Process Biochemistry 44 (2009) 668–677

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Functional, bioactive and antioxidative properties of hydrolysates obtained from cod (*Gadus morhua*) backbones

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ARTICLE INFO

Article history:
Received 2 July 2008
Received in revised form 5 February 2009
Accepted 17 February 2009

Keywords:
Hydrolysates
Cod
By-products
Functional
Antioxidative properties
CGRP
Gastrin
Cholecystokinin

ABSTRACT

Fish protein hydrolysates (FPH) have good and well documented functional properties. Peptides obtained from various fish protein hydrolysates have also shown bioactive and antioxidative activities.

The aim of this study was to evaluate how storage and preparation of cod (*Gadus morhua*) backbones influence the yield, functionality, bioactivity (CGRP and gastrin/CCK related molecules) and antioxidative properties of fish protein hydrolysates. A series of hydrolysis trials have been carried out using backbones from cod that were initially fresh or frozen and further hydrolysed for different times (10, 25, 45 and 60 min). Use of fresh raw material significantly increased yield of dry FPH, gave lighter and less yellow powders with better emulsification properties. Longer time of hydrolysis gave higher FPH yield, increased degree of hydrolysis and decreased water holding capacity of the powders. Among the hydrolysis times tested, 25 and 45 min hydrolysis demonstrated the best emulsification properties.

FPH have potential to enhance product stability by preventing oxidative deterioration. The DPPH scavenging activity showed that antioxidative activity of hydrolysates could be due to the ability to scavenge lipid radicals. The ability of hydrolysates to inhibit iron induced lipid oxidation was not influenced by time of hydrolysis.

This work also shows that it is possible to obtain bioactive molecules from cod backbones by protein hydrolysis. The content of bioactive peptides (gastrin/CCK- and CGRP-like peptides) could make the cod hydrolysates useful for incorporation in functional foods.

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1. Introduction

Seafood processing discards and by-products account for approx. three-quarters of the total weight of the catch [1,2]. Fish processing by-products are usually regarded as residuals left after filleting and when viscera is included this can represent up to 2/3 of the round cod [3–6]. Valuable components such as fish oil, proteins, collagen and gelatine, enzymes and minerals can be obtained from this rest raw material. Recent studies have identified a number of bioactive compounds from fish by-products and shellfish and crustacean shells [7]. These compounds can be extracted and purified with technologies of varying complexity. Development of new technologies to extract new bioactive compounds from marine processing by-products may bring more value out of what is today considered a waste.

Enzymatic hydrolysis is one of the methods for recovery of valuable components from fish by-products [4,8–10]. Fish protein hydrolysates (FPH) have good solubility over a wide range of ionic strength and pH and usually tolerate strong heat without precipitating [11]. FPH have good functional properties and can contribute to water holding, texture, gelling, whipping and emulsification properties when added to food [12]. Some studies have shown that FPH can contribute to increased water holding capacity in food formulations [13–16]; and addition of FPH from salmon reduced water loss after freezing [17]. Fish protein hydrolysates (FPH) have good foaming and emulsifying properties, thus may be used as emulsifying and emulsion stabilizing ingredients in a variety of products as well as aid in the formation and stabilisation of foam-based products. Because the size of the peptides is very important for interfacial/surface activity of FPH,

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doi:10.1016/j.procbio.2009.02.010

the degree of hydrolysis is important [18]. Several reports have suggested that there is an optimum molecular size or chain length for peptides to provide good foaming and emulsifying properties, and that limited hydrolysis resulting in larger peptides generally leads to improved emulsification and foaming properties, while extensive hydrolysis resulting in small peptides reduce these properties [18–22]. In addition, except for the deficit of a few amino acids, hydrolysates have a high nutritional value [9,13].

Several studies have indicated that peptides derived from fish proteins have antioxidative properties in different oxidative systems [12,18,23–28]. The antioxidant activity of proteins and peptides can be the result of specific scavenging of radicals formed during peroxidation, scavenging of oxygen containing compounds, or metal-chelating ability [12,29]. Production of fish protein hydrolysates with antioxidant properties will enable production of protein enriched and oxidatively stable seafood.

The hydrolysis process can lead to the production of various peptides bearing a structural resemblance to hormones. These newly formed peptides can retain the biological properties of the native protein, or can show new properties. The calcitonin-gene related peptide (CGRP) is a 37-residue peptide widely distributed in the central nervous system and peripheral nerves. Different biological functions have been described for CGRP such as vasodilation [30] and induction of satiety [31,32]. On the other hand, different authors have reported the presence of gastrin/CCK-like molecules in protein hydrolysates from fish by-products [33,34]. These molecules are the only known members of the gastrin family in humans, and could have a positive effect on food intake in humans and fish species in aquaculture. Gastrin is a gastric hormone which stimulates postpandrial gastric acid secretion and epithelial cell proliferation. In humans there are two different gastrins, one with 17 and one with 34 amino acids residues. Cholecystokinin (CCK) is a group of peptides which controls the emptying of the gallbladder, as well as pancreatic enzyme secretion. It is also a growth factor, and regulates intestinal motility, satiety signaling and the inhibition of gastric acid secretion [35]. Both gastrin and CCK inhibit food intake and share a common COOH-terminal pentapeptide amide that also includes the sequences essential for biological activity.

The aim of the present study was to find how state (fresh versus frozen, pre-rigor versus post-rigor filleted, whole versus cut) of raw material and time of hydrolysis influence functional, antioxidative and certain bioactive (gastrin/CCK- and CGRP-like peptides) properties of fish protein hydrolysates obtained from cod backbones.

2. Materials and methods

2.1. Raw material

Backbones from farmed Atlantic cod (*Gadus morhua*) obtained from a fish farm located in Central Norway were used for experiments. Weight and length of the fish was 2.7 ± 0.4 kg and 57.5 ± 2.8 cm. After hand filleting, one part of the bones after post-rigor filleting were frozen (−20 °C) and stored for approx. 1 month—part A, while fresh backbones were used for the other part of the experiment—part B.

Frozen backbones were thawed overnight in a cold room, placed in plastic bags or were cut into 1–2 cm pieces with a knife and placed in plastic bags. For the second part of the experiment (part B) fresh backbones from pre-rigor and post-rigor filleted fish were used (Fig. 1). In order to have more uniform cutting of backbones it was decided to mince backbones in a HOBART mincer (model AE 200) using large (10 mm diameter) holes.

2.2. Enzyme and chemicals

Protamex™ (Novozymes A/S, Bagsvaerd, Denmark) was used for the hydrolysis. This enzyme was kindly delivered by Novozymes and complied with the recommend purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) [36,37]. Protamex™ is shown as effective proteolytic enzyme in hydrolysis of cod backbones [8] and are commonly used in industrial application.

Human CGRP was obtained from Bachem (Weil am Rhein, Germany). Labeled hormones (specific activity: 2000 Ci/mmole) were from GE Healthcare (Chalfont St. Giles, UK). The anti human CGRP antibody was a generous gift of Dr. A. Jullienne, Paris, France. Male Wistar rats were obtained from Janvier breeding (Le Genest sur Isle, France). Bovine serum albumin (Sigma–Aldrich) was heat-inactivated before radioreceptorassays. Other chemicals were of reagent grade. Formaldehyde (Merck, Darmstad, Germany) was used for the chemical analysis.

A fish protein powder MariPep CP® (Danish Fish Protein, Denmark) was used as commercial available reference. The powder contained 97.5% dry matter, where 77.8% was proteins, 19.4% salt and less than 0.3% lipids.

2.3. Hydrolysis process

The hydrolysis was performed in a 4 l closed glass vessel stirred with a marine impeller (150 rpm). Thawed (or fresh in part B) backbones were mixed with warm (55 °C) water at a weight ratio 1:1. When the temperature of the mixture was 55 °C, the enzymatic hydrolysis was started by adding 0.1% (by weight of raw material) Protamex™. After different hydrolysis times: 10, 25, 45 and 60 min (A part) and 10 and 60 min (B part), enzyme inactivation was done by microwave heating for 5 min at a temperature higher than 90 °C. The bones were separated from the hydrolysate mixtures by sieving and the hot mixtures were centrifuged in 1 l batches at 2250 × *g* for 15 min. Two fractions were obtained after centrifugation: the sludge (non-water-soluble part) on the bottom and fish protein hydrolysate (FPH, water-soluble compounds). The fractions were separated by decanting. Both fractions were freeze-dried. The hydrolysis was performed in duplicate.

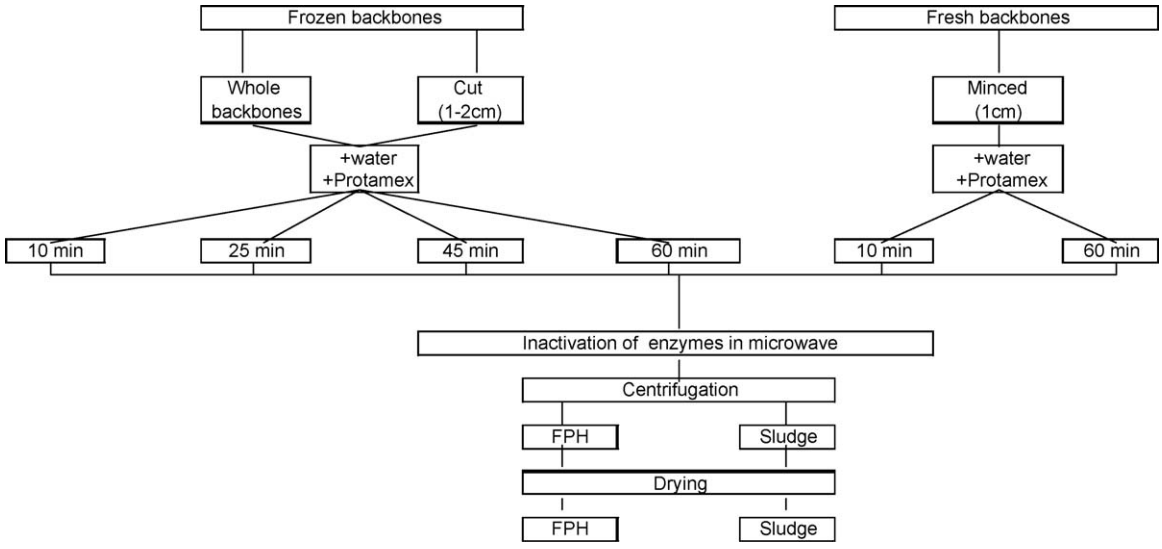

Fig. 1. Flow sheet of hydrolysis.

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