Stability to thermal treatment of dipeptidyl peptidase IV (DPP-IV) inhibitory activity of a boarfish (*Capros aper*) protein hydrolysate when incorporated into tomato-based products

Running title: Heat-stable biofunctional tomato-based beverages

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Summary

Biofunctional peptide ingredients should retain their stability following standard processing operations in food-based delivery vehicles. A boarfish protein hydrolysate, exhibiting anti-diabetic activity was subjected to a range of thermal treatments following incorporation into tomato-based soup and juice products. The dipeptidyl peptidase-IV (DPP-IV) inhibitory activity and peptide profile of the hydrolysate within the products were assessed before and after thermal treatment. The treatments applied had no effect on the DPP-IV inhibitory activity or peptide profile of the protein hydrolysate. The heat-treated (90°C x 1 min and 121°C x 42 s) juice-fortified beverage had microbial counts within the acceptable limits for consumption when stored at 4°C for 30 days. Furthermore, the hydrolysate within the beverage products was resistant to simulated gastrointestinal digestion (SGID) regardless of whether it was heat or non-heat treated, or stored for 30 days at 4°C. Therefore, tomato-based beverages are suitable delivery vehicles for biofunctional peptide ingredients.

Keywords: antidiabetic; boarfish; dipeptidyl peptidase IV inhibition; functional food ingredient; peptide; protein hydrolysate; simulated gastrointestinal digestion; stability; thermal treatment.

1. Introduction

There is an established consumer demand and a rapidly growing market for foods and/or food ingredients which provide health benefits beyond their basic nutritional properties, i.e., functional foods. Food proteins contain a diverse array of short peptides (2-20 amino acids) which are released during food processing (by enzymatic hydrolysis or fermentation) or during gastrointestinal transit with the potential to beneficially modulate human health (Harnedy & FitzGerald, 2012 & 2013). Protein hydrolysates or peptides mediating such properties have potential applications as functional food ingredients. The selection of an appropriate food vehicle for delivery of a functional ingredient is of major importance as foods contain many components that can interact with peptides and mediate a reduction in peptide bioactivity and bioavailability (Kamdem & Tsopmo, 2019). Furthermore, one of the primary challenges associated with the incorporation of bioactive peptides into commercial products is the susceptibility of the peptides to modification during conventional food processing operations, e.g., thermal treatment. Due to its ability to inactivate microorganisms and spoilage enzymes thermal treatment is the most commonly used processing operation in the food industry (Rawson *et al.*, 2011). Thermal treatment of protein/peptide containing foods may induce substantial changes in the structure of the protein or protein hydrolysate. These include protein/peptide denaturation or aggregation, interactions between protein/peptide/amino acids and other components within the food matrix e.g., the generation of Maillard reaction products, destruction of heat sensitive amino acids and the formation of dehydroalanine-derived cross-links (Singh, 1991; Korhonen *et al*., 1998; Gerrard, 2002; Vasbinder *et al*., 2003; Rao *et al*., 2016).

To date, information regarding the influence of routinely used industrial processing and storage conditions on the bioactivity, in particular the potential anti-diabetic activity, of protein hydrolysates when incorporated into different food matrices is limited. The majority of the research reported to date in this area has been performed with aqueous solutions of protein hydrolysates/peptides and not with delivery vehicles containing the functional protein hydrolysate/peptide ingredient (Hwang, 2010; Wu *et al*., 2014; Zhu *et al.*, 2014; Lai *et al*., 2016; Wali *et al*., 2017; Rivero-Pino *et al*. 2020).

In a previous study we have shown that a boarfish-derived protein hydrolysate (BPH) exhibits promising anti-diabetic activity both *in vitro* (DPP-IV inhibition and pancreatic β cells and enteroendocrine cells in culture) and in a small animal study (Parthsarathy *et al*., 2019). While the hydrolysate had no effect on biomarkers of glycaemic control and satiety at the dose given in a randomised controlled human intervention crossover study with healthy adults, a significant increase in satiety rating was reported at 180 min following consumption of a beverage fortified with the hydrolysate compared to the unfortified control (Crowe *et al*., 2018). Furthermore, a number of peptides with *in vitro* and *in situ* cell-based (Caco-2) DPP-IV inhibitory activity and *in situ* insulin secretory activity have been identified within the BPH (Harnedy-Rothwell *et al*., 2020). Based on the above results the BPH has potential applications as an antidiabetic functional food ingredient.

In order for protein hydrolysates to be utilised as functional food ingredients they must retain their activity when incorporated into food matrices which experience various processing and storage conditions. Therefore, this study determined the effect of thermal treatment (pasteurisation and sterilisation) and storage conditions on the *in vitro* dipeptidyl peptidase-IV (DPP-IV) inhibitory activity of a BPH when incorporated into two tomato-based products (a soup and a beverage). Furthermore, the effect of fortification of a tomato-based matrix, heat treatment and storage conditions on the susceptibility of the BPH to simulated gastrointestinal digestion (SGID) was investigated.

2. Materials and methods

2.1. Materials and chemicals

H-Gly-Pro-7-amino-4–methyl coumarin (AMC) and Diprotin A were obtained from Bachem Feinchemikalien (Bubendorf, Switzerland). Kjeldahl catalyst tablets were obtained from VWR International (Dublin, Ireland). Low nitrogen sodium hydroxide (40% (w/v)) was obtained from TE Laboratories Ltd. (Carlow, Ireland). Sulphuric acid (low nitrogen) was purchased from Lennox Laboratory Supplies Ltd (Dublin, Ireland). Corolase® PP was provided by AB Enzymes (Darmstadt, Germany) and BC pepsin was kindly provided by Biocatalysts Ltd (Cardiff, Wales, United Kingdom). All

other reagents including Alcalase® 2.4L and Flavourzyme® 500L and DPP-IV, from porcine kidney (≥10 units/mg protein) were supplied by Sigma Chemical Company Ltd. (Wicklow, Ireland). Samples of minced deboned boarfish (*Capros aper*) meat was kindly provided by Bio-Marine Ingredients Ireland, Ltd., Lough Egish Food Park, Castleblaney, Co. Monaghan, Ireland. A tomato based chilli soup, containing tomatoes, red peppers, red chillies and chilli flakes, garlic, olive oil, fresh basil and vegetable stock was prepared at Ulster University, Coleraine, Co. Derry, Northern Ireland.

2.2. Generation of BPH at semi-pilot scale

The BPH was generated with Alcalase 2.4L and Flavourzyme 500L at semi-pilot scale as described previously (Harnedy-Rothwell *et al*., 2020).

2.3 Quantification of protein equivalent content

The protein equivalent content of the BPH was measured by the macro-Kjeldahl procedure (Connolly *et al*., 2013) using a nitrogen to protein conversion factor of 6.25 (Kristinsson and Rasco, 2000).

2.4. Preparation, processing and storage of tomato soup and juice products and control samples.

Tomato based chilli soup samples with (5.83% (w/w)) and without BPH were prepared. An equivalent aqueous solution of the hydrolysate was also prepared (e.g., 5.83 % (w/w)) at pH 5.26) and used as a control. The samples containing the hydrolysate were heated at 80 and 95 ^{o}C for 3 min and at 121 ^{o}C for 42 s (sterilisation conditions) in an oil bath and were then rapidly cooled by placing on ice (Gould, 1992). A second set of samples which received no heat treatment was also prepared.

V8® vegetable juice (Kelsen A/S Group, Denmark) was purchased from a health food shop (Limerick, Ireland). Vegetable juice samples with and without BPH (2.33% (w/v) powder equivalent to 1.64% (w/v) protein/hydrolysate) were prepared. An equivalent aqueous solution containing the hydrolysate was also prepared (e.g., 2.33% (w/w) and pH 4.71) and used as a control. The samples containing the hydrolysate were heated at 90 ^{o}C for 1 min (pasteurisation conditions) and 121 ^{o}C for 42 s (sterilisation

conditions) in an oil bath and then rapidly cooled by placing on ice (Gould, 1992). Samples were stored for 30 days at 4 °C and were subsampled on Day 0, 1, 3, 7, 10, 15, 20, 25 and 30. A second set of samples, which received no heat treatment (and which were not stored for 30 days at 4 °C) were also prepared to investigate the effect of heat treatment on the DPP-IV inhibitory activity and peptide profile of the hydrolysate on Day 0.

2.5. Simulated gastrointestinal digestion (SGID)

SGID was performed as described by Walsh *et al*. (2004) with modifications. In brief, the pH of the water and soup/juice products containing 2.0 % (w/v) hydrolysate was altered to pH 2.0 using 6M HCl and were then incubated at 37˚C for 90 min with pepsin at an E:S of 2.5% (w/w). The pH of the samples were then adjusted to pH 7.0 using 5N NaOH and incubated at 37˚C with Corolase PP (E:S of 1.0 % (w/w)) for a further 150 min. Enzyme activity was inactivated by heating at 80˚C for 20 min.

2.6. Peptide profile

The peptide profiles of the samples were determined by reverse-phase ultra-performance liquid chromatography (RP-UPLC) as described by Nongonierma & FitzGerald (2012) at a flow rate of 0.2 ml/min.

2.7. Quantification of DPP- IV inhibitory activity

DPP-IV inhibition was determined as described by Harnedy *et al.* (2015). All assays were performed in triplicate (n=3). Activity results were expressed as % inhibition at a BPH concentration of 2 mg/ml. Diprotin A was used as a positive control.

2.8. Microbial analysis

Samples of fortified juice which underwent thermal treatment and were stored at 4˚C for 30 days, were diluted using Ringers solution and plated onto standard plate count agar (PCA) to determine the

153 total plate count (TPC). The plates were incubated at 37˚C for 48 h and the colonies were then
154 counted.
155
156 **2.9. Statistical analysis**
157 All statistical analysis was performed using the SPSS statistical software program (Version 22, IBM Inc.,
158 Chicago, IL, USA). Statistical significance ($p<0.05$) was determined using one-way analysis of variance
159 (ANOVA) followed by Tukey's and Games–Howell post-hoc tests, where applicable.
160
161 **3.0 Results and Discussion**
162 **3.1 Selection of hydrolysate delivery vehicle and assessment of thermal heat treatment conditions**
163 The nature and type of food products that can act as delivery vehicles for biofunctional peptide
164 ingredients depends to a large extent on the stability of the active peptides within the food matrix
165 selected, the processing conditions employed and the stability of the peptides during storage. Two
166 tomato-based products, a soup and juice which in general undergo pasteurisation and sterilisation
167 treatments to enhance shelf-life, were selected as the delivery vehicles in this study. Selection of these
168 formats was based on the following: (a) the savoury nature of the soup/juice could mask the
169 odour/taste associated with the BPH, (b) the low glycaemic load (GL: 4 for V8® vegetable juice
170 (Atkinson *et al*., 2008)) and negligible lipid content makes it suitable for delivery of a functional
171 ingredient for glycaemic management and (c) its intrinsically low protein content (approximately 0.9
172 % (w/v)). The protein equivalent content of the BPH generated at semi-pilot scale used herein was
173 71.35 % (w/w). The quantity of hydrolysate incorporated into the soup and juice product in this study
174 was based on the amount of hydrolysate powder (3.5g) which mediated an anti-diabetic response *in*
175 *vivo* (Parthsarathy *et al*., 2019). Taking into account a typical serving of soup (60 ml) and juice (150
176 ml), the hydrolysate fortification level utilised was 5.83 and 2.33 % (w/v) for the soup and juice
177 product, respectively. This in turn equates to a protein equivalent content of 4.16 and 1.64 % (w/v),

178 respectively, and an overall dose of 2.50 and 2.46 g protein equivalent, respectively. The fortified
179 tomato-based soup sample utilised in this study was the same as that used in a randomised controlled
180 human intervention crossover study designed to assess the effect of hydrolysate intake (3.50 g)
181 equivalent to 2.50 g protein on biomarkers of glycaemic control and satiety in healthy adults (Crowe
182 *et al.,* 2018). Thermal processing is conventionally used in the food industry to inactivate
183 microorganisms and enzymes and to thereby extend the shelf-life of food products. In the tomato
184 processing industry, common conditions used for pasteurisation and sterilisation of tomato-based
185 products involve heating at 90^{o} C x 1 min and 121 ^{o}C x 42 s, respectively (Gould, 1992). Furthermore,
186 the recommended instructions for heating tomato-based soup products prior to consumption is to
187 heat at 80^{o} C x 3 min, however, the common practice of heating soup involves heating at 95^{o} C x 3 min.
188 Studies were therefore performed to determine if the routine thermal treatment conditions used in
189 industry to preserve tomato-based products (soup and juice) and the suggested or actual conditions
190 used to heat soup prior to consumption had an effect on the *in vitro* DPP-IV inhibitory activity and
191 peptide profile of the BPH when incorporated into these delivery formats.
192
193 3.2 **Effect of heat treatment on the DPP-IV inhibitory activity and peptide profiles of the boarfish**
194 **hydrolysate when incorporated into tomato-based soup and juice products.**
195 As shown in Tables 1 and 2, none of heat treatments employed/tested had any effect on the DPP-IV
196 inhibitory activity of the aqueous BPH solutions. Furthermore, no differences were observed in the
197 RP-UPLC profiles of the aqueous hydrolysate solutions irrespective of whether they were subjected or
198 not subjected to thermal treatment (data not shown). This indicates that the hydrolysate was
199 thermostable and that the recommended pasteurization (90°C x 1 min) and the recommended and
200 commonly used reheating conditions for soup (80^{o} C x 3 min and 95^{o} C x 3 min, respectively) and the
201 sterilization (121°C x 42 s) conditions employed had no effect on the hydrolysate peptide profile and
202 the DPP-IV inhibitory activity.

The results reported herein are consistent with the findings of Lai *et al*. (2016), who showed that walnut protein hydrolysates were stable and retained their antioxidant activity following heat treatment at 65°C x 30 min and 121°C x 20 min. Furthermore, the bioactivity exhibited by rapeseed, tuna cooking juice and bovine casein-derived protein hydrolysate/peptides was reported to be stable when subjected to temperatures in the range 0-100 °C (Hwang, 2010; Wu *et al*., 2014; Wali *et al*., 2017). However, the angiotensin converting enzyme inhibitory activity mediated by the rapeseed and casein-derived protein hydrolysate/peptides significantly decreased when heated at temperatures above 100 °C. However, it should be noted that the duration of thermal treatment was 1 and 2 h, respectively (Wu *et al*., 2014; Wali *et al*., 2017). Antioxidant peptides derived from Jinhua ham were shown to be less thermostable than the protein hydrolysate/peptides described above (Zhu *et al*., 2014). The ham peptides were shown to lose activity when heated above 60°C.

A similar result to that observed with aqueous hydrolysate solutions herein was seen with the tomato-based soup and juice products containing the BPH. The thermal treatments employed were shown to have no effect on the DPP-IV inhibitory activity (Table 1 and 2). As shown in Table 1 no significant difference ($p>0.05$) was observed in the *in vitro* DPP-IV inhibitory activity of the fortified soup samples which underwent no heat treatment (72.29 ± 1.18 %) or which were heated at 80 °C x 3 min (72.34 ± 1.71 %), 95° C x 3 min (73.47 ± 2.97 %) and 121°C x 42 s (72.45 ± 2.66 %) when tested at 2 mg/ml. Similarly, no significant difference was observed in the DPP-IV inhibitory activity (Table 2) of the fortified juice samples which received no heat treatment (74.23 ± 2.56 %) or were treated at 90° C x 1 min (73.67 ± 4.39 %) and 121°C x 42 s (72.74 ± 4.63 %). Similar RP-UPLC profiles were observed for the non-heat treated aqueous and tomato beverage samples containing the hydrolysate (data not shown). This indicates that there was no negative interaction between the tomato-based matrix and the hydrolysate. Furthermore, no differences were observed in the RP-UPLC profiles of the hydrolysate incorporated into soup and juice matrices pre- and post-heat treatment (Fig 1a and 2). This indicates that that there was no interaction between the tomato-based matrix and the hydrolysate during the heat treatments employed. This suggests that the biofunctional BPH is stable under the thermal

conditions tested and could be incorporated into a tomato-based matrix and withstand the standard industrial thermal processing conditions required for the preservation and reheating of these commercially available products.

Food products commonly undergo heat treatment to improve their safety and/or extent their shelf-life (Rawson *et al.*, 2011). Therefore, the effect of storage (4 °C for 30 days) on the bioactivity and the peptide profile of heat treated (90^{o} C x 1 min and 121°C x 42 s) hydrolysate containing juice samples was assessed. A similar study with an aqueous suspension of the hydrolysate was performed for comparative purposes. Firstly, microbial analysis of the fortified juice samples which underwent pasteurisation and sterilisation and which was stored for 30 days at 4 °C shows that the heat treatments applied were effective in inhibiting the growth of potential spoilage organisms during the course of 30 days' storage. The sterilised sample was free from bacteria, while the pasteurised sample had a total count of 400 cfu/ml at day 30, which is within the acceptable limits for human consumption (FSA, 2016). As shown in Table 2, the DPP-IV inhibitory activity of the aqueous solution did not change over the storage period assessed irrespective of the thermal treatment applied. Furthermore, the DPP-IV inhibitory activity exhibited by the juice product fortified with the hydrolysate also remained the same over the storage period assessed again irrespective of the thermal treatment applied. This indicates that the hydrolysate was stable over the storage period assessed (as seen with the aqueous hydrolysate solution) and that the juice vehicle had no negative effect on the *in vitro* anti-diabetic activity of the hydrolysates during storage. This was also seen following RP-UPLC analysis of the samples where no difference was observed in the peptide profiles obtained for the thermally and non-thermally treated fortified juice samples stored for 30 days at 4 °C (Figure 2). Similar results were reported for a round scad protein hydrolysate stored at 4 and 25 °C for 6 weeks where Thiansilakul *et al*. (2007) showed that the hydrolysate retained its antioxidant activity during the storage period. While a small decrease in DPPH radical-scavenging activity was observed within the first week of storage at 4 and 25 °C, no further change in DPPH radical-scavenging activity was observed thereafter. No significant difference in the reducing power or the metal-chelating activity of the protein

hydrolysate was observed within the first 2 weeks of storage at 4 and 25 °C, however, a slight decrease in activity was observed between weeks 2-6. It was shown that the antioxidant activity exhibited by the round scad protein hydrolysate was more stable when stored at 4 than at 25 °C (Thiansilakul *et al*., 2007). Milk protein derived antihypertensive peptides, RYLGY and AYFYPEL, were also shown to be stable when incorporated into a yoghurt product and stored at 4 °C for 28 days (Contreras *et al.,* 2011).

While the storage temperature selected in this study (4 °C) represents that commonly utilised for a pasteurised product, a sterilised product would usually be stored at room temperature and for a longer period of time (>30 days). It would be interesting to investigate if the sterilised hydrolysate containing product could retain their activity following storage at ambient temperature for extended periods of storage.

3.3 Effect of SGID on the DPP-IV inhibitory activity and RP-UPLC profiles of a boarfish hydrolysate when incorporated into tomato-based soup and juice products

In order to exert a biological effect *in vivo*, the integrity of active peptides within the food vehicle must be maintained during digestion and transport. In a previous study we have shown that the BPH was resistant to SGID (Harnedy-Rothwell *et al*., 2020). This was also observed herein in Table 1 where no significant differences (P>0.05) were observed in the DPP-IV inhibitory activity by the non-heat treated aqueous hydrolysate samples pre- and post-SGID.

As shown in Table 1 and 2, no significant differences (P>0.05) were observed in the DPP-IV inhibitory activity pre- and post-SGID for the non-heat and heat-treated soup and juice samples containing the hydrolysate. The fact that similar DPP-IV inhibitory activity and peptide profiles were observed for the non-heat treated soup and juice samples before and after SGID (Tables 1 and 2, Figures 1, 2 and 3) indicates that components within the tomato-based vehicles did not alter the hydrolysate's resistance to SGID. Furthermore, similar results were seen with the heat treated soup and juice samples, which

would indicate that the heat treatments employed had no effect on the *in vitro* digestion status of the hydrolysate (Tables 1 and 2, Figures 1, 2 and 3).

To our knowledge there is no information available on the effect of food formulation and processing on the SGID status of foods containing protein hydrolysates. However, it is possible that the bioactivity of other hydrolysates, when incorporated into a food matrix and/or subjected to various processing conditions may be improved or reduced following SGID. Modifications to peptide structure induced during processing may alter the digestibility of the peptides producing breakdown products which may be more or less potent than the original peptides. Furthermore, peptides that would otherwise be rendered inactive by exposure to the SGID enzymes when taken in aqueous format may be protected when incorporated in a food matrix. However, *in vivo* investigations would be required to confirm the observations herein.

4. Conclusion

This study has shown that the thermal processing and storage conditions used routinely to enhance the shelf-life of tomato-based juice and soup products had no effect on the *in vitro* anti-diabetic activity and peptide profile of a BPH incorporated therein. Furthermore, the tomato-based juice and soup vehicles and the processing and storage conditions assessed had no effect on the SGID status of the BPH. Therefore, the present study highlights the potential of tomato-based products as vehicles for delivery of protein hydrolysates. While preliminary sensory analyses performed prior to the human study indicated that the savoury flavour of the tomato-based chilli soup had the ability to mask the fishy taste/odour of the BPH within, comprehensive organoleptic and acceptability studies need to be performed to ensure that the fortified products are accepted by a broad range of consumers. It must also be noted that while the tomato-based beverages used herein had low glycaemic loads, in general, commercially produced tomato soup can contain sugar which would add significantly to the glycaemic load and render it unsuitable as a delivery vehicle for antidiabetic agents. Furthermore, fortification of foods with the BPH presents a unique opportunity to increase the commercial value of a low-value

underutilised fish species through the production of nutritionally and biofunctionally enriched beverage products.

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Conflict of Interest

The authors would like to declare that there are no conflicts of interest.

Ethical Guidelines

Ethics approval was not required for this research.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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425 **Table 1.** Impact of different thermal treatments and simulated gastrointestinal digestion (SGID) on the
426 dipeptidyl peptidase-IV (DPP-IV) inhibitory activity of a boarfish (*Capros aper*) protein hydrolysate
427 (BPH) in an aqueous and soup matrix.
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Sample	Heat treatment	DPP-IV inhibition (%)	
		Before	After SGID
BPH + H_2O	None	63.27 ± 1.18[a]	62.07 ± 1.82[a]*
	80 °C x 3 min	62.13 ± 1.96[a]	62.25 ± 2.13[a]*
	95 °C x 3 min	63.72 ± 2.39[a]	65.39 ± 0.11[a]*
	121 °C x 42 s	61.56 ± 1.02[a]	61.68 ± 1.71[a]*
BPH + Soup	None	72.79 ± 1.18[a]	76.11 ± 2.64[a]*
	80 °C x 3 min	72.34 ± 1.71[a]	76.84 ± 0.17[a]*
	95 °C x 3 min	73.47 ± 2.97[a]	75.43 ± 2.26[a]*
	121 °C x 42 s	72.45 ± 2.66[a]	75.06 ± 0.39[a]*
Soup + H_2O	None	12.70 ± 1.96[a]	nd
	80 °C x 3 min	12.47 ± 1.42[a]	nd
	95 °C x 3 min	10.20 ± 0.96[a]	nd
	121 °C x 42 s	10.01 ± 1.04[a]	nd

429 Mean ± SD (n=3). The concentration of hydrolysate used in all cases was 2 mg/ml. For each sample
430 values with different letters are significantly different at $p<0.05$. *: indicates that no significant
431 difference ($p>0.05$) was observed before and after SGID. nd: not determined
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Table 2. Impact of heat treatment, storage time and simulated gastrointestinal digestion (SGID) on the dipeptidyl peptidase-IV (DPP-IV) inhibitory activity of a boarfish (*Capros aper*) protein hydrolysate (BPH) in an aqueous and tomato-based juice matrix.

Sample	Heat treatment	Storage time (Days)	DPP-IV inhibition (%)	
			Before SGID	After SGID
BPH + H_2O	None	0	61.72 ± 2.47[a]	66.66 ± 2.71[a]*
	90 °C x 1 min	0	63.05 ± 3.71[a]	67.59 ± 1.06[a]*
	90 °C x 1 min	15	62.49 ± 3.60[a]	64.81 ± 1.49[a]*
	90 °C x 1 min	30	64.21 ± 1.56[a]	66.58 ± 1.94[a]*
	121 °C x 42 s	0	62.80 ± 1.44[a]	64.26 ± 1.72[a]*
	121 °C x 42 s	15	63.00 ± 2.39[a]	66.98 ± 0.63[a]*
	121 °C x 42 s	30	62.09 ± 3.61[a]	66.30 ± 0.34[a]*
BPH + Juice	None	0	74.23 ± 2.56[a]	80.34 ± 3.03[a]*
	90 °C x 1 min	0	73.67 ± 4.39[a]	76.23 ± 1.25[a]*
	90 °C x 1 min	15	73.93 ± 2.52[a]	76.46 ± 1.91[a]*
	90 °C x 1 min	30	74.40 ± 2.85[a]	77.58 ± 1.85[a]*
	121 °C x 42 s	0	72.74 ± 4.63[a]	75.03 ± 1.60[a]*
	121 °C x 42 s	15	74.49 ± 4.08[a]	75.73 ± 2.38[a]*
	121 °C x 42 s	30	75.56 ± 3.21[a]	80.39 ± 1.91[a]*
Juice + H_2O	None	0	31.46 ± 0.42[a]	nd
	90 °C x 1 min	0	30.09 ± 1.85[a]	nd
	90 °C x 1 min	15	31.12 ± 2.84[a]	nd
	90 °C x 1 min	30	29.53 ± 5.14[a]	nd
	121 °C x 42 s	0	27.36 ± 3.16[a]	nd
	121 °C x 42 s	15	29.39 ± 5.14[a]	nd
	121 °C x 42 s	30	29.85 ± 3.16[a]	nd

Mean ± SD (n=3), The concentration of hydrolysate used in all cases was 2 mg/ml. For each sample values with different letters are significantly different $p<0.05$. *: indicates that no significant difference ($p>0.05$) was observed before and after SGID. nd: not determined

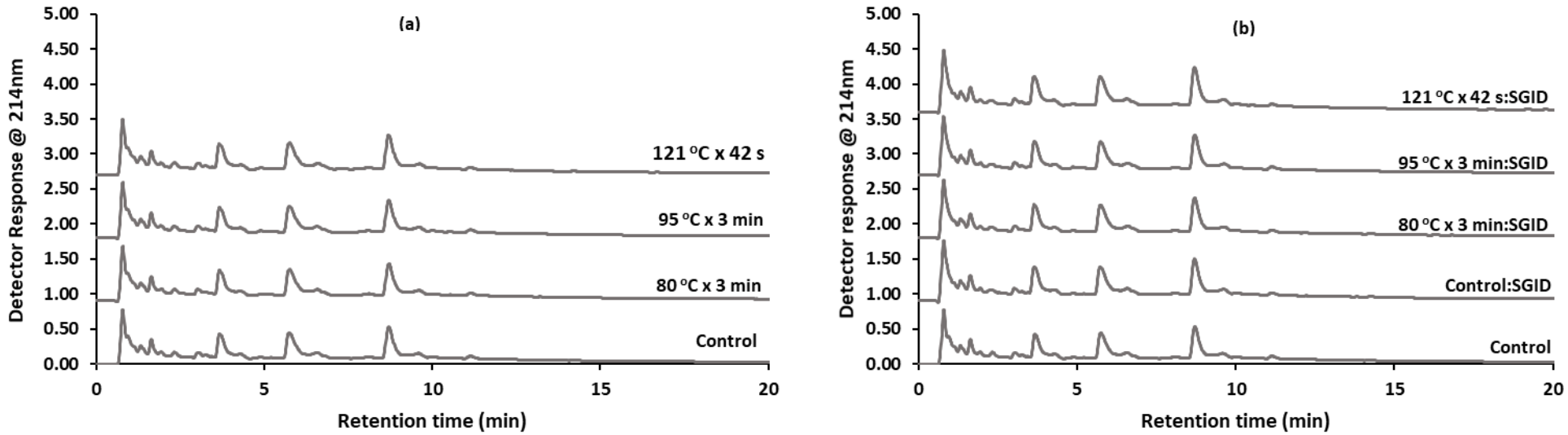

Fig. 1. Reverse-phase ultra-performance liquid chromatography profiles of soup samples fortified with a boarfish (*Capros aper*) protein hydrolysate (5.83 % (w/w)) (a) before and after different heat treatments and (b) before and after simulated gastrointestinal digestion (SGID). Control: No heat treatment

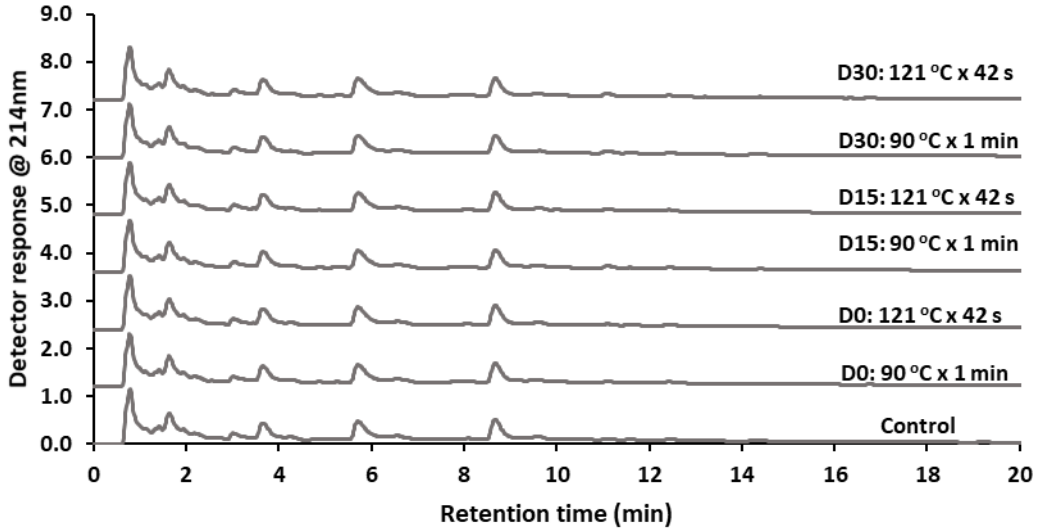

Fig. 2. Reverse-phase ultra-performance liquid chromatography profiles of juice samples containing a boarfish (*Capros aper*) protein hydrolysate (2.33% (w/v)) following two different heat treatments (90 °C x 1 min and 121 °C x 42 s) and subsequent storage at 4 °C. Control: No heat treatment, D0: Day 0, D15: Day 15, D30: Day 30.

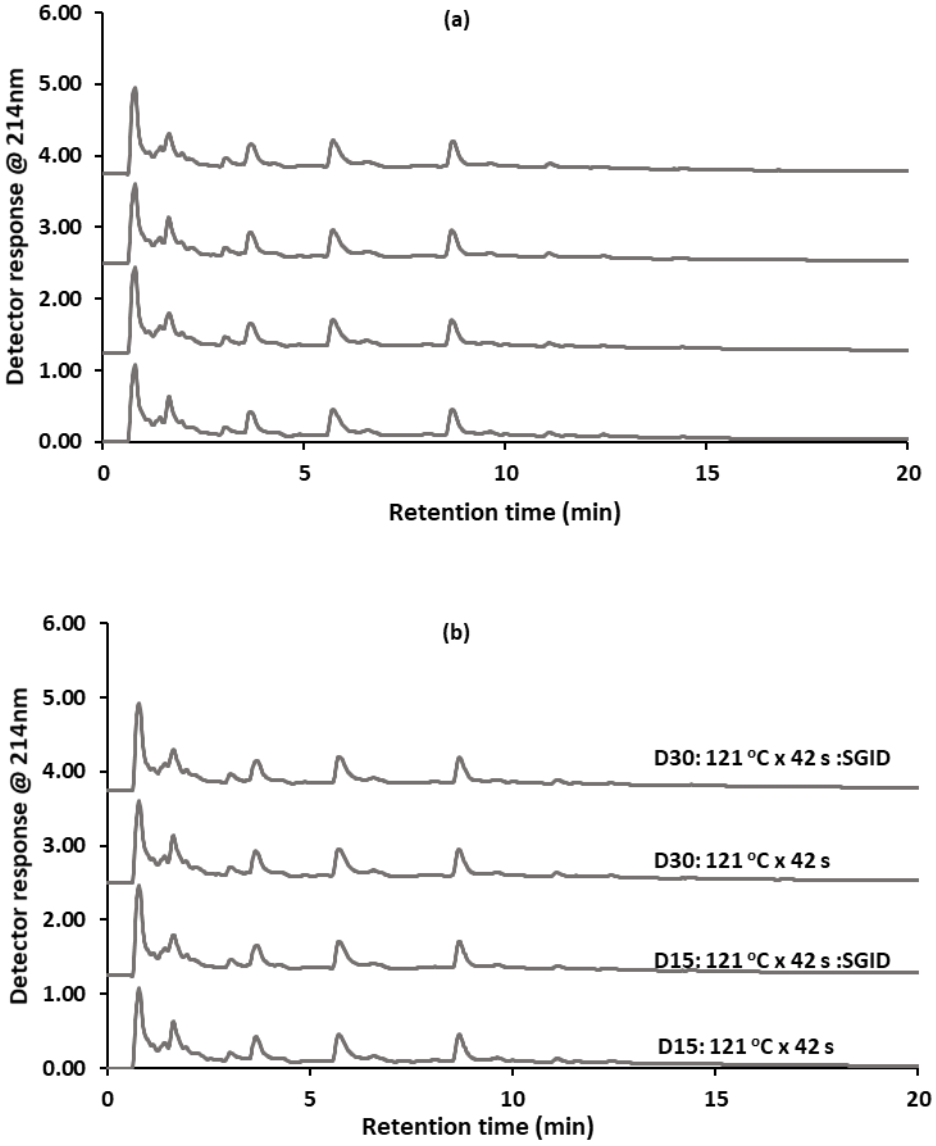

Fig. 3. Reverse-phase ultra-performance liquid chromatography profiles of juice samples containing a boarfish (*Capros aper*) protein hydrolysate (2.33% (w/v)) before and after simulated gastrointestinal digestion (SGID) following different heat treatments (a) 90 °C x 1 min and (b) 121 °C x 42 s and subsequent storage at 4 °C for 15 (D15) and 30 (D30) days.

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