive value to aid its sustainability development and food security.

Seal meat is of high quality and high nutritional value based on its bioactive components and nutrients. It is lean meat with very low-fat content (<2% fat) which is predominantly monounsaturated fatty acids (MUFAs), and long chain omega-3 polyunsaturated fatty acids (PUFAs). The meat has a high protein content with a well-balanced amino acid composition (Anderson, Priest, Collins, & MacIsaac, 2019). Furthermore, seal meat is rich in vitamins and minerals, especially vitamin B_{12}, iron (Fe), and zinc (Zn) (Brunborg, Julshamn, Nortvedt, & Frøyland, 2006). High amount of Fe is always of significant interest, and among the two forms of Fe in meat, i.e., heme iron (Fe^{2+}) and nonheme iron (Fe^{3+}), the Fe^{2+} state is more readily absorbed in human gastrointestinal (GI) tract (Goff, 2018) and thus considered to be of high health benefits.

Seal meat is tender and easy to be hydrolyzed or digested by enzymes. Its texture, flavor, and taste are not appealing to some consumers, and thus seal meat hydrolysates to be used as a food supplement may be a solution to utilize seal meat. The previously reported methods to produce seal meat hydrolysates included use of microbial proteases to investigate the hydrolysis, proximate composition and flavors (Hwang, Shahidi, Onodenalore, & Ho, 1997; Shahidi, Synowiecki, & Balejko, 1994). However, there is no research found on the effect of digestion on

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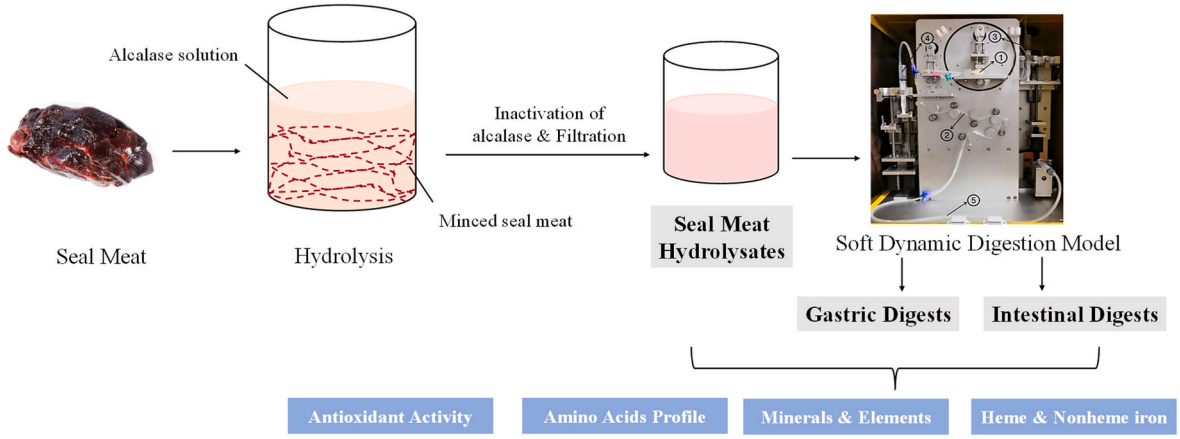


Fig. 1. Scheme of the generation, digestion, and analyses of seal meat hydrolysates.

the protein profiles, minerals, elements, and bioactivities of seal meat hydrolysates. Thus, the objectives of this study were to (i) determine the amino acid and mineral compositions of harp seal meat hydrolysates before and after a dynamic digestion model, and (ii) investigate the effect of antioxidant bioactivity of seal meat hydrolysates on the forms of Fe ions. The present study, as shown in Fig. 1, provides new knowledge on how digestion can influence the nutritional value and the health benefits of seal meat hydrolysates as potential healthy food supplement.

2. Methods and materials

2.1. Preparation of seal meat hydrolysates

Fresh harp seal (*Pagophilus groenlandicus*) adult loins were provided by Les entreprises SeaDNA Canada Inc., Québec, Canada. Seal meat hydrolysates were prepared via enzymatic catalysis using alcalase (FoodPro alkaline protease) prepared in pH 7.5 phosphate buffer (1 mL enzyme in 50 mL buffer). Seal meat was minced and added to the alcalase solution (at a ratio of 1:2, w/v) with continuous stirring at 25 °C for 1 h. Subsequently, the mixture was boiled at 100 °C for 1 min to inactivate the enzyme and stop the enzymatic hydrolysis. Hydrolysates were filtered using 0.22 μm vacuum filtration membrane, and the filtrates were collected and designated as seal meat hydrolysates. Part of the hydrolysate was subjected to biochemical analyses, and some of it were freeze-dried for simulated digestion and analyses.

2.2. Determination of antioxidant activities

Antioxidant activity of seal meat hydrolysates was assayed by (i) DPPH free radical scavenging activity, and measured spectrophotometrically at 517 nm, and (ii) hydroxyl radical scavenging activity, and measured spectrophotometrically at 536 nm (Zhang, He, Bonneil, & Simpson, 2020).

2.3. Simulated gastrointestinal digestion

Freeze-dried seal meat hydrolysates were digested in a dynamic soft rat stomach model established by Pongmalai et al. (2018). The model was equipped with dynamic stomach, duodenum, gastric juice pump, and intestinal juice pump, etc. The excretions after stomach and intestine digestions were collected and referred to as “gastric digests” and “intestinal digests”, respectively, for subsequent analyses. Some of the digests were freeze-dried for further analyses.

2.4. Protein content & amino acids analyses

Protein contents were measured spectrophotometrically by BCA (bicinchoninic acid) assay with a Pierce BCA protein assay kit (Pierce, Rockford, IL, USA), where bovine serum albumin was used as standard protein. Amino acid composition of both the seal meat hydrolysates and the digests in freeze-dried powder forms, were measured using an ultra-performance liquid chromatography (UPLC) system using the methods developed previously (Zhang, Dutilleul, Orsat, & Simpson, 2018; Zhang et al., 2020).

2.5. Determination of minerals

The composition of minerals in both seal meat hydrolysates and digests of hydrolysates were measured on inductively coupled plasma - mass spectrometry (ICP-MS) (Agilent 7700×, Santa Clara, CA, USA), according to the methods described by Lemmens, Deleu, De Brier, Smolders, and Delcour (2021).

2.6. Measurements of Fe^{2+} and Fe^{3+} contents

The freeze-dried hydrolysate samples were first dissolved in distilled water and incubated for 30 min at 25 °C, followed by centrifugation at 4000 rpm for 1 min. The supernatants were collected and the Fe^{2+} contents were measured using the 1,10-phenanthroline method. To measure the Fe^{3+} content, total content of Fe ions was determined using 1,10-phenanthroline method after converting Fe^{3+} to Fe^{2+} using hydroxylammonium chloride, and the content of Fe^{3+} ions was back calculated (Balavandy et al., 2020). The contents of Fe ions were calculated using a standard curve.

2.7. Statistical analyses

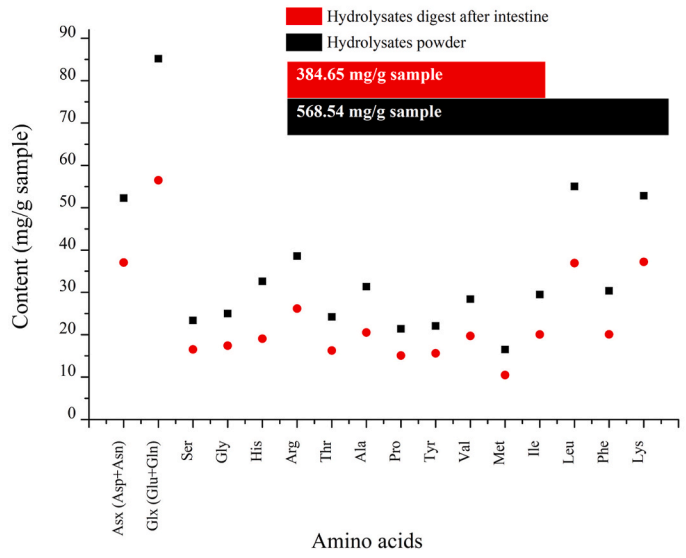
Triplicate measurements were performed for enzyme inhibition determinations. Statistical significance was analyzed using SPSS 22.0 (IL, USA).

3. Results and discussion

3.1. Yield and antioxidant activities of seal meat hydrolysates

The yield of the seal meat hydrolysates generated using alcalase enzyme was determined based on the protein content. The enzymatic hydrolysis produced 49.3 mg hydrolysates per 1 g of fresh harp seal meat under the conditions described in subsection 2.1. The seal meat hydrolysates were adjusted to 5 mg/mL and subjected to free radicals scavenging assays. The hydrolysates showed DPPH free radical scavenging activity of 33.55 ± 0.26% and hydroxyl free radical scavenging activity of 40.76 ± 0.01%. The data showed that the seal meat hydrolysates had antioxidant activities, which suggest the hydrolysate has potential

A



B

Amino Acids	Numbers (per 1000 amino acids)	
	Seal meat hydrolysates	Hydrolysates digests from intestine
Non-essential amino acids (NEAA)		
Ala	86	83
Arg	48	48
Asx (Asp+Asn)	89	93
Glx (Glu+Gln)	129	126
Gly	86	88
Pro	43	45
Ser	52	55
Tyr	26	28
Total NEAA	*559*	*566*
Essential amino acids (EAA)		
His	46	40
Ile	51	51
Leu	95	94
Lys	81	84
Met	25	23
Phe	40	39
Thr	47	46
Val	56	57
Total EAA	*441*	*434*
EAA/NEAA	0.789	0.767

Fig. 2. Protein and amino acids profiles. (**A**) Content of amino acids (mg/g sample) of seal meat hydrolysates and its intestinal digests. (**B**) Amino acids composition (number per 1000 amino acids) of seal meat hydrolysates and its intestinal digests.

benefits for gut health (Kumar Singh et al., 2019; Million et al., 2020).

3.2. Amino acids profiles of hydrolysates and their digests

Amino acids composition is a key indicator of the nutritive value of food protein products. They are mainly absorbed in the intestinal tract of humans; thus, the amino acids profiles of seal meat hydrolysates and their digests from intestine were determined, as shown in Fig. 2, to verify their potential nutritive value. The total amino acids content of seal meat hydrolysates was determined as 568.54 mg/g powder sample, while the hydrolysates digests from intestine showed 384.65 mg/g powder sample (Fig. 2A). Seal meat hydrolysates generally had a higher amount of each amino acid than their intestinal digests, but the amino acids profiles for both hydrolysates and their digests followed a similar trend. In general, seal meat hydrolysates were rich in Glx (Glu + Gln), Leu, and Lys with contents of 85.18, 55.02, and 52.83 mg/g sample, respectively, (Fig. 2A), of which Leu and Lys are essential amino acids (EAA).

Fig. 2B shows the composition of amino acids (i.e., number of each amino acid per 1000 amino acids). Even though there were differences

A

Minerals	Gastric digests of seal meat hydrolysates (mg/kg)	Intestinal digests of seal meat hydrolysates (mg/kg)
Ag	<0.50	<0.50
Al	27.65	7.45
As	<0.50	<0.50
Au	<0.50	<0.50
B	<0.50	<0.50
Ba	<0.50	<0.50
Be	<0.50	<0.50
Bi	<0.50	<0.50
Ca	1089.56	1063.64
Cr	45.98	41.47
Cd	<0.50	<0.50
Co	<0.50	<0.50
Cu	1.88	3.00
Fe	548.49	602.00
Ga	<0.50	<0.50
Ge	<0.50	<0.50
Hf	<0.50	<0.50
Hg	<0.50	<0.50
K	7332.17	11703.57
Li	<0.50	<0.50
Mg	1029.28	651.66
Mn	0.84	1.12
Mo	<0.50	<0.50
Na	55055.19	83511.03
Ni	<0.50	<0.50
P	18667.98	9659.13
Pb	<0.50	<0.50
Pd	<0.50	<0.50
Pt	<0.50	<0.50
S	4335.01	3409.87
Sb	<0.50	<0.50
Se	<0.50	<0.50
Sn	<0.50	<0.50
Sr	12.95	12.16
Ti	1.18	0.80
Tl	<0.50	<0.50
V	<0.50	<0.50
W	<0.50	<0.50
Zn	54.18	69.52
Zr	<0.50	<0.50

B

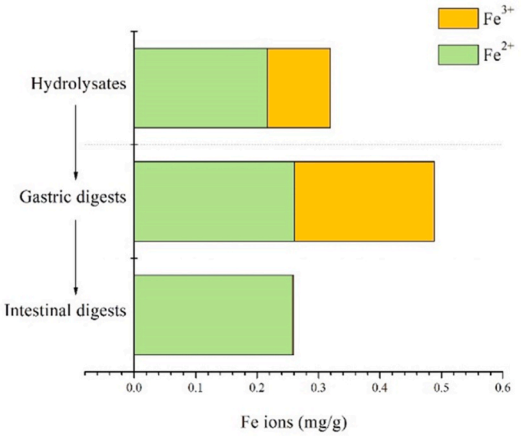


Fig. 3. (**A**) Amounts of minerals (mg/kg) in gastric and intestinal digests of seal meat hydrolysates. (**B**) Amounts of Fe^{2+} and Fe^{3+} ions (mg/g) in seal meat hydrolysates and its gastric and intestinal digests.

observed in the content of amino acids between the two samples (Fig. 2A), there was no significant difference observed between the hydrolysates and their digests after intestinal digestion in terms of amino acids composition (Fig. 2B). However, the digestion process reduced His by 6 per 1000 compared to the original hydrolysates, suggesting that His might be utilized for various physiological effects such as carnosine synthesis, neuroprotection, and improving metabolic syndrome during digestion (Moro, Tomé, Schmidely, Demersay, & Azzout-Marniche, 2020). The ratio of EAA/non-essential amino acids (NEAA) is important to indicate the nutritive quality of protein foods. The EAA/NEAA values of the hydrolysates and the digests were 0.789 and 0.767, respectively, suggesting that some EAA is used up in the digestion process (Moro et al., 2020). The EAA are vital in the process of building proteins, synthesis of hormones/neurotransmitters, and in many metabolic pathways (Wu, Imhoff-Kunsch, & Girard, 2012). Some essential amino acids such as His and Lys are found in high amounts in seal meat hydrolysates. His is important in the production of histamine, a neurotransmitter necessary for immune response, digestion and the sleep-wake cycle (Kovacova-Hanuskova, Buday, Gavliakova, & Plevkova, 2015). Lys plays major roles in protein synthesis, hormone and enzyme production, as well as the absorption of calcium (Liao, Wang, & Regmi, 2015).

Fig. 2 shows that, in general, seal meat hydrolysates had relatively higher amino acids and EAA/NEAA contents compared to their intestinal digests, suggesting that amino acids may likely be lost during the digestion process or during the quantification assay. Nonetheless, the results on amino acids profiles suggest that both seal meat and its hydrolysates are good sources of EAA and can be used as EAA-rich food ingredients.

3.3. Minerals profiles of hydrolysates digests

Minerals can be absorbed from any portion of the GI tract. However, the bulk of absorption for most minerals takes place in the small intestine (Goff, 2018). The content of various minerals of both the gastric digests and the intestinal digests (in the freeze-dried powders) of seal meat hydrolysates are shown in Fig. 3A. There were no harmful minerals (e.g., Ag, As, Au, B, Ba, Be, Bi, Cd, Co, Ga, Ge, Hf, Hg, Li, Mn, Mo, Ni, Pb, Pb, Pt, Sb, Se, Sn, TI, V, W, Zr) found in both digests. There were differences in the contents of nutritious elements and minerals between the gastric and intestinal digests. In the gastric and intestinal digests, K showed 7332 and 11,704 mg/kg, P showed 18,668 and 9659 mg/kg, Ca showed 1090 and 1064 mg/kg, Fe showed 548 and 602 mg/kg, Mg showed 1029 and 652 mg/kg, Zn showed 54 and 70 mg/kg, respectively. It was reported previously that seal meat is a good source of trace-elements and minerals including Fe, Zn, K and P, and the content of Fe in seal meat was approximately 15 times higher than beef (Brunborg et al., 2006). The present finding on minerals and elements of seal meat hydrolysates showed that the seal meat hydrolysates are good sources of beneficial minerals.

3.4. Fe ions forms in hydrolysates and the digests

The seal meat hydrolysates have great potential to be used as Fe enriched functional food ingredients to people suffering from low intake of Fe diets and Fe deficiency anemia (Mantadakis, Chatzimichael, & Zikidou, 2020). The amounts (mg/g sample) of Fe^{2+} and Fe^{3+} ions in the hydrolysates, the gastric digests, and the intestinal digests are shown in Fig. 3B. The amount of Fe^{3+} ions were declined drastically in the intestinal digests versus the original hydrolysate and the gastric digest, while Fe^{2+} ions content remained almost the same (~0.26 mg/g sample). It is expected that the low pH of gastric acid would facilitate the conversion of the ferric (Fe^{3+}) to absorbable ferrous (Fe^{2+}) ions (Omar et al., 2021). However, seal meat hydrolysates exhibited antioxidant activities (as shown in Subsection 3.1.), which might be ascribed to the low-molecular peptides and hydrophobic amino acids, that could either prevent the oxidation of Fe^{2+} ions or chelate the Fe^{3+} ions in the *in vitro* GI digestion system (Paul, Osoniyi, Eghianruwa, Oparinde, & Adesina, 2021; Wang et al., 2021). It is also possible that Fe^{3+} ions formed complexes due to the chelating with other compounds such as Vitamin A during the digestion process (Briguglio et al., 2020). Since only the Fe^{2+} form can be absorbed by the intestines, the amount of Fe^{2+} in digests that remained practically unchanged suggested good health benefits of the seal meat hydrolysates as Fe^{2+} ion source.

4. Conclusions

Proteolysis of seal meat using exogenous enzymes produced hydrolysates rich in nutrients and endowed with biological activities that could potentially be used to modulate physiological functions in the body. The study suggests that seal meat hydrolysate could be explored as bioingredients in health foods based on its high antioxidant capacity, essential amino acids content, and essential minerals such as Fe. Nonetheless, further studies are warranted to verify the safety of seal meat hydrolysates in terms of non-toxicity and non-allergenicity, to justify their commercial production and use as food ingredients to supplement the limited number of similar products currently available on the market.

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Declaration of competing interest

None.

CRediT authorship contribution statement

Yi Zhang: Investigation, Methodology, Writing – original draft. **Xin Rui:** Investigation, Methodology. **Romy Vaugeois:** Resources. **Benjamin K. Simpson:** Supervision, Writing – review & editing.

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