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Peptides from fish and crustacean by-products hydrolysates stimulate cholecystokinin release in STC-1 cells

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ABSTRACT

Fish protein hydrolysates (FPH) are of significant interest, due to their potential application as a source of bioactive peptides in nutraceutical and pharmaceutical domains. Here, we investigated the action of FPH from blue whiting (*Micromesistius poutassou*) and brown shrimp (*Penaeus aztecus)* on cholecystokinin release from intestinal endocrine cells (STC-1). We demonstrated for the first time that FPH were able to highly stimulate CCK-releasing activity from STC-1 cells and that this stimulation was mainly due to peptide molecules. The partial purification of CCK-stimulating peptides showed that their apparent molecular weight ranged between 1000 and 1500 Da for fish and crustacean FPH, respectively. Finally, in an aim to industrially produce hydrolysates enriched in CCK-stimulating molecules, we tested the effects of membrane processes (ultrafiltration and nanofiltration) on active peptide enrichments.

1. Introduction

Excess of food intake largely contributes to the growing world prevalence of body weight increase and obesity that predispose several pathologies, such as diabetes, hypertension, coronary heart diseases and respiratory problems (Arora & Anubhuti, 2006). The long-term regulation of maintenance of body weight is integrated by several endocrinal signals, such as insulin and leptin (Schwartz, Woods, Porte, Seeley, & Baskin, 2000). This regulation is coupled with short-term signals related to the meal size, the most efficient of these is given by cholecystokinin (CCK). This contributes in maintaining an appropriate meal size and to regulate the daily energy intake, which is coordinated with long-term body weight regulation (Morton, Cummings, Baskin, Barsh, & Schwartz, 2006). CCK is the most studied satiety signal and is primarily secreted in two forms (CCK 33 and CCK 8) from I-cells located in the upper portion of the small intestine (Liddle, 1997). CCK is also synthesised within the central nervous system, in the form of CCK 8 (Herranz, 2003). I-cell CCK secretion is stimulated in the intestine, in response to a meal stimulation. The main stimulants (fatty acids and peptides) appear to be species specific (Douglas, Woutersen, Jansen, de Jong, & Lamers, 1988; Lewis & Williams, 1990). The release of luminal CCK leads to several gastrointestinal effects, like intestinal mobility, gall bladder contraction, inhibition of gastric emptying, and stimulation of pancreatic secretion (Strader & Woods, 2005). Several *in vitro* studies reported that peptones were able to stimulate CCK secretion (Sufian et al., 2006) and gene transcription in STC-1 cell line (Cordier-Bussat et al., 1997; Nemoz-Gaillard et al., 1998). This cell line harbours a high similarity with human CCK-secreting intestinal I cells and was described as a suitable model for *in vitro* CCK secretion study (Cordier-Bussat et al., 1997; Nemoz-Gaillard et al., 1998; Wang, Prpic, Green, Reeve, & Liddle, 2002). Reports also demonstrated that in STC-1 cells, CCK-stimulating action of soybean and pork peptones could be linked to appetite suppression effects in rats (Nishi, Hara, Asano, & Tomita, 2003; Nishi, Hara, & Tomita, 2003; Sufian et al., 2006). However, the action of fish protein by-products hydrolysates (FPH) on CCK secretion has not been studied yet.

We previously described that FPH could harbour secretagogue (Guerard & Ravallec-Ple, 2001; Ravallec-Plé et al., 2001; Ravallec-Plé, Gilmartin, Van Wormhoudt, & Le Gal, 2000; Ravallec-Ple & Van Wormhoudt, 2003) and calciotropic-like activities (Fouchereau-Peron et al., 1999; Ravallec-Plé et al., 2001; Rousseau, Batista, Le Gal, & Fouchereau-Peron, 2001). In the present work, we investigated the effects of three FPH from blue whiting (*Micromesistius poutassou*) and brown shrimp (*Penaeus aztecus)* on CCK secretion in STC-1 cells, and partially purified the CCK-stimulating peptides. Secondly, we analysed the effect of ultrafiltration and

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nanofiltration on active peptides enrichments of FPH performed on an industrial scale.

2. Materials and methods

2.1. Material and chemicals

Blue whiting (*M. poutassou*) were caught in the North Atlantic Ocean and brown shrimp (*P. aztecus)* in the waters of French Guyana by the Compagnie des Pêches Saint Malo (France). Raw material was hand prepared and stored at −20 °C on board during the fishing year and then kept at −20 °C until use. Dulbecco's modified Eagle's Medium (DMEM) for STC-1 cell line, foetal calf serum, and all other cell culture reagents were purchased from PAN-Biotech GmbH (Aidenbach, Germany). Amino acid mixture (AAM) was prepared in accordance with hyperamine 25 (HA25, B.Braun Medical, Boulogne, France). The free amino acid composition was: L-isoleucine 6.4 g l^{-1}, L-leucine 11.2 g l^{-1}, L-lysine 8.8 g l^{-1}, L-methionine 4 g l^{-1}, L-phenylalanine 9.05 g l^{-1}, L-threonine 6.4 g l^{-1}, L-tryptophan 1.6 g l^{-1}, L-valine 8 g l^{-1}, L-arginine 27.65 g l^{-1}, L-histidine 7.2 g l^{-1}, L-alanine 9.6 g l^{-1}, L-aspartic acid 15.05 g l^{-1}, L-cysteine 1.6 g l^{-1}, L-glutamic acid 15.05 g l^{-1}, glycine 12.8 g l^{-1}, L-proline 5.3 g l^{-1}, L-serine 2.55 g $l^{-1}$and L-tyrosine 0.55 g l^{-1}. Bovine serum albumin (BSA) and albumin egg hydrolysate (AEH) were purchased from Sigma (St. Louis, MO). The gastrin/CCK radioimmunoassay kit was from CIS Bio International (Cèze, France), and all other chemicals were of reagent grade.

2.2. Preparation of fish protein hydrolysate (FPH)

Blue whiting muscle and shrimp head were thawed at room temperature and hydrolysed on a laboratory scale (1 l batch) under controlled hydrolysis conditions (pH, temperature, and stirring speed) according to the pH-stat method (Diniz & Martin, 1996). Two different blue whiting hydrolysates (A and B) and also a brown shrimp (C) hydrolysate were prepared. Enzymatic inactivation was accomplished by heating at 90 °C for 20 min. The slurry was centrifuged at 7000g for 30 min, and the supernatant was further freeze-dried and kept at −20 °C. Hydrolysates were re-suspended in distilled water (0.1 g ml^{-1} dry weight) and filtered (0.22 μm) before being used. These hydrolysates were industrially prepared, so technical details were limited.

2.3. Membrane processes, ultrafiltration and nanofiltration

In order to industrially concentrate peptides of interest and avoid excessive concentration of salt, FPH B and C were prepared on a pilot scale in a 5 l batch, following the same protocol as described in Section 2.2 and submitted to membrane processes. A UF/NF Microlab 40c pilot plant (VMA – Getzmann GmbH, Reichshof, Germany) was used for FPH fractionation steps using tubular organic membranes of a surface area of 0.033 m^2. Hydrolysates were successively filtrated on three different molecular weight cut off (MWCO) membranes, following the protocol previously described (Vandanjon, Johannsson, Derouiniot, Bourseau, & Jaouen, 2007). Briefly, the FPH supernatant was loaded into the pilot plant for a first peptide fractionation using an MT68 in polysulfon membrane of 8000 Da MWCO. The retentate (R1) was kept and the permeate was loaded into the pilot for a second ultrafiltration step using an MTP04 in modified polyethersulfone membrane of 4000 Da MWCO. The retentate (R2) was kept and the permeate was used for the nanofiltration step using an MT04 in polyamide/polyethersulfone membrane of 300 Da MWCO. Finally the retentate (R3) was kept. Retentates were lyophilised and stored at −20 °C before use.

2.4. Cell line, culture condition and CCK secretion studies

The STC-1 cell line was a gratefully received gift from Dr C. Roche (INSERM U865, Lyon, France). STC-1 plurihormonal cell line is derived from an endocrine tumour developed in the small intestine of double transgenic mice (Rindi et al., 1990). The STC-1 cells were grown at 37 °C, 5% CO_2 atmosphere in Dulbecco's modified Eagle's Medium (DMEM, 4.5 g l^{-1} glucose) supplemented with 10% of foetal calf serum, 2 mM glutamine, 100 U ml^{-1} penicillin, and 100 μg ml^{-1} of streptomycin. Two days before the experiment, cells were seeded into 24-well culture plates. When sub-confluence was reached, after approximately 2 days of culture, the medium was replaced by the incubation buffer (4.5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM glucose, 140 mM NaCl, and 20 mM Hepes-Tris, pH 7.4) containing increasing concentrations of the tested FPH or control effectors. The cells were then incubated at 37 °C, 5% CO_2 atmosphere for 2 h; the incubation medium was collected on ice, centrifuged (2000*g* for 7 min) and the supernatants kept at −20 °C before CCK determination. The amounts of CCK released in the medium were determined using a gastrin/CCK radioimmunoassay kit (CisBio International, France).

2.5. Proliferation studies

Effects of FPH on cell proliferation were measured using XTT II assay (Roche Applied Science, Indianapolis, IN). This assay was based on the reduction of a tetrazolium salt (XTT) into yellow formazan salt by active mitochondria. For proliferation tests, cells were transferred into a 96-well microtitre plate at a density of 7000 cells by well in 100 μl of medium (DMEM, 4.5 g l^{-1} glucose supplemented with 10% of foetal calf serum, 2 mM glutamine, 100 U ml^{-1} penicillin, and 100 μg ml^{-1} of streptomycin) and in the presence or in the absence of FPH at different concentrations. After 48 h at 37 °C, 5% CO_2 atmosphere, cells were incubated in the presence of XTT for 4 h. Absorbance (490 nm, against 655 nm reference) in each well was measured in a microplate reader spectrophotometer (Benchmark, Biorad Laboratories, Hercules, CA). Results were expressed as percentages of basal growth activity.

2.6. Size exclusion chromatography by fast protein liquid chromatography (SEC–FPLC)

The peptidic molecular weight distribution of hydrolysates was analysed by SEC–FPLC. The procedure was performed at 25 °C using a Superdex Peptide HR 10/30 column (GE Healthcare Biosciences AB, Uppsala, Sweden), with acetonitrile, H_2O, TFA (30:70:0.1) as eluant. The flow rate was 0.5 ml min^{-1} and the absorbance was monitored at 214 nm. The liquid chromatographic system consisted of a Dionex P-680 pump and a Dionex UVD-170 U (Dionex Corporation, Sunnyvale, CA). The column was calibrated with standard peptides of known molecular weight (cytochrome C, 12,400 Da; aprotinin, 6511 Da; gastrin I, 2126 Da; substance P, 1348 Da; 1–7 substance P, 900 Da; glycine, 75 Da). The relationship between the retention time and the log of the molecular weight of standards was used as the basis for the Cirrus GPC software (Polymer Laboratories, Amherst, MA) to calculate molecular weight distribution of hydrolysates. The freeze-dried samples were diluted (20 mg ml^{-1}) in distilled water and filtered (0.22 μm) before injection (10 μl).

2.7. Molecular sieving

Size exclusion chromatography gel permeation was performed on a Toyopearl HW-40F column (Tosoh Bioscience, Stuttgart, Germany) equilibrated in ammonium acetate buffer (200 mM, pH 5.0) at 4 °C. A quantity of 3.5 g of freeze-dried hydrolysate was

homogenised in 10 ml of the equilibration buffer before elution. Fractions were separated in different molecular weight classes, calculated beforehand with markers of known molecular weight: aprotinin (6511 Da), CGRP (3750 Da), bacitracin (1422 Da) and hexaglycine (360 Da).

3. Results

3.1. Effects of marine by-product hydrolysates on CCK-releasing activity

As shown in Fig. 1, STC-1 basal CCK mean secretion was 4.0 pM after 2 h of incubation; cell exposure to blue whiting (A and B) and shrimp (C) by-products hydrolysates induced a dose-dependent increase in CCK release. Thus, after incubation at 0.2% (wt/vol), the amounts of CCK secreted by STC-1 cells were 40.9, 49.5 and 78.3 pM for FPH A, B, and C, respectively. At the highest concentration assayed (1%, wt/vol), the CCK secretion induced by the hydrolysates B and C (122.0 and 160.7 pM, respectively) was significantly higher than that of the secretion obtained with the FPH A and the commercial albumin egg hydrolysate (AEH) (71.1 and 67.6 pM, respectively). This represented, for FPH A and AEH, a 17-fold increase over the basal value, and for FPH B and C, a 30 and a 40-fold increase, respectively. This led us to select FPH B (blue whiting hydrolysate) and C (shrimp hydrolysate) for the subsequent purification and enrichment steps. In contrast, STC-1 cells exposure to undigested protein (BSA, 1%) resulted in a slight effect on CCK release (31.2 pM); in the same way amino acid mixture (AAM, 1%) weakly stimulated CCK release (8.6 pM).

The effect of FPH B and C was tested at 0.5% and 1% (wt/vol) on STC-1 cell growth (Fig. 2). Results shown that FPH B and C were not toxic, moreover FPH B had no significant effect on STC-1 cell growth, while FPH C exerted a positive effect (92% increase) at 0.5% (wt/vol), and an inhibitory effect (28% decrease) at 1.0% (wt/vol).

3.2. Partial purification of CCK-stimulating peptides from blue whiting and brown shrimp hydrolysates (FPH B and C)

To partially purify CCK-releasing peptides contained in fish protein hydrolysates and characterise their apparent molecular weight, FPH B and C were subjected to size exclusion chromatography using a Toyopearl HW-40F column. Nine fractions, defined from the calibration profile, were collected according to their molecular weight (F1: 7000–5000 Da, F2: 5000–4000 Da, F3: 4000–3000 Da, F4: 3000–2000 Da, F5: 2000–1500 Da, F6: 1500–1000 Da, F7:1000–500 Da, F8: 500–300 Da and F9: <300 Da). The CCK-releasing efficiency of the different fractions was then assayed at 0.5% (wt/vol). The molecular weight distributions of the two hydrolysates were further confirmed by SEC–FPLC, using a Superdex Peptide column. Results obtained for processed B showed that the nine molecular weight fractions stimulated CCK secretion on STC-1 cells (Fig. 3, panel A). However, the effects obtained with the F1 to F5 fraction were lower than that obtained with FPH B before molecular sieving. The best CCK-stimulating effects, compared to the effect of crude extract (60.9 pM of CCK secreted), were obtained with F6 and F8 fractions (90.8 and 77.7 pM of CCK secreted), that is for peptides with a molecular weight smaller than 1500 Da. The most efficient fraction was F6, obtained with a purification factor of 1.5. This fraction was characterised by peptides of molecular weight ranging between 1000 and 1500 Da and representing 13.9% of total area under the absorbance curve (AUC), as calculated from the peptidic profile after SEC–FPLC (Fig. 3, panel B).

Concerning the CCK-stimulating effects of FPH C (Fig. 4), the nine molecular weight fractions also stimulated CCK secretion on

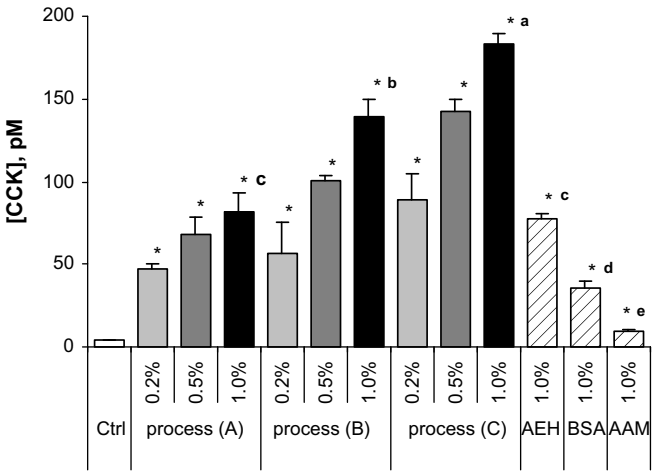

Fig. 1. Effect of hydrolysates obtained from blue whiting (A and B) and shrimp (C) by-products on CCK-releasing activity of STC-1 cells. Hydrolysates were tested at different concentrations (0.2–1.0% wt/vol) on CCK release and compared to those obtained with albumin egg hydrolysate (AEH, a positive control), bovine serum albumin (BSA) and free amino acid mixture (AAM). Values are means ± SEM of four repeated measurement. (∗): $p < 0.05$ versus control. CCK releases at 1.0% concentration (wt/vol) were compared: means without a common letter are different ($p < 0.05$).

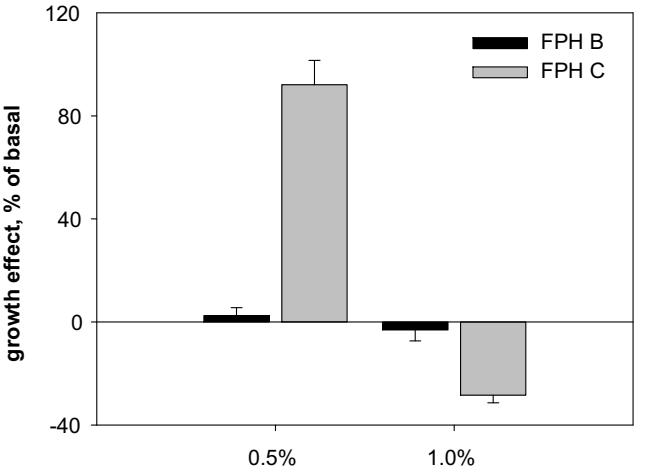

Fig. 2. Effect of FPH B and C tested at different concentrations (0.5 and 1.0% wt/vol) on the STC-1 cell proliferation using a colorimetric assay (XTT-based).

STC-1 cells. Results were similar to data for FPH B with a lower CCK-stimulating effect observed with the F1 to F4 fractions, in comparison with the crude extract. The molecular weights of the most active peptides (F5 to F7 fractions) were in the range of 500–2000 Da (Fig. 4, panel A). CCK secretions obtained with the different fractions compared to the effect of crude extract (78.9 pM) revealed that the stimulation was significantly higher only for the F6 fraction (145 pM), with a purification factor of 1.8. This fraction was characterised by peptides of molecular weight ranging between 1000 and 1500 Da; this represented 6.3% of the peptidic population of the FPH C (Fig. 4, panel B).

3.3. Fractionation of FPH by ultrafiltration and nanofiltration for active peptides enrichment

Ultrafiltration and nanofiltration are two well-known methods used for peptide concentration enrichment or separation of protein

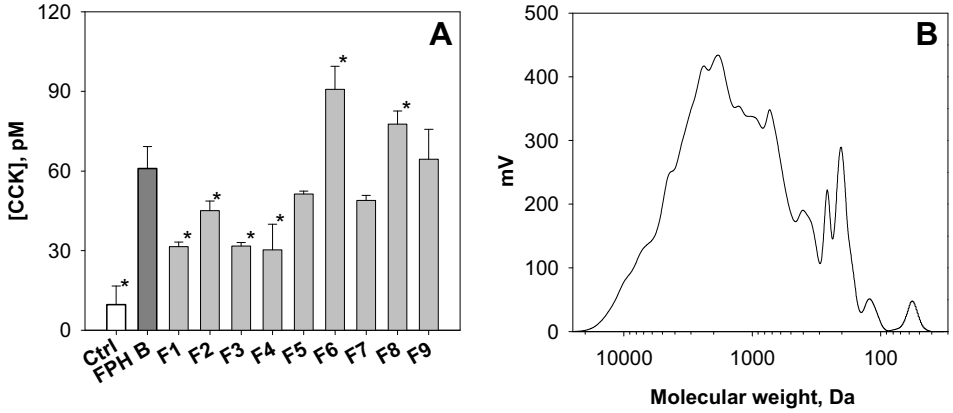

Fig. 3. Molecular sieving of FPH B. Panel A: CCK-releasing activity of STC-1 cells following exposure to different molecular weight fractions (0.5% wt/vol) obtained after size exclusion chromatography (Toyopearl HW-40F column) of FPH B. (∗): $p < 0.05$ versus FPH before molecular sieving. Panel B: molecular weight distribution (SEC–FPLC) of FPH B obtained using a Superdex Peptide HR 10/30 calculated with GPC software.

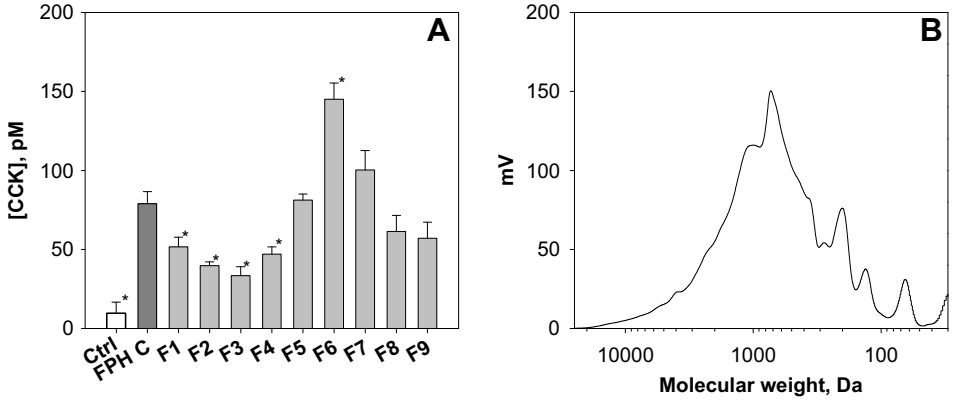

Fig. 4. Molecular sieving of FPH C. Panel A: CCK-releasing activity of STC-1 cells following exposure to different molecular weight fractions (tested at 0.5% wt/vol) obtained after size exclusion chromatography (Toyopearl HW-40F column) of FPH C. ∗: $p < 0.05$ versus FPH before molecular sieving. Panel B: molecular weight distribution (SEC–FPLC) of FPH C obtained using a Superdex Peptide HR 10/30 calculated with GPC software.

hydrolysate at the industrial level. In the present study we tested, on a pilot scale, the effect of ultrafiltration and nanofiltration separation on the enrichment of peptides able to stimulate CCK secretion in STC-1 cells. FPH B and C were produced in a 5 l batch and then fractionated by successive steps of ultrafiltration and nanofiltration, using 8000, 4000 and 300 Da MWCO membranes. In this study, only retentates were used on STC-1 cells, as the high salt concentration of permeates excluded their use for this biological assay. The three retentates successively obtained (R1, R2, and R3) were then assayed on STC-1 cells at 0.5% wt/vol and compared to the effect obtained with the crude extracts at the same concentration (Fig. 5). For FPH B, results showed that only R1 (8000 Da MWCO membrane) exerted a slight significant increase on CCK secretion (52.0 pM) when compared to FPH B crude extract (44.6 pM). For FPH C, no significant increase in CCK secretion was observed when compared to the crude extract (94.6 pM) after ultrafiltration and nanofiltration. The only significant effect was a decrease of the CCK-stimulating activity observed with the retentate R3 (79.3 pM) obtained after nanofiltration (300 Da MWCO membrane) (Fig. 5). With the purpose of visualising the impact of the successive filtration steps on the peptidic population, the molecular weight profiles of the crude extract and of the three different retentates were measured and expressed as a percentage of the total AUC for each FPH. As seen in Table 1, the separation of different filtration steps were not totally effective, thus peptidic populations were not significantly different between retentates and

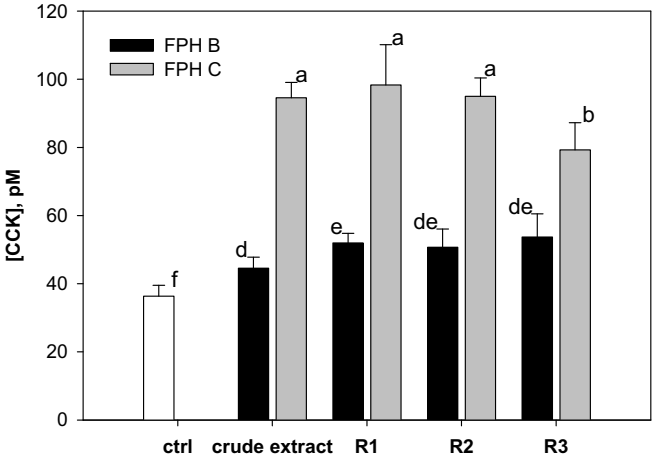

Fig. 5. CCK-releasing activity of STC-1 cells following exposure to retentates (0.5-% wt/vol) obtained after ultrafiltration (R1, 8000 Da MWCO and R2, 4000 Da MWCO) and nanofiltration (R3, 300 Da MWCO) of FPH B and C. Values are means ± SEM of four repeated measurements. Means without a common letter are different ($p < 0.05$).

crude extracts, which corroborated the weak difference obtained on STC-1 CCK secretion. In fact, for the FPH B, the retentate

Table 1
Molecular weight distributions of FPH B and C obtained before and after ultrafiltration and nanofiltration steps

Molecular weight (kDa)	% of total area							
	FPH B				FPH C			
	Crude extract	R1	R2	R3	Crude extract	R1	R2	R3
7–5	1.7	5.6	0.8	0.2	0.2	0.8	0.0	0.0
5–4	2.7	5.1	1.4	0.5	0.3	1.0	0.1	0.0
4–3	4.7	8.0	3.2	1.3	0.7	1.8	0.3	0.1
3–2	10.4	15.5	9.2	4.7	2.0	4.3	1.4	0.5
2–1.5	9.9	12.8	10.1	5.9	2.6	4.8	2.4	1.1
1.5–1	13.9	15.8	15.7	10.5	6.3	9.8	6.5	3.5
1–0.5	19.7	16.8	23.5	22.2	20.1	24.3	23.1	16.0
0.5–0.3	12.8	8.2	14.9	19.2	21.5	20.3	24.0	21.5
<0.3	22.5	9.8	21.0	35.5	46.2	32.8	42.3	57.2

Results were expressed in percentage of the total AUC of molecular weight profile obtained by SEC–FPLC on a Superdex Peptide HR 10/30.

obtained after the first ultrafiltration step (R1, 8000 Da MWCO membrane) was enriched in peptides larger than 1000 Da; the R2 (4000 Da MWCO membrane) was enriched in peptides smaller than 2000 Da, and the R3 (300 Da MWCO membrane), in peptides smaller than 1000 Da. For FPH C, R1 was enriched in peptides larger than 500 Da, R2 in peptides ranging from 300 to 1500 Da and the R3 in dipeptides and tripeptides smaller than 300 Da.

4. Discussion

In order to find appetite-suppressive molecules derived from fish protein by-products hydrolysates from industrial origin, we searched for their ability to stimulate CCK-releasing activity in the enteroendocrine STC-1 cell line. In this work we demonstrated for the first time that two hydrolysates from marine species by-products (blue whiting and brown shrimp) highly stimulated CCK secretion in the STC-1 cell line in a dose-dependent manner. The effect obtained with the hydrolysates was compared to a commercial albumin egg hydrolysate (AEH) as a positive control (Nemoz-Gaillard et al., 1998). Among the three FPH assayed, two of them showed a high CCK-stimulating activity: the hydrolysates from blue whiting and brown shrimp (FPH B and C) induced 1.8 and 2.4-fold increases over the effect obtained with AEH. STC-1 exposure to undigested protein (BSA) and free amino acid mixture (AAM) resulted in only a mild effect on CCK release. Toxicity of these two FPH were assayed and demonstrated that FPH B had no significant effect on STC-1 cell growth, whereas FPH C could highly stimulate cell proliferation at 0.5% wt/vol, or exert a significant inhibitory but non-toxic effect at 1.0% wt/vol. A recent study corroborated these results, reporting that black soybean protein hydrolysate with adipogenesis inhibitory activity, exerted (0.01–0.1% wt/vol) a non-toxic inhibitory effect on the cell growth of 3T3-L1 cells, in the same range as that obtained with FPH C (Kim, Bae, Ahn, Lee, & Lee, 2007).

Several studies showed that peptones from animal and vegetable origin were able to stimulate CCK release in STC-1 cells; among stimulant peptones, soybean β-conglycin, pork and country beans peptone were identified as CCK-releasing stimulants (Sufian, Hira, Asano, & Hara, 2007; Sufian et al., 2006). The weak effects obtained in the present work with BSA and AAM suggested, in accordance with preceding reports (Nemoz-Gaillard et al., 1998; Sufian et al., 2007), that the strong CCK-stimulating effects obtained with brown shrimp and blue whiting hydrolysates were mainly due to peptide molecules. The molecular sieving of blue whiting and brown shrimp hydrolysates showed that CCK-stimulating peptides were contained in different proportions in all the molecular weight fractions. That could be explained by the existence of different molecular weight peptides, generated from the same parent proteins, which possess common sequences, and/or by the ability of peptidic molecules to stimulate CCK secretion. Nevertheless, results showed that in both hydrolysates, small peptides (<1500 Da) exerted a greater CCK-stimulating effect than larger molecular weight peptides. Moreover, for the two FPH obtained from crustacean and fish, the more potent CCK-stimulating peptides had an apparent molecular weight ranging from 1000 to 1500 Da. Further studies are in progress to characterise the peptide sequence of active peptides from brown shrimp and blue whiting hydrolysates and identify if common peptide structures are involved in both FPH.

On the other hand, it has been reported that membrane processes (ultrafiltration and nanofiltration) could be used to de-salt and concentrate hydrolysate solutions on an industrial scale (Vandanjon et al., 2007). The preparation of both brown shrimp and blue whiting hydrolysates on a pilot scale was performed in order to evaluate the effect of ultrafiltration and nanofiltration on CCK-stimulating peptides concentration and molecular weight peptide distribution. Our results showed that these three filtration steps did not significantly increase the CCK-stimulating effects of FPH. This, in accordance with the observation that after each filtration step, and despite a variable enrichment of different molecular weight peptides, peptidic profiles were quite similar in the crude extract and in the different retentates. This low enrichment could be explained by the high retention rate of the membranes (Vandanjon et al., 2007). In this context, membrane processes could not be applied to industrially enrich our FPH with CCK-stimulating peptides.

In summary, we demonstrated for the first time that peptide molecules from fish and crustacean by-products hydrolysates were able to highly stimulate CCK secretion in intestinal endocrine STC-1 cells. This stimulating effect was mainly due to peptides of apparent molecular weight ranging from 1000 to 1500 Da. For the industrial utilisation of this FPH as potential appetite-suppressive products, further studies should be performed in both the rat (*in vivo*) and human (clinical), to confirm FPH effects on satiety.

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