The effects of cooking salmon sous-vide on its antithrombotic properties, lipid profile and sensory characteristics

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Abstract

Fish contains bioactive polar lipids (PL) and is mainly consumed cooked. The aim of this study was to evaluate the sensory characteristics of sous-vide cooked salmon and the in vitro cardio-protective properties of its PL. PL were extracted from brined and un-brined sous-vide preparations in 52 °C, 65 °C, and 80 °C, while their antithrombotic cardio-protective properties were assessed in human platelets and their fatty acid (FA) content was evaluated by LC-MS. Sensory tests were performed using rating followed by check-all-that-apply (CATA). Mild temperatures (52 °C, 65 °C) did not affect the inhibitory effect of PL from brined and un-brined salmon, against human platelet aggregation induced by platelet-activating factor (PAF), thrombin, adenosine diphosphate (ADP) or collagen. In higher temperatures used for pasteurization (80 °C), a reduction of antithrombotic properties was observed in PL from both un-brined and brined salmon samples. This reduction was accompanied by a decrease of their n3 eicosapentaenoic acid (EPA) and overall polyunsaturated FA (PUFA) content, but only in the PL of the un-brined salmon preparations. Thus, changes in the fatty acid content of PL of all sous-vide preparations, and especially of specific PUFA, seem to be associated with the observed changes in their antithrombotic potency. Changes in the content of the n-3 docosapentaenoic acid (DPA), a precursor of EPA and docosahexaenoic acid (DHA), seem to be associated with differences observed in the antithrombotic potency of PL from different sous-vide salmon preparations. Taste attributes were not affected by the conditions of sous-vide preparations, whereas slight textural differences were observed in samples treated at 65 °C and 80 °C. These outcomes, if combined with the observed low values of the n-6/n-3 PUFA ratio in PL of all sous-vide preparations, further suggest a beneficial role for such a mild cooking procedure for preserving the antithrombotic and cardio-protective properties of salmon without affecting its sensory characteristics.

1. Introduction

Salmon has been characterized as an oily fish (Gil et al., 2015). It is rich in bioactive compounds and especially in cardio-protective lipids, thus is widely recommended as beneficial to consume fish intake in several dietary guidelines. Indeed, the nutritional guidelines of healthy dietary patterns favor oily fish such as salmon to be consumed once per week (Goel et al., 2018). Consumption of oily fish such as salmon regularly has been shown to have cardio-protective effects (Goel et al., 2018). Initially this was attributed to the high content of salmon in omega-3 polyunsaturated fatty acids (n-3 PUFA), such as the eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids (FA) that displaces the intakes of pro-atherosclerotic foods higher in omega-6 PUFA (n-6 PUFA), saturated fatty acids (SFA) and trans fatty acids (trans-FA) in the diet (Gil et al., 2015, Goel et al., 2018, Simopoulos, 2008).

Thus, significant research in salmon has focused on its EPA and DHA n-3 PUFA content, which have been shown to demonstrate several beneficial properties for human health and especially improved platelet functionality and cardiovascular health (Adili et al., 2018; Mori et al., 2002).
In salmon, its polar lipids (PL), and especially those rich in n-3 PUFA, have exhibited strong antithrombogenic properties against platelet aggregation induced by the potent inflammatory mediator, platelet-activating factor (PAF), but also against other thrombogenic mediators and classic platelet agonists such as thrombin, collagen and ADP (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019). These platelet agonists act through specific membrane receptors, while even though their thrombogenic pathways were initially thought to be independent, recent findings have emphasized that they are interconnected, and especially the pathways between PAF, thrombin and collagen (Lordan et al., 2020; Tsoupras et al., 2009). We have previously described the importance of both PAF and thrombin in several thrombogenic manifestations implicated in the onset and development of both chronic disorders (Lordan et al., 2020; Tsoupras et al., 2009; Tsoupras, Lordan, & Zabetakis, 2019) and severe thrombogenic complications, such as those observed during persistent infections, like the COVID-19 (Tsoupras, Lordan, & Zabetakis, 2020). Thus understanding the crosstalk between strong anti-PAF and anti-thrombin compounds found in foods, and especially in salmon (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019), against the PAF and thrombin pathways involved in platelet-related thrombus formation, is necessary to identify potential targets for the development of new antiplatelet functional foods/compounds.

Marine PL, including those found in salmon, possess a plethora of beneficial bioactivities against inflammation-related disorders and high bioavailability of their bioactive n-3 PUFA into plasma lipoproteins, cell membranes, and several tissues, including those with difficult accessibility such as the brain, including their abilities to beneficially affect the activities, levels and metabolism of PAF towards homeostasis and cardio-protection (Bjørndal et al., 2014; Burri et al., 2012; Davidson et al., 2012; Lordan et al., 2020; Nasopoulou, Tsoupras, et al., 2011; Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019). It is generally accepted that due to consumers requirements, fish including salmon is generally consumed cooked and sometimes smoked or cured. The effects of cooking on fish PL bioactivities against platelet aggregation has been previously studied in fried cod (Panayiotou et al., 2000) and grilled sardines (Nasopoulou, Psani, et al., 2011). For salmon, there are studies focusing mostly to the effects of cooking on its fatty acid composition, while reduction of its health benefits are mostly correlated with any changes in the n-3 PUFA content of the overall lipid content of cooked salmon (Leung et al., 2018). Harsh traditional cooking methods such as frying, steaming and pan-frying are used worldwide and they have been found to degrade the n-3 PUFA content of fish due to the high temperatures generated which consequently leads to the breaking of double bonds leading to oxidation and highly oxidised molecules related to oxidative stress (Leung et al., 2018).

Sous-vide cooking has gained popularity because of its mild cooking conditions on meat and fish preparations, nut also because it allows the infusion of various flavours. Sous-vide is a French term meaning ‘under vacuum’ and the foodstuff, including fish, is cooked using precise temperatures and times by utilising heat-stable vacuumed pouches” (Sidney, 2012). Vacuuming preserves the nutrients and taste of the foodstuff. The use of vacuum sealing in sous-vide provides a very efficient and consistent transfer of heat from water to food product and increase the shelf life of products due to the absence of oxygen in the vacuum sealed pouches, therefore inhibition of lipid oxidation occurs. From the sensory analysis point of view, the sous-vide process conserves the food stuffs volatile compounds and due to the anaerobic environment of the foodstuff, oxidative deterioration is avoided which is widely regarded as the principal cause of off-flavours and odours in other cooking techniques. In addition, the benefits of sous-vide from a human health perspective are correlated to the conservation of the nutritional value of the foodstuff in comparison to conventional cooking methods such as boiling and frying, due to the use of low cooking temperatures which in turn inhibit the loss of compounds such as vitamins and anti-oxidants through solubilisation and volatilisation (Baldwin, 2012; Ibora-Bernad et al., 2013; Rosewski et al., 2018). To the best of our knowledge, there aren’t any studies reporting the effects of cooking, and especially of mild cooking techniques such as sous vide on the bioactivities and fatty acid composition of salmon PLs.

The aim of this study is to elucidate for the first time the effects of cooking sous-vide salmon, at different temperatures and brining conditions, on the biofunctionality and fatty acid content of its PL, while also evaluating potential alterations of the sensory characteristic of sous-vide cooked salmon at these conditions. This will allow the determination of the optimum conditions of the sous-vide cooking procedures that result in minimal effects on the antithrombogenic and cardio-protective properties and sensory attributes of salmon.

To achieve this goal, the antithrombogenic properties of PL extracted from brined and un-brined sous-vide cooked salmon in several temperatures (52 °C, 65 °C and 80 °C) were assessed in human platelets and their structures and fatty acid (FA) content was elucidated by LC-MS analysis (Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019). In addition, sensory analysis on all sous-vide salmon preparations was also performed using napping followed by check-all-that-apply (CATA) methodology, in order to detect the effects of this emerging cooking process in sensory characteristics of cooked salmon such as smell, taste, colour, texture as well as the formation of off-flavours and off-odours (Bennett & Hayes, 2012; Dehlholm et al., 2012; Diaz et al., 2009; Reinbach et al., 2014). Napping and CATA are two sensory profiling methods which are based on consumers’ perception (Varela & Ares, 2012) and as research shows they can correlate with findings from descriptive profile conducted by trained assessors (Ares et al., 2010). They are both quick and relatively easy to use, while capturing the voice of the consumers (Reinbach et al., 2014). Napping quantifies overall sensory similarities and dissimilarities between products, and because in some cases these can be difficult to describe CATA, was also employed in the current study to add information on the description of the products (Ares et al., 2010).

2. Materials and methods

2.1. Materials and instruments

All plastic and glass consumables, as well as reagents and solvents of analytical grade, were purchased from Fischer Scientific Ltd. (Dublin, Ireland). Evacuated sodium citrate 5-monovettes and 20G safety needles for blood sampling were purchased from Sarstedt Ltd (Wexford, Ireland). The platelet aggregation bioassay was carried out on a Chronolog-490 two-channel turbidimetric platelet aggregometer (Havertown, PA, USA), coupled to the accompanying AGGRO/LINK software package. All platelet aggregation consumables were purchased from Labmedics LLP (Abingdon on Thames, UK). Standard PAF, thrombin, and bovine serum albumin (BSA) were purchased from Sigma Aldrich (Wicklow, Ireland), while collagen and ADP were from Chronolog (Havertown, PA, USA). Centrifugations were carried out on an Eppendorf 5702R centrifuge (Eppendorf Ltd., Stevenage, UK). Spectrophotometric analysis was carried out on a Shimadzu UV-1800 spectro-photometer (Kyoto, Japan). All salmon samples were placed in vacuum pouches and a Multivac Vacuum machine (Sepp Haggenmuller GmBh, Germany) was used. The salmon samples were cooked in a Grant JB Nova water bath (Grant Instruments, Cambridge, UK).

2.2. Sous-vide process

All samples (n = 21) of salmon (Irish organic farmed Atlantic salmon, Salmo salar) fillets were provided by the same provider (Marine Harvest,
Co. Donegal, Ireland) in specific conditions that are not altered throughout year. All fish were reared in a natural environment ensuring that the fish possessed favourable body shape and sufficient muscle tone. Salmon fillets were prepared in approximately 50 g portions. A 10% sodium chloride to water solution was created. Various sous vide cooking temperatures (52 °C, 65 °C and 80 °C, as measured by thermometer embedded within each fillet during cooking) were used for 15 min by using a chronometer, and for each different temperature samples were either brined (10% sodium chloride in water) or un-brined. The sous-vide cooking at each temperature and brining conditions and all other experimental procedures were performed in triplicate samples of salmon fillets. Raw salmon fillets were also used as control samples, again in triplicates, in all procedures.

2.3. Extraction and separation of bioactive polar lipid compounds from cooked salmon

Extraction and separation of bioactive PL from cooked salmon of all sous-vide preparations (n = 21), were performed as previously described (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, O’Keeffe, et al., 2019). Briefly, after each sous-vide cooking process, the 50–60 g of each sous-vide cooked salmon sample was homogenized mechanically using a Waring blender (Fisher Scientific Ltd) and its total lipids (TL) were extracted into chloroform according to the method of Bligh and Dyer (1959) and flash vapourised under N₂ stream, weighed, re-dissolved in 2 mL of CHCl₃/MeOH 1:1 v/v, and stored at –20 °C. For each sample, approximately 1/10 of the TL was stored in sealed vials at –20 °C, whilst the remainder of TL was further separated into total neutral lipids (NL) fraction and the total polar lipids (PL) fraction using the counter-current distribution method developed by Galanos and Kapoulas (1962). The PL and NL recovered from this method for each sous-vide salmon preparation were weighed and stored for further analysis under a nitrogen stream at –20 °C.

2.4. Platelet aggregometry bioassay

Blood collection from several healthy donors (n = 12) and preparation of human platelet-rich plasma (hPRP), along with evaluation of the antithrombotic properties of PL from each salmon sous-vide preparation against human platelet aggregation induced by PAF, ADP, thrombin and collagen in hPRP, were conducted as previously described (Tsoupras, O’Keeffe, et al., 2019). The Ethics Committee of the University of Limerick approved the protocol, which was performed in accordance with the Declaration of Helsinki. Healthy donors were young (age: 33.7 ± 4.9 years, range 28–49 years), non-obese male subjects, and none of them was taking drugs or dietary supplements. Subjects were fully aware that their blood samples were used in our study and written consent was provided. The concentration (µg) of the PL samples from each sous-vide salmon preparation that led to 50% of platelet aggregation induced by PAF, ADP, collagen and thrombin in hPRP, was calculated as the 50% inhibitory concentration value also known as the IC₅₀ value (half-maximal inhibitory concentration) for each sample. The resulting IC₅₀ values were expressed as a mean value of the mass of PL (µg) in the aggregometer cuvette ± standard deviation (SD). All experiments were performed several times (n ≥ 10), using a different donors’ blood samples for each PL replicate to ensure reproducibility.

2.5. LC-MS analysis

FA content and structural elucidation of PL from each sous vide salmon preparations were performed by LC-MS analysis as previously described (Tsoupras, Lordan, Shiels, et al., 2019).

2.6. Sensory analysis

Six salmon samples and a replicate (control Brined 80 °C) were all tested by 30 panelists using napping followed by check-all-that-apply. Tests were conducted in designated individual booths under artificial daylight. 30 panelists (age 18–60, 19 female) with experience in sensory evaluation but no previous experience in napping evaluated the products. The panelists were asked to provide information in relation to consumption of salmon (40% would consume salmon weekly, 27% monthly and the rest occasionally) and the majority of them would consume it baked (42%) or smoked (31%). This study does not rely on subjective measurement of the salmon samples but objective measurements of the attributes, and the frequency of consumption is not expected to affect the sensory profiling results. In other words, the assessors were not asked to express preference or liking which would be affected by the frequency of consumption. For the napping test they were given the following instructions in the beginning of the test:

“Please taste all the samples, in the order presented to you. Place the samples by dragging the code across into the rectangle according to the strategy that two samples placed closer to each other are more alike than two samples placed further apart. The criteria for how to separate the samples just has to make sense to you. There is no right or wrong answer.”

Furthermore, a scientist was present during the testing in order to answer the assessors’ questions in the event that there were any. The seven salmon samples (six samples and the replicate) were simultaneously presented in a random order to each assessor.

The assessors were requested to taste the samples and to lay them out in a rectangular space on the screen in such a way that two salmon samples were very near if they seemed similar and that two salmon samples were distant from one another if they seemed different. For each salmon product, both X co-ordinate and Y co-ordinate were collected through Fizz Biosciences software (version 2.51 (a 86) Couternon, France). Assessors were also asked to give reasons for creating their groups. The frequencies of the terms used for the group creation were calculated.

The next stage of testing included a check-all-that-apply (CATA) test were the participants were asked to check a number of terms for each of the samples they were presented. The 17 attribute terms used for the CATA session (Appearance (A)- pink/salmon, discoloured, fatty/oily; Odour (O)- strong fishy, mild fishy; Texture (Tx)- soft, firm, dry, moist, flaky; Taste (T)- sweet, salty, umami and Aftertaste (At) – strong fishy, mouth drying, oily and sweet) were developed during a vocabulary session with untrained participants, where all six samples (without the replicate) were presented monadically to the panelists and they were asked to write down as many terms as possible in relation to the appearance (A), texture (Tx), taste (T), odour (O) and aftertaste (AT) of the samples.

2.7. Statistical analysis

One-way analysis of variance (ANOVA) was applied to determine significant statistical differences between analyses if present for all data concerning the yield of extraction and the IC₅₀ values of the antithrombotic properties of PL from sous-vide salmon preparations, while Kruskal–Wallis nonparametric multiple comparison test was used for comparisons in the FA composition acquired from the LC-MS analysis (SPSS Inc. 26, Chicago, 215 IL, USA).

For the data generated from napping, Multiple Factor Analysis (Le Dien & Pagès, 2003) was used to generate the bi plots of the products using XL Stat software. Factosmine-R software was used to generate the factor map/score with confidence ellipses as described by Christian Dehblom (2014). Although CATA results are usually considered exploratory or descriptive (Fleming et al., 2015) for this study CATA data were analysed using a Cochran’s Q test in order to find differences between samples and pairwise comparisons were performed using the McNemar approach. XLStat (2019.2.1.58716) was used to conduct the
above tests and to generate the symmetric plots.

3. Results and discussion

Several nutritional guidelines have included salmon as an oily fish in healthy diets (Lara et al., 2007; Zhang et al., 2012). However, due to food safety and hygiene reasons, salmon is commonly cooked prior to consumption. The majority of the studies related to the effects of cooking to salmon are related mainly to the effects on its fatty acid composition and reduction of its health benefits are mostly attributed to any changes observed in the n-3 PUFA content due to cooking (Al-Saghir et al., 2004, Brookmire et al., 2013, Echarte et al., 2001, Gladyshev et al., 2006, Larsen et al., 2010, Leung et al., 2018, Zhang et al., 2012). This is the first study examining the effects of mild sous-vide cooking procedures not only on the fatty acid content, but also on the bio-functionalities of antithrombotic salmon PL, along with sensory properties of thus cooked salmon, in different conditions applied.

More specifically, different temperatures and brining conditions were applied in order to evaluate the optimum conditions of sous-vide that has the minimum effects on the functionality and fatty acid content of salmon PL without affecting its sensory properties. Salmon fillets were cooked by the sous-vide technique in 52°C, 65°C and 80°C, while for each different temperature a sample was either brined or un-brined by a 10% sodium chloride to water solution. The 52°C temperature was chosen as it is reported as the optimum sous-vide cooking temperature for salmon, in order to maximize taste and texture qualities (Brookmire et al., 2013). The 80°C temperature was used as this is a standard temperature for the pasteurization of food (González-Fandos, 2005).

The intermediate temperature of 65°C was used in order to evaluate the effects of an intermediate temperature of sous-vide cooking on salmon anti-thrombotic properties. Furthermore, brining process was also applied in all temperatures since it is a common technique used by chefs worldwide to improve the texture of cooked fish and to prevent it from flaking upon cooking (Jittinandana et al., 2002).

3.1. Yield of extraction and separation of bioactive lipids from sous-vide cooked salmon

The Bligh and Dyer method of extraction used in the present study is one of the most well established methods for high yield of extraction of lipids in combination with the high stability of key bioactive lipid substances extracted, since it does not need heat treatment during extraction (Bligh & Dyer, 1959). In several fish species, including salmon, when the Bligh and Dyer extraction method is accompanied by an appropriate counter-current distribution technique, such as the Galanos and Kapoulas method (Galanos & Kapoulas, 1962), the PL fractions can be separated with the highest efficacy from the TL extract, while highly bioactive marine polar lipids have been preserved within such PL fractions (Nasopoulou et al., 2007, ; Nasopoulou, Psani, et al., 2011; Nasopoulou, Tsoupras, et al., 2011; Panayiotou et al., 2000, Tsoupras, Lordan, Demuru, et al., 2018, Tsoupras, O’Keeffe, et al., 2019).

Within the present study, the same procedures applied for raw salmon (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, O’Keeffe, et al., 2019) was also implemented for extracting the TL extracts from all salmon samples subsequent to the various different sous-vide cooked preparations, and for separating their bioactive PL fractions. The content for TL, PL and NL is presented in Table 1 and is expressed as g of lipids per 100 g of fish tissue for all samples, with the PL and NL also being displayed as a percentage of the TL.

Both raw and cooked salmon had high amounts of TL. This is in agreement with the classification of salmon as an oily fish species, which has been reported to contain high levels of TL as opposed to other fish species (Zhang et al., 2012). The effect of brining exhibited no differences in the yield of TL in all sous vide preparations and in comparison to previously reported outcomes for raw salmon (Tsoupras, Lordan, Demuru, et al., 2018). The majority of the lipids that were extracted were of neutral lipid nature which is also in agreement with previously reported results in raw salmon (Tsoupras, Lordan, Demuru, et al., 2018). The NL fraction of raw salmon fillets generally comprises of approximately 60–84% of the TL, whilst the PL fraction contributes to approximately 16–40% of the TL (Tsoupras, Lordan, Demuru, et al., 2018).

All sous-vide cooked salmon samples exhibited higher % of NL and lower % PL in comparison to raw salmon (Tsoupras, Lordan, Demuru, et al., 2018). It seems that a higher release of neutral lipids during the cooking process lead to an increase in lipid extraction in NL and subsequently in TL, whereas the absolute values of PL (g/100 g) did not change between the raw salmon and the sous-vide cooked salmon. This may be due to a more effective extraction of the neutral lipids from cooked fish samples rather than raw fish due to the bound neutral lipids being released as free lipids during the cooking process therefore, leading to an increased lipid extraction, as it was also suggested by Larsen et al. (2010). It is also reported that an increase in TL from cooked fish samples may be due to the loss and changes in other lipid materials in the fish muscles (Ma et al., 2006) and polymerisation and/or oxidation of the triglycerides (Bakar et al., 2008).

3.2. The effect of sous-vide cooking at several conditions on the biofunctionality of salmon lipids against platelet aggregation

It is well established that platelet activation and aggregation is implicated in thrombotic and inflammatory processes during the onset, development and progression of several inflammation related chronic disorders, including atherosclerosis and cardiovascular diseases (CVD) (Lordan et al., 2020; Tsoupras, Lordan, & Zabetakis, 2018, 2019). The consumption of fish and fish oils has been proposed to improve platelet function, human thrombosis and haemostasis (Adili et al., 2018; Mori et al., 1997). Marine PL, including salmon PL, and especially those bearing n3 PUFA within their structure, apart from their higher bioavailability of their n-3 PUFA content (Burri et al., 2012, Lordan et al., 2020, 2019, 2019), have also been found to possess potent antithrombotic properties against platelet aggregation (Lordan et al., 2020; Nasopoulou, Psani, et al., 2011, Tsoupras, Lordan, Demuru, et al., 2018, 2020, Tsoupras, Lordan, Shiels, et al., 2019, Tsoupras, O’Keeffe, et al., 2019). More specifically, PLs from raw salmon fillets and heads have been recently found to possess

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The content of Total lipids (TL), neutral lipids (NL) and polar lipids (PL) expressed as g/100 g of sample with NL and PL also being expressed as a percentage of TL (range values, n = 3).</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSF%</td>
<td>3.61–7.41</td>
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<tr>
<td>52°C CSVSNB</td>
<td>4.81–8.73</td>
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<tr>
<td>52°C CSVSB</td>
<td>5.87–8.21</td>
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<tr>
<td>65°C CSVSNB</td>
<td>7.27–7.61</td>
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<tr>
<td>65°C CSVSB</td>
<td>7.47–8.19</td>
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<tr>
<td>80°C CSVSNB</td>
<td>7.25–8.37</td>
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<tr>
<td>80°C CSVSB</td>
<td>7.25–8.37</td>
</tr>
<tr>
<td>RSF%</td>
<td>2.58–6.74</td>
</tr>
<tr>
<td>52°C CSVSNB</td>
<td>4.21–8.01</td>
</tr>
<tr>
<td>52°C CSVSB</td>
<td>5.09–7.35</td>
</tr>
<tr>
<td>65°C CSVSNB</td>
<td>6.54–6.84</td>
</tr>
<tr>
<td>65°C CSVSB</td>
<td>6.76–7.36</td>
</tr>
<tr>
<td>80°C CSVSNB</td>
<td>6.30–7.32</td>
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<tr>
<td>80°C CSVSB</td>
<td>7.25–8.77</td>
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<tr>
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<tr>
<td>52°C CSVSB</td>
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<tr>
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<tr>
<td>80°C CSVSNB</td>
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<tr>
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<tr>
<td>80°C CSVSB</td>
<td>9.97–12.49</td>
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</table>

* Results for raw salmon are reproduced from Tsoupras, Lordan, Demuru, et al. (2018).
strong anti-inflammatory, antithrombotic, and cardio-protective activities against platelet activation and aggregation induced by inflammatory and thrombotic mediators such as PAF and thrombin (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019). Salmon by-products (salmon heads) have also been found to possess strong antiplatelet effects against platelet aggregation induced by other platelet agonists, such as collagen and ADP (Tsoupras, O’Keeffe, et al., 2019).

As aforementioned, fish, including salmon, is mostly consumed cooked. The effects of cooking on the antiplatelet bioactivities of PL fractions derived from fish-species other than salmon, using with the same procedures have been previously studied in cod (Panayiotou et al., 2000) and sardines (Nasopoulou, Psani, et al., 2011). However, in these previous studies the PL fractions obtained from these fish species were assessed against aggregation of washed rabbit platelets induced solely by the inflammatory and thrombotic mediator PAF (Nasopoulou, Psani, et al., 2011, Panayiotou et al., 2000).

The novelty of the present study lies on studying the effects of sous-vide cooking on the biofunctionality of PL obtained from salmon, a native Irish oily fish, against platelet aggregation of human platelets (hPRP), induced not only by PAF, but also by other thrombotic mediators and well established platelet agonists, such as thrombin, collagen and ADP. The use of hPRP provide the benefit to investigate the bioactive salmon PL in closer to the in vivo conditions, while assessing the antiplatelet properties of salmon PL against a range of well-established agonists (PAF, thrombin, collagen and ADP) provide a more complete understanding of the mechanisms and pathways involved in the antithrombotic properties of both raw and cooked salmon (Tsoupras, O’Keeffe, et al., 2019).

This is the first study examining in general the effect of cooking on the biofunctionality of bioactive PL from a food source in relation to their anti-thrombin effects but also against collagen and ADP induced platelet aggregation. Platelets are endowed with a repertoire of surface receptors that enable them to adhere, activate and aggregate upon vascular injury by several mediators and their related pathways, such as PAF (Lordan et al., 2020; Tsoupras, Lordan, & Zabetakis, 2018, 2019). Apart from PAF, platelet adhesion is governed by the interaction between vascular collagen and its related receptors on platelet membranes and the engagement of other soluble agonists, predominantly ADP, Thromboxane A2 (TXA2) and thrombin, with their related Gq-protein coupled receptors (Lordan et al., 2020; Tsoupras, Lordan, & Zabetakis, 2018, 2019). Therefore, an understanding of the platelet signaling mechanism involved in thrombus formation by all these agonists and their related pathways is necessary to identify the overall antithrombotic properties of food related antiplatelet agents, such as the bioactive salmon PL.

In order to facilitate comparisons between the cooked and raw salmon fillets, the antiplatelet properties of PL from raw salmon fillets against both ADP and collagen induced aggregation of platelets were also studied for the first time in the present study. In addition, milder cooking preparations according to the sous-vide technique where applied which differs from the cooking techniques used in cod and sardine cooking studies (frying and grilled) (Nasopoulou, Psani, et al., 2011, Panayiotou et al., 2000), in order to evaluate if in these milder conditions have better preservation of the bioactivities of salmon PL.

The results are shown in Fig. 1 and are expressed in IC50 values (half-maximal inhibitory concentrations), measured as the mass of lipid measured in micrograms (µg) for each PL that inhibit by half (50%) the

![Fig. 1](image-url)
maximal-reversible aggregation of platelets induced by each one of the platelet agonists assessed (PAF, collagen, thrombin or ADP). It should be noted that the lower the IC_{50} value, the stronger the inhibition against the platelet agonist tested. The IC_{50} values obtained against platelet aggregation, induced by all these inflammatory and thrombotic platelet agonists, showed that all of the PL extracts assessed exhibited strong antithrombotic effects.

More specifically, PL from brined and un-brined mild sous-vide salmon preparations (52 °C CSVSNB, 52 °C CSVSB, 65 °CSVNB and 65 °CSVSB) were found to strongly inhibit the PAF pathway of platelet aggregation (Fig. 1A), with similar IC_{50} values to the ones previously reported for PL of raw salmon fillets (SF) (Tsoupras, Lordan, Demuru, et al., 2018) (p > 0.05 in all these comparisons) and of other edible fish sources (Nasopoulos et al., 2007, Nasopoulos, Psani, et al., 2011, Panayiotou et al., 2000). By applying higher temperatures, such as those used for pasteurization, the anti-PAF effects of thus cooked salmon were significantly decreased, in both brined and un-brined sous-vide salmon preparations (80 °CSVSNB and 80 °CSVSB) (p < 0.05 in all comparisons), with IC_{50} values being approximately one order of magnitude lower than those of the previously reported for raw salmon fillets (Tsoupras, Lordan, Demuru, et al., 2018) (p < 0.05). These results are in accordance with those previously reported in cooking preparations (grilling) in another oily fish, namely sardines, in which a decrease in the anti-PAF activities of sardine PL was also observed in high temperature of grilling in comparison to raw sardines (Nasopoulos, Psani, et al., 2011). Even though a significant reduction of the anti-PAF activities of the PL in these pasteurized sous vide salmon preparations was observed, still these antithrombotic properties remained within a range of potency comparable to salmon by-products, such as salmon heads, and other fish sources and related by-products, such as boarfish and herring fillets and heads (Tsoupras, O’Keeffe, et al., 2019). This emphasizes the efficacy of sous-vide and the nutritional value of salmon with respect to its antithrombotic potency, even when sous-vide cooking techniques are performed in higher temperatures than the optimum proposed ones of 52 °C.

Similar pattern was also observed in the anti-thrombin effects of the PL of cooked salmon compared to raw SF. PL derived from mild sous-vide salmon preparations (52 °CSVSNB, 52 °CSVSB and 65 °CSVSB) exhibited the strongest inhibitory effects against thrombin-induced platelet aggregation in comparison to those of PL extracted from all the other sous vide preparations in higher temperatures (Fig. 1B). These anti-thrombin effects were also found similar to previously reported anti-thrombin effects of the PL extracts of raw SF (Tsoupras, Lordan, Demuru, et al., 2018) (p > 0.05 in all these comparisons), while when using higher temperatures (80 °CSVSNB and 80 °CSVSB) the anti-thrombin effects of cooked salmon were significantly lower (p < 0.05). Interestingly, similarly to PL extracted from raw SF in the same way (Tsoupras, Lordan, Demuru, et al., 2018), the anti-thrombin effects of cooked salmon PL were significantly lower than their relative anti-PAF effects in all sous-vide preparations, suggesting a higher specificity of thus extracted salmon PL against the PAF pathway, rather than the thrombin pathway.

Concerning the anti-ADP effects of salmon PL, by applying mild temperatures in the sous-vide preparations (52 °C and 65 °C) either brined or un-brined, the strong inhibitory effects of salmon PL against ADP-induced platelet aggregation were preserved in similar potency with those of the raw SF. Again, the anti-ADP effects of salmon PL were significantly decreased when higher temperatures were applied (80 °C brined and un-brined) during sous vide preparations, as it happened for the PAF and thrombin pathways too. Nevertheless, the anti-ADP effects of PL form all sous vide preparations were stronger than their anti-thrombin and lower than their anti-PAF effects (p < 0.05 in all these comparisons), respectively, apart from the PL of both the 80 °CSVSNB and 80 °CSVSB samples that had similar anti-ADP and anti-PAF effects (p > 0.05 in all these comparisons).

The PAF pathway of platelet aggregation is related to the intracellular changes within platelets after the binding of PAF on its specific for PAF G-coupled protein receptor, namely PAF-receptor (Tsoupras, Lordan, & Zabetakis, 2018). Intracellular signalling triggered in platelets by an initial PAF-related signal is that of the eicosanoids and of ADP, which concludes in release and secretion of ADP from platelet granules to enhance the initial signal (related to PAF) by a further activation of platelets by the ADP-pathway too, resulting in platelet aggregation and thrombus formation (Lordan et al., 2020; Tsoupras, Lordan, & Zabetakis, 2018, 2019). Thus, PAF, eicosanoids and ADP pathways are inter-related, and the anti-PAF effects of food-derived bioactive PL including salmon PL seem to be related with their anti-ADP effects. This may explain the observed similarities between the observed changes in both the anti-PAF and anti-ADP activities of salmon PL after cooking in several temperatures.

Another interesting pathway for inducing platelet aggregation that recently has received plenty of attention as a target for drug and food supplement development, is that related to collagen (Lordan et al., 2020; Tsoupras, Lordan, & Zabetakis, 2018, 2019). One of the events that triggers platelet tethering to the site of vascular injury occurs upon subendothelial collagen exposure, mostly fibrillar types I and III, to the circulation. Collagen mediates platelet adhesion by interacting with a range of unique surface platelet receptors. Binding of Collagen on these receptors activates by specific intracellular signalling the aggregation of platelets by specific pathways. The ability of salmon PL to inhibit the collagen pathway is of great importance for the endothelium platelets interaction during atherosclerosis and atherothrombosis.

Notably, the bioactive PL from raw salmon were found for the first time to exhibit strong anti-collagen effects. Differently than the effects of the PL from sous vide preparations against all the other platelet agonists, the anti-collagen effects of the PL from all sous vide preparations exhibited different pattern (Fig. 1D). More specifically, the PL from both 65 °CSVSNB and 65 °CSVSB samples had the strongest inhibitory effects against collagen-induced platelet aggregation than all samples tested. The anti-collagen effect of these two sous vide preparations were significantly stronger not only when compared with the anti-collagen effects of the PL from 80 °CSVSNB and 80 °CSVSB, but also when compared with the anti-collagen effects of the PL from 52 °C sous vide salmon preparations and that of the raw salmon fillets. The anti-collagen effects of PL from both 65 °CSVSNB and 65 °CSVSB samples were also stronger from their anti-PAF, anti-ADP and anti-thrombin effects in these conditions applied. This suggests that both 65 °CSVSNB and 65 °CSVSB seem to contain PL molecules with higher specificity against the collagen pathway than all the other samples. The stronger antithrombotic properties of the PL of these two sous vide preparations at 65 °C against the collagen pathway seem to be associated with changes observed in their fatty acid content, and especially in the n-3 DPA (22:5n3) content, which is a precursor of EPA and DHA, and is related to more potent inhibition of the collagen pathway than either EPA or DHA (Akiba et al., 2000).

Since recent findings have exhibited that platelet-activation induced by pathways associated with PAF, thrombin, collagen, and ADP, seems to be inter-connected and interrelated in several manifestations related to inflammation and thrombosis (Lordan et al., 2020; Tsoupras et al., 2009; Tsoupras, Lordan, & Zabetakis, 2018, 2019), the overall beneficial antiplatelet effects of salmon PL against these platelet agonists further support their antithrombotic properties, which seem to be well preserved when salmon is cooked in mild cooking conditions, such as those of the sous-vide technique.

3.3. The effect of sous-vide cooking at several conditions on the fatty acid content and structure activity relationships of bioactive salmon polar lipids.

The variance on the changes observed in the antiplatelet effects of the bioactive salmon PL against not only PAF, but also against all the other platelet agonists studied (thrombin, collagen and ADP), during sous-vide processing in different temperatures and brining conditions,
These changes in the fatty acid composition of salmon PL also seem to be related to alterations of the fatty acid composition observed in these salmon PL too. In order to evaluate such changes in the fatty acid composition of salmon PL from all sous-vide preparations, all PL samples were saponified and further analysed by LC-MS analysis as previously described (Tsoupras, Lordan, Shiels, et al., 2019). Representative chromatograms of such an analysis for the saponified salmon PL are shown in Fig. 2. In addition, the changes of the obtained fatty acid profile of salmon PL during all sous-vide preparations can be seen in Table 2.

More specifically, the SFA were the most abundant fatty acid class in PL of raw salmon and all sous-vide preparations followed by the monounsaturated fatty acids (MUFAs) and the PUFA. In PL of raw salmon and all sous-vide preparations the most abundant of the SFA was the palmitic acid (PA) (16:0), followed by lower but considerable amounts of stearic acid (18:0). The most abundant of the MUFAs was found to be oleic acid (OA) (18:1n9), followed by lower but considerable amounts of gadoleic acid (20:1n9).

In addition, raw salmon and all sous-vide preparations have PL rich in PUFAs with a beneficial low ratio of n-6/n-3 (Simopoulos, 2006; Simopoulos, 2008). These results are in agreement with previously reported outcomes in raw salmon samples of the same batch (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019) and further emphasizes the potential cardio-protective properties of salmon PL, even when cooked with mild procedures such as the sous-vide technique, since the lower this ratio the better the health benefits in CVD and other chronic disorders (Simopoulos, 2006; Simopoulos, 2008). The most abundant of the n-3 PUFA were the DHA (22:6n3) and the EPA (20:5n3), while considerably less quantities of their precursors, n-3 DPA (22:5n3) and α-linolenic acid (ALA; 18:3n3), were also present. In n-6 PUFA the most abundant were linoleic acid (LA; 18:2n6) followed by much less amounts of arachidonic acid (ARA; 20:4n6) in PL from raw salmon and from all sous vide preparations. These results are also in accordance with previously reported ones for raw salmon fillets and heads (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019), as shown in Table 2.

The PUFA content of the un-brined and pasteurized sous vide preparations (80 °C-SVSNB) was significantly reduced, in comparison to all the other sous vide preparations and in comparison to the previously reported raw salmon samples by Tsoupras, Lordan, Demuru, et al. (2018), Tsoupras, Lordan, Shiels, et al. (2019), Tsoupras, O’Keeffe, et al. (2019), as shown in Table 2. The reduction of the PUFA content in the PL of this sous vide preparation seem to be associated with a subsequent observed increase in both their SFA and MUFa content, in comparison to the PL of all the other sous vide preparations, but also when compared to the previously reported raw salmon samples (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019), as shown in Table 2. It seems that in higher temperatures, such as the one applied for pasteurization there is thermal modification of the double bonds of the PUFA producing more saturated forms such as the MUFa and SFA.

However, none statistical difference was observed in the n-6/n-3 ratio in all un-brined sous vide preparations, which was preserved in lower levels than the much higher levels of 5:1–20:1 for this ratio in unhealthy westernized diets (Simopoulos, 2006; Simopoulos, 2008). It has been reported that high values of this ratio are correlated with a higher risk in cardiovascular disease and other chronic diseases (Simopoulos, 2006; Simopoulos, 2008). Taking this into account, the observed favorable n-6/n-3 ratio in the bioactive PL of all sous vide preparations further supports the preservation of the cardio-protective properties for PL and emphasizes the benefits of the sous vide mild cooking technique. This also comes in accordance with some studies stating that these PUFA in certain fish species remained unchanged after several cooking processes (Echert et al., 2001; Gladyshev et al., 2006; González-Fandos, 2005; Jittinândana et al., 2002; Leung et al., 2018).

In addition, all sous-vide preparations, this ratio was found to be lower in the brined cooked salmon than the relative ratio for the un-brined preparation of the same temperature applied. In all temperatures tested the overall n-3 PUFA contents in the PL of the brined preparations, and especially those of the DHA and EPA contents, were significantly higher than the relative n-3 PUFA, DHA and EPA contents in the PL of un-brined preparations for each one of the temperatures applied. The above further suggest that brining may beneficially preserve more the n-3 PUFA content of salmon PL during mild cooking procedures such as the sous vide technique.

Moreover, it was previously described in raw salmon samples that the most bioactive salmon PL against platelet aggregation induced by PAF and thrombin belong to PL fractions of PC and PE that are rich in n-3 PUFA, and especially in EPA and DHA (Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019). Moreover, a standard PC containing DHA at its sn-2 position of its glycerol backbone was recently reported to potently inhibit human platelet aggregation induced by PAF, thrombin, ADP and collagen, with higher specificity against the PAF pathway (Tsoupras, Lordan, Harrington, et al., 2020). In the present study, sous vide preparations in low temperatures were not found to affect the n3 PUFA content of the PL of cooked salmon (Table 2), and subsequently they did not affect their antithrombotic properties against platelet aggregation induced by PAF and thrombin, but also by ADP and collagen. It should be noted that the n-3 PUFA content, the n6/n3 PUFA ratio and subsequently the antithrombotic properties of bioactive salmon TPL in human platelets remained in beneficial levels with respect to their antithrombotic and cardio-protection, when compared to other fish samples, including salmon heads (Nasopoulou et al., 2007, Nasopoulou, Psani, et al., 2011, Panayiotou et al., 2000, Tsoupras, O’Keeffe, et al., 2019).

On the other hand, significant reduction of the antithrombotic properties against thrombin, PAF and ADP was of similar magnitude in PL from brined and un-brined sous-vide preparations in high temperatures, whereas the observed changes in the EPA and overall PUFA content in these two preparations were not of the same magnitude (more intense reduction were observed in the un-brined case). Taking into account that the most bioactive salmon PL against PAF and thrombin pathways were previously found to be those baring EPA [9], this result further suggest that the reduction observed in the EPA content of the PL from un-brined sous-vide preparations when higher temperatures were applied seem to be correlated with the observed reduced antithrombotic properties of these preparations’ PL. On the other hand, reduction of antithrombotic properties was also observed in TPL of brined preparations in these temperatures, without observed changes in the EPA content. This implies that apart from the rich in EPA and DHA bioactive salmon PC and PE, other types of bioactive PL seem also to be present in salmon, such as bioactive marine PL of the sphingomyelin family and/or glycolipids (Nasopoulou, Psani, et al., 2011; Nasopoulou, Tsoupras, et al., 2011; Tsoupras, Lordan, Demuru, et al., 2018; Tsoupras, Lordan, Shiels, et al., 2019; Tsoupras, O’Keeffe, et al., 2019), which may also be more affected by the higher temperatures applied. However, more studies are needed in order to reveal such a structure activity relationships for marine glycolipids with different conditions of cooking applied.

In addition, changes in the content of the n-3 DPA of the bioactive salmon PL in different temperatures may be related to the differences in their anti-collagen activities, since n-3 DPA is related to more potent inhibition of the collagen pathway than EPA and DHA (Akiba et al., 2000). n-3 DPA content was increased in the PL from sous-vide salmon prepared in 65 °C, compared to the other temperatures, which may explain the stronger anti-collagen effects of the PL derived from sous-vide salmon in this temperature, rather than those in raw salmon fillets or when lower (52 °C) and higher (80 °C) temperatures were applied.

Nevertheless, more in depth lipidomic approaches are needed in order to fully elucidate the effects of cooking and especially of the sous-vide technique in the structure activity relationship of antithrombotic salmon PL, with respect to their FA content and overall structures.
Fig. 2. Representative chromatograms of the LC-Ms analysis of the saponified salmon PL from raw salmon and all sous-vide preparations. A represents raw salmon samples, A1 represents salmon cooked using the sous-vide process at 52 °C brined, A2 represents salmon cooked using the sous-vide process at 52 °C un-brined. B1 represents salmon cooked using the sous-vide process at 65 °C brined, B2 represents salmon cooked using the sous-vide process at 65 °C un-brined, C1 represents salmon cooked using the sous-vide process at 80 °C brined, C2 represents salmon cooked using the sous-vide process at 80 °C un-brined.
1.386* 0.759 0.0007 0.0041 0.0113 0.0004 0.0283* 0.089 0.0352 0.0103 0.0004 0.0041 0.0007 0.0004 0.0283 0.0041 0.0004 0.0103 0.0004 0.0041 0.0004 0.0283

### 3.4. Sensory analysis

The sous-vide cooking process allows for food stuffs of high nutritional quality and high sensory attributes to be preserved with a longer shelf life than other cooking-cooking processes. Therefore, in this study, we performed sensory analysis on all sous vide salmon preparations in order to detect the changes in smell, taste, colour, juiciness as well as 2016). The muscle of the fish separates into flakes at temperatures around 60°C and above 65 °C is transformed into gelatine. When salmon and cod fillets were previously cooked between the temperatures of 50–70 °C the maximum water loss from salmon flesh was at 50 °C (Ofstad et al., 1995). It has also been reported that after this point at temperatures above 60 °C, various structural changes in the conjunctive tissue of the fish take place which can explain the changes in structure that were also observed from the judges (Ofstad et al., 1995). Moreover, the texture of fish cooked under sous-vide at temperatures higher than 65°C is significantly different from the raw fish (Kato et al., 2016). The previously observed changes in texture were measured using a texture analyser and no sensory data were collected for these samples (Kato et al., 2016).

Interestingly, in the present study there were no significant differences due to taste as it would be expected between the brined and unbrined samples. This was the case for both the CATA data and the napping data as seen in Figs. 3 and 4.
Fig. 3. Principal coordinate analysis (PoCA) biplots from multiple factor analysis on the napping data of the seven sous-vide samples.

More specifically, in the present study, data collected from napping were analysed using multiple factor analysis. The first two factors from the principal coordinate analysis can explain the 57.10% of variance. As seen in Fig. 3, factor 1 separates the sous-vide samples at 52 °C from the rest of the samples. Factor 3 separates the brined samples from the unbrined ones. The panelists were asked to write down the attributes of the products which led to their groupings and it was found that 25 out of the 30 judges created their groups based on the texture of the products. Whereas only eleven times taste was mentioned and only one panelist mentioned saltiness in specific. The appearance was mentioned seven times as a contributing factor for creating groups. Groupings generated from napping can be further explained in CATA data are taken into consideration.

Fig. 4 represents the symmetric plot of the products and the attributes as resulted from the principal coordinate analysis (PoCA) of the CATA data. The first two dimensions explain 83.99% of the variation and the scree plot indicates the two first dimensions are sufficient to interpret the relationships between the attributes and the products. In this figure, it is observed that the two sous-vide samples at 52 °C were perceived more similar and were characterized by attributes such as Fatty/Oily Appearance, Strong Fishy Odour, Moist Texture. Whereas the control samples and the samples sous-vide at 65 °C were closer in the PCA plot and they were characterized as flaky, firm. This was expected since these samples were cooked at temperatures above 60 °C.

Table 3 shows the proportions of 1 (I choose vs 0 not choose) across assessors for each combination of products and attributes. A high proportion means the attribute is frequently ticked by the panelist for the product under investigation. For a given attribute, the Cochran’s Q test allowed to test for a given product whether the panelists feel the attribute or not. For the cases of significant p-values multiple pairwise comparison where conducted by applying the McNemar test using XLstat 2018. Products with the same letter do not differ significantly. As seen on the table the main differences across samples are observed in the attributes related to texture, such as firm, soft, dry and moist. There were also differences observed in the appearance attribute fatty/oily and in the after-effect attributes mouth-drying and oily.

In Table 3, it can be observed that the two sous-vide samples at 52 °C were perceived to be more similar and were characterized by attributes such as fatty/oily appearance, strong fishy odour, moist texture. On the other hand, the control samples (80 °C) and the samples sous-vide at 65 °C were closer in the PCA plot and they were characterized as flaky, firm. This was expected since these samples were cooked at temperatures above 60 °C.

Previous research has shown that CATA results obtained from semi-trained panelists on CATA terms can be comparable to results from descriptive profiling (Alexi et al., 2018). However, in the present study, although the panelists had conducted other sensory tests were not trained in the attributes used for CATA therefore they cannot be compared with the semi-trained panelists from previous studies (Alexi et al., 2018). On the other hand, the findings from CATA are comparable to the findings from napping as seen in Fig. 4. Indeed, as seen in Fig. 3 samples cooked at 52 °C were perceived different from the rest of the samples. Furthermore, the CATA and napping findings of the present study were in agreement to previous reported findings on comparable information from CATA and napping on beer samples (Reinbach et al., 2014).

Finally, there are some limitations to this study. The data presented show that the most favourable results were found with mild sous-vide cooking (52 °C and 65 °C). In view of these temperatures and the importance of heat treatment to guarantee food safety, one limitation of this study was the lack of microbiological tests to guarantee safety against microorganisms such as Clostridium botulinum and Listeria monocytogenes in these mild conditions. As restaurants do actively use these techniques on fresh produce, further microbiological safety experiments
Fig. 4. Symmetric plot of products as revealed from the principle coordinate analysis on the CATA data. Abbreviations: At: Aftertaste; Tx: Texture; O: Odour; A: Aroma; T: Taste; Sousvide 52: salmon cooked sous-vide at 52 °C no brine; Sousvide B 52: salmon cooked sous-vide at 52 °C brined; Sousvide 65: salmon cooked sous-vide at 65 °C no brine; Sousvide B 65: salmon cooked at sous-vide at 65 °C brined; Control 80: salmon cooked sous-vide at 80 °C no brine; Control B80: salmon cooked sous-vide at 80 °C brined.

Table 3
Proportion of 1 s across assessors for each combination of products and attributes. Multiple pairwise comparisons of products applying the McNemar test on the data from CATA.

<table>
<thead>
<tr>
<th>Attributes</th>
<th>p-values</th>
<th>52CSVSNB</th>
<th>52SVSB</th>
<th>65SVSNB</th>
<th>65SVSB</th>
<th>80CSVSNB</th>
<th>80SVSB</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-Pink/Salmon</td>
<td>0.991</td>
<td>0.600 (a)</td>
<td>0.567 (a)</td>
<td>0.533 (a)</td>
<td>0.533 (a)</td>
<td>0.567 (a)</td>
<td>0.600 (a)</td>
</tr>
<tr>
<td>A-Discoloured</td>
<td>0.290</td>
<td>0.200 (a)</td>
<td>0.100 (a)</td>
<td>0.200 (a)</td>
<td>0.267 (a)</td>
<td>0.100 (a)</td>
<td>0.233 (a)</td>
</tr>
<tr>
<td>A-Fatty/Oily</td>
<td>0.000</td>
<td>0.633 (b)</td>
<td>0.500 (b)</td>
<td>0.200 (ab)</td>
<td>0.200 (ab)</td>
<td>0.067 (a)</td>
<td>0.233 (ab)</td>
</tr>
<tr>
<td>O-Strong Fishy</td>
<td>0.342</td>
<td>0.300 (a)</td>
<td>0.233 (a)</td>
<td>0.167 (a)</td>
<td>0.100 (a)</td>
<td>0.100 (a)</td>
<td>0.167 (a)</td>
</tr>
<tr>
<td>O-Mild Fishy</td>
<td>0.791</td>
<td>0.400 (a)</td>
<td>0.467 (a)</td>
<td>0.567 (a)</td>
<td>0.500 (a)</td>
<td>0.533 (a)</td>
<td>0.433 (a)</td>
</tr>
<tr>
<td>Tx-Soft</td>
<td>0.000</td>
<td>0.567 (ab)</td>
<td>0.667 (b)</td>
<td>0.433 (ab)</td>
<td>0.367 (ab)</td>
<td>0.200 (ab)</td>
<td>0.267 (ab)</td>
</tr>
<tr>
<td>Tx-Firm</td>
<td>0.000</td>
<td>0.133 (ab)</td>
<td>0.033 (a)</td>
<td>0.533 (bc)</td>
<td>0.267 (abc)</td>
<td>0.600 (c)</td>
<td>0.567 (c)</td>
</tr>
<tr>
<td>Tx-Dry</td>
<td>0.000</td>
<td>0.033 (a)</td>
<td>0.067 (ab)</td>
<td>0.200 (ab)</td>
<td>0.167 (ab)</td>
<td>0.500 (b)</td>
<td>0.233 (ab)</td>
</tr>
<tr>
<td>Tx-Moist</td>
<td>0.001</td>
<td>0.767 (b)</td>
<td>0.667 (ab)</td>
<td>0.367 (ab)</td>
<td>0.533 (ab)</td>
<td>0.333 (a)</td>
<td>0.400 (ab)</td>
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<tr>
<td>Tx-Flaky</td>
<td>0.060</td>
<td>0.100 (a)</td>
<td>0.100 (a)</td>
<td>0.300 (a)</td>
<td>0.300 (a)</td>
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<tr>
<td>T-Sweet</td>
<td>0.498</td>
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<td>0.267 (a)</td>
<td>0.133 (a)</td>
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<td>0.200 (a)</td>
<td>0.100 (a)</td>
<td>0.200 (a)</td>
<td>0.200 (a)</td>
<td>0.233 (a)</td>
</tr>
<tr>
<td>At-Strong Fishy</td>
<td>0.107</td>
<td>0.367 (a)</td>
<td>0.300 (a)</td>
<td>0.200 (a)</td>
<td>0.167 (a)</td>
<td>0.233 (a)</td>
<td>0.067 (a)</td>
</tr>
<tr>
<td>At-Mouth Drying</td>
<td>0.000</td>
<td>0 (a)</td>
<td>0.067 (ab)</td>
<td>0.367 (ab)</td>
<td>0.133 (ab)</td>
<td>0.433 (b)</td>
<td>0.233 (ab)</td>
</tr>
<tr>
<td>At-Oily</td>
<td>0.000</td>
<td>0.533 (b)</td>
<td>0.267 (ab)</td>
<td>0.100 (a)</td>
<td>0.233 (ab)</td>
<td>0.067 (a)</td>
<td>0.233 (ab)</td>
</tr>
<tr>
<td>At-Sweet</td>
<td>0.321</td>
<td>0.200 (a)</td>
<td>0.100 (a)</td>
<td>0.100 (a)</td>
<td>0.200 (a)</td>
<td>0.133 (a)</td>
<td>0.133 (a)</td>
</tr>
</tbody>
</table>

Abbreviations: A: Aroma; O: Odour; Tx: Texture; T: Taste; At: Aftertaste. 52CSVSNB = 52 °C sous-vide cooked salmon no brine; 52CSVSB = 52 °C sous-vide cooked salmon brined; 65CSVSNB = 65 °C sous-vide cooked salmon no brine; 65CSVSB = 65 °C sous-vide cooked salmon brined; 80CSVSNB = 80 °C sous-vide cooked salmon no brine; 80CSVSB = 80 °C sous-vide cooked salmon brined. *values across the rows sharing the same letter do not differ significantly.
are required to ensure safe consumption of sous-vide fish products. However, it should also be noted that salmon is widely consumed raw as sushi and cooked in sous-vide globally, but of course this does not mean that raw salmon is also safe to be consumed in sushi dishes and there is inherent risk in eating any food product that is not handled, cooked or stored correctly.

Another limitation of the study is the small number of healthy participants – blood donors (n = 12), for the platelet preparations that were used to evaluate the antithrombolic properties of PL from salmon sous-vide preparations. This affects the variability (increased standard deviation) of the experimental data, as everybody has a slightly different platelet response to agonists and many factors can affect platelet function, even within the same person from time to time. In order to decrease the variability (standard deviation) of these outcomes, the participants, were fasted the night before and were all deemed healthy prior to volunteering. However, a much larger sample would be optimal for platelet research needed (n = 50–100), which however, could not be achieved within the design of this study.

4. Conclusions

Sous-vide salmon preparations cooked at mild conditions (52–65 °C) of both brined and un-brined salmon resulted in preservation of the antithrombotic properties of bioactive salmon PL against human platelet aggregation induced by the inflammatory and thrombotic mediators such as PAF and thrombin, but also by the well-established platelet agonists ADP and collagen. However, higher temperatures such as those applied for pasteurization (80 °C) resulted in reduction of these antithrombotic properties for salmon PL, which was accompanied by a reduction in their EPA and overall PUFA content in the absence of brining. FA compositional analysis of all sous vide salmon preparations, showed that even though there were variances observed on the FA content in the PL of sous vide cooked salmon preparations in different temperatures, especially for the un-brined salmon samples, their overall n-6/n-3 PUFA ratio was also preserved in beneficially low values for CVD and other chronic disorders. Furthermore, sous vide salmon preparations in all conditions did not affect the taste, whereas in 65 °C and 80 °C slight textural differences were observed. Emerging cooking techniques, such as sous vide seem to be the optimal procedures for preserving the beneficial antithrombotic bioactivities and cardioprotective fatty acid composition of salmon PL, without affecting its taste and texture. Further studies are needed in order to fully evaluate the benefits and drawbacks of sous vide in fish cookery and functional food processing.

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CRediT authorship contribution statement

Shane Redfern: Methodology, Software, Formal analysis, Data curation, Writing - review & editing. Maria Dermiki: Methodology, Validation, Formal analysis, Resources, Data curation, Writing - review & editing, Supervision. Shelley Fox: Methodology, Formal analysis, Resources. Ronan Lordan: Conceptualization, Methodology, Formal analysis, Writing - review & editing, Supervision, Project administration, Funding acquisition. Katie Shields: Software, Formal analysis. Sushanta Kumar Saha: Methodology, Software, Formal analysis, Resources, Writing - review & editing, Supervision. Alexandros Tsoupras: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. Ioannis Zabetakis: Conceptualization, Investigation, Resources, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition, Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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