Role of Extracts Obtained from Rainbow Trout Side Streams by Accelerated Solvent Extraction and Pulsed Electric Fields on Modulating Bacterial and Anti-inflammatory Activities

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Abstract: In this study, accelerated solvent extraction (ASE) and pulsed electric field (PEF) were used as innovative approaches to recover extracts from rainbow trout side streams rich in high-added-value compounds. Then, after aseptic filtration, the impact of the obtained extracts on bacterial growth and anti-inflammatory potential was evaluated. Moreover, the protein content and the total antioxidant capacity of the samples were determined. The results showed that some extracts could inhibit the growth of pathogenic bacteria, such as the ASE rainbow trout skin extracts, which showed significant antibacterial activity on *Staphylococcus aureus*. In addition, some extracts promoted probiotic bacteria growth. For example, the PEF rainbow trout head and skin extracts promoted *Lactobacillus casei* growth, while the ASE rainbow trout head and skin extracts promoted *Bifidobacterium lactis* growth. In addition, some samples, such as ASE rainbow trout viscera extracts had interesting anti-inflammatory properties. Therefore, the use of ASE and PEF can be considered as useful strategies to recover antimicrobial, prebiotic, and anti-inflammatory extracts from rainbow trout side streams although it is necessary to evaluate each specific side stream.

Keywords: fish side streams; rainbow trout; accelerated solvent extraction; pulsed electric fields; antimicrobial; prebiotic; anti-inflammatory

1. Introduction

Over the last decades, growing attention has been paid to the development of natural and alternative antibiotics, especially due to the large use of the traditional ones, which has led to the increase of drug-resistant bacteria [1]. At the same time, food safety problems caused by food-borne pathogens are also concerned by consumers, which makes researchers urgently seek new natural anti-bacterial compounds from food and side streams [2]. There are thousands of naturally active compounds or foods that are thought to have anti-bacterial potential, being marine species of the oceans an interesting potential source of these antimicrobial compounds [3,4].

For instance, around 70% of the earth's surface is covered by water, representing the marine species ≈50% of the global biodiversity, among which fish resources occupy an important position. As the annual output of fish products increases, some by-products from processing side streams are produced. In the industrial processing of fish, each ton of fish processed produces ≈350~600 kg of waste, including head, viscera, bones, and so on [5,6]. These side stream by-products may be used as fertilizers, livestock feeds or directly discarded. The high-value-added bioactive compounds in the side streams have not been utilized very well, causing the waste of resources [7].

These side streams contain several biologically active ingredients, such as protein, fish oil, gelatin, *etc*., which have high application value. For example, fish protein can be used as an important source of high-quality protein, in addition to containing a large amount of collagen. Moreover, it is an important source of bioactive peptides with antioxidant properties [8]. In addition, previous studies also have shown that some components in fish and their side streams show

interesting antibacterial and antiviral capacities. For example, Beaulieu et al. [9] confirmed that the enzymatic hydrolysates of mackerel by-products show antibacterial effects on *Listeria* and *Escherichia coli*; Fuochi et al. [10] also found that the skin mucus of the *Dasyatis pastinaca* (Linnaeus, 1758) showed anti-bacterial and anti-fungal effects. So, at this stage of development, there is a growing interest regarding the valorization of these side streams as potential sources of high-added-value compounds for the development of antioxidants, antimicrobials or antiviral compounds.

Traditionally, heat treatment and/or organic solvent extraction, *etc*., have been used as conventional extraction methodologies to recover valuable compounds from the food side streams. However, these techniques are not in full correspondence with the green extraction concept as they use great amounts of solvents, which in some cases are toxic, long extraction times and can have negative effects on the thermolabile valuable compounds due to the high temperatures used, among other drawbacks [11,12].

In this study, two innovative non-thermal approaches, such as pulsed electric fields (PEF) and accelerated solvent extraction (ASE) were applied to improve the extraction rate according to the green extraction concept. As a short-time pulse effect, PEF has been widely studied in non-thermal food processing. The application of PEF will disintegrate the biological cell membrane of the food matrix and form temporary or permanent membrane pores, which can retain the nutritional and health characteristics of the food to a large extent, ensure the taste and improve the extraction rate [13]. The use of PEF to pretreat fish and algae to extract bioactive compounds has been reported [14,15]. ASE is also a green and efficient extraction method, which works in a high-pressure environment, it can increase the extraction rate of the samples through the accumulation of heat and pressure. Due to its characteristics of being environmentally friendly and safe, ASE has been widely used in the extraction of a variety of high-added value compounds [16,17] and has been recently shown as a useful technique to recover bioactive peptides with antioxidant and antimicrobial properties from salmon side streams [18].

Therefore, in the present work, based on a previous study [19], the fish side streams of rainbow trout with high nutritional value were selected as the target matrices to recover high-value-added compounds with potential antioxidant, antimicrobial, and prebiotic activities. For this purpose, PEF and ASE were used to recover the bioactive compounds from fish side streams (head, skin, and viscera), then protein content and total antioxidant capacity of the recovered were evaluated. Afterwards, the effect of these recovered on bacterial growth (pathogenic and probiotic) and anti-inflammatory activity was explored.

2. Materials and Methods

2.1. Chemicals and reagents

Sodium carbonate (Na_2CO_3) was purchased from VWR (Saint-Prix, France). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), AAPH (2,2'-azobis-2-methyl-propanimidamide), ABTS (2,2'-azinobis (3-ethylbenzothiazoline 6-sulfonic acid)), fluorescein sodium salt, potassium persulfate ($K_2S_2O_8$) was purchased from Sigma-Aldrich (Steinheim, Baden-Württemberg, Germany). Ethanol (99%) was acquired from Baker (Deventer, Overijssel, The Netherlands). Potassium dihydrogen phosphate, sodium phosphate dibasic were purchased from VWR International Eurolab S.L. (Barcelona, Spain). Deionized water was obtained by a Milli-Q SP Reagent Water System (Millipore Corporation, Bedford, MA, USA).

2.2. Sample preparation

The rainbow trout (*Oncorhynchus mykiss*) samples used in the experiments were purchased from a local market in Valencia (Spain). The whole fish was separated in the laboratory to obtain the different fish side streams, including fish head, skin, and viscera for extraction. For PEF treatments, fresh samples were used while for the ASE experiments, samples were pretreated stored at -20 ºC for more than 12 h, then freeze-dried at -48 ºC for 72 h. The freeze-dried samples were stored at -20 ºC until needed.

2.3. Extraction conditions

2.3.1. PEF-assisted extraction

A PEF-Cellcrack Ⅲ (German Institute of Food Technologies (DIL)) equipment (ELEA, Quakenbrück, Osnabrück, Germany) was used for extractions. Specifically, fish side streams were placed in the treatment chamber and tap water was added. Then, the conductivity was measured to be between 1000~2000 μs/cm. The samples were pretreated according to the best conditions previously obtained in the laboratory (**Table 1 a**). Samples were transferred to a beaker and were kept under agitation using a magnetic stirrer for a certain period at room temperature. Then, the supernatant was filtered through a 0.22 μm sterile filter membrane to obtain the samples. The control group was obtained under the same conditions but without applying PEF pretreatment.

2.3.2. ASE-assisted extraction

Similarly, the selection of ASE conditions was also based on the optimal conditions obtained previously in the laboratory [20]. An ASE-200 accelerated solvent extractor (Sunnyvale, California, USA) was used in this study. According to the different samples, it was modified as the ratio of diatomaceous earth: sample utilized being 1.0:2.0 g/g, 1.5:3.0 g/g, and 2.0:2.0 g/g for head, skin and viscera, respectively. The samples and diatomaceous earth were mixed in a mortar and transferred to the extraction tank. The standard parameters used for ASE extraction were: preheating time (1 min), heating time (5 min), flushing volume (60%), nitrogen scanning (60 s) and pumping pressure (103.4 bar). Other conditions are shown in **Table 1 b**. The samples processed by ASE were filtered through a 0.22 μm sterile filter membrane and the control groups were also prepared.

Table 1 (a). Pulsed electric fields (PEF)-assisted extraction experimental conditions.

Sample	Weight (g)	Field strength (kV/cm)	H_2O (mL)	Specific energy (kJ/kg)	Time (h)[1]
Head	100.25	1.00	1500	219.76	21.33
Skin	45.30	3.00	675	300.00	24.00
Viscera	45.30	3.00	675	123.75	15.17

[1] time of supplementary extraction

Table 1 (b). Accelerated solvent extraction (ASE) experimental conditions.

Sample	T (℃)	Time (min)	pH	Pressure
Head	55	15	5.2	103.4
Skin	45	15	6.5	103.4
Viscera	50	15	6.8	103.4

2.4. Chemicals analyses

2.4.1. Protein content

The BCA (Bicinchoninic acid) assay was used to determine the protein content of the extracts [21]. The working solution was prepared according to the BCA kit. Bovine serum albumin (0~2000 mg/L) was used as a standard to prepare the standard curve. Ten microliters of sample/standard and 200 μL of BCA working solution were added to the microplate, then the mixture was mixed well and incubated at 37 ºC for 30 min, the absorbance of the samples was measured at 562 nm.

2.4.2. Total antioxidant capacity

2.4.2.1. Oxygen radical absorbance capacity assay (ORAC)

The determination of ORAC values was carried out according to the previously described method [18,22]. Phosphate buffer (pH 7.0~7.4) was used as the blank group and 1 mM Trolox solution was the standard. Fifty microliters of sample and the 50 μL fluorescein sodium salt were added respectively to a 96-well plate, then the 25 μL AAPH was added, and the plate was kept under 37 ºC for 10 min. Wavelengths of emission at 520 nm and excitation at 480 nm were established to record the results within 60 min. Then, the antioxidant capacity of the sample was calculated according to the formula:

$$\text{ORAC (trolox unit)} = \frac{A_{sample} - A_{blank}}{A_{trolox} - A_{blank}} \quad (1)$$

A measured ORAC value of 1 unit indicates that the antioxidant capacity of the sample solution is equivalent to 100 μM Trolox solution.

2.4.2.2. Trolox equivalent antioxidant capacity assay (TEAC)

The TEAC assay was used to determine the ABTS free radical scavenging capacity of the extracts. According to De la Fuente's method and with some modifications, 25 mL ABTS (7 mM) and 440 μL $K_2S_2O_8$ (140 mM) were mixed to obtain an $ABTS^+$ working solution, which was stored at room temperature in dark about 12~16 h for use [18]. The $ABTS^+$ working solution was diluted with 96% (*v*/*v*) ethanol to maintain the absorbance of 0.700±0.020 at 734 nm. During the test, the samples were diluted to obtain a 50% free radical inhibition rate. The absorbance of 2 mL of the working solution was recorded as the initial value, then 100 μL of the correct dilution of the samples were added, the absorbance was recorded after 3 min of reaction. Different concentrations of Trolox (0~250 μM) were used as the standard to prepare the standard curve and calculate the total antioxidant capacity of the samples.

2.4.3. Impact of extracts on bacterial growth

The impact of the different extracts obtained on several pathogenic and probiotic bacteria was investigated. The culture conditions of the different bacteria are shown in **Table 2**.

Table 2. The culture conditions of the bacteria used in this study

Bacterial	Collection number	Culture medium	Culture conditions
Listeria innocua	(CECT 910)	BHI [1]	37 ℃, 24 h, aerobic
Escherichia coli	(CECT 99)		
Staphylococcus aureus	(CECT 86)		
Salmonella enterica	(CECT 4138)		
Lactobacillus casei	(BB 12)	MRS	37 ℃, 48 h, anaerobic
Bifidobacterium lactis	(NCC 2818)	MRS+0.05% *L*-cys	

[1] BHI: Brain heart infusion medium. [2] MRS: Man rogosa sharpe medium; *L*-cys: *L*-cysteine hydrochloride; CECT: Spanish National Culture Collection (www.cect.org).

In this study, four common pathogenic and two probiotic bacteria modulating human health were selected to evaluate growth patterns in the presence or absence of extracts. Bacterial cultures were collected by centrifugation and inoculated

in the corresponding medium at a final optical density at 595 nm of 0.05. The culture medium (200 μL) and sample (20 μL) were added to 96-well microplates and incubated in a POLARstar (BMG, Labtech, Offenburg, Germany) equipment at 37 ºC for 20 h and optical density was recorded. Gompertz equation was used to describe the bacterial growth rate and maximum optical density:

$$y = K + A \exp\left[-\exp\left(-\frac{\mu_{max}e}{A}(\lambda - t) + 1\right)\right] \quad (2)$$

where y is the extent of growth at time t (h), K is an initial cell number, A is the change in the number of cells between the inoculum and the stationary phase, μ_{max} is the maximum growth rate (the variation in the number of cells per unit of time), λ is the length of the lag phase (h) and e is a constant (2.7182).

2.4.4. Anti-inflammatory analysis

2.4.4.1. Cell culture

To investigate the anti-inflammatory potential of the extracts, a reporter gene assay to analyze the activation of the pro-inflammatory transcription factor NF-κB was performed. Therefore, the human colon tumorigenic cell line HT-29 was previously stably transfected with the plasmid pNiFty2-SEAP (Invivogen, California, USA) containing a secreted alkaline phosphatase (SEAP) reporter gene [23]. The cell line was routinely cultured in DMEM high glucose medium, supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 μg/mL streptomycin and 150 μg/mL zeocin. Cells were cultured at 37 ºC, 5% CO_2 under a humidified atmosphere.

2.4.4.2. Analysis of NF-κB activation

In the experiment, 65.000 cells/well were seeded into 96-well plates and grown for 24 hours. Then, to investigate the activation of NF-κB, 10 μL of pro-inflammatory cytokine TNF-α was added to achieve a final concentration of 10 ng/ml, and 10 μL of extracts to achieve a total volume of 100 μL in each well. After 24 h of stimulation, the supernatant was collected and cells were lysed in PBS containing 1% Triton, 1 mM PMSF (phenylmethylsulfonyl fluorid) and 1 mM EDTA (ethylenediaminetetraacetic acid). The protein content of each well was determined using the Bradford Protein Assay (Biorad). SEAP activity in the supernatant was measured using p-nitrophenyl phosphate as the phosphatase substrate and was normalized to the protein content of each well. The absorbance at 414 nm was measured with a microplate analyzer, the activity of NF-κB induced by TNF-α was recognized to be 100%.

2.5. Statistical analysis

Significant differences between the results were analysed by analysis of variance (ANOVA). A Tukey's Multiple Comparison Test was used to indicate the significant differences in the means. All statistical analyses were performed using the software Statgraphics Centurion XV (Statpoint Technologies, Inc., USA).

3. Results and Discussion

3.1. Protein content

Although the antimicrobial, prebiotic, and anti-inflammatory activities were the main focus of this study, first of all, the protein content was evaluated. Figure 1 shows the effect of the two selected alternative technologies (PEF and ASE) to assist the recovery of protein from rainbow trout side streams. As can be seen in the table, when ASE-assisted extraction was used, the protein content of fish head extracts was significantly increased (p<0.05), and the content reached almost 2-fold higher values compared to the control group. ASE also significantly (p<0.05) increased the protein content of fish skin and viscera extracts. Compared with ASE, PEF-assisted extraction had less effect on the protein content of fish side streams extracts. For rainbow trout, PEF-assisted treatment had no significant (p>0.05) effect on the increase of protein content compared with the control group, being the protein content of fish head and skin extracts slightly lower than that observed for the control group, while no significant effect was observed for viscera extracts (p>0.05).

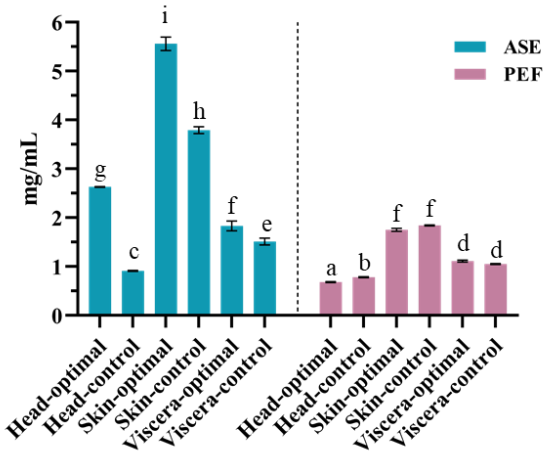

Figure 1. Protein content in control and optimal PEF/ASE assisted extracts from fish by-products. PEF: pulsed electric fields; ASE: accelerated solvent extraction; Different letters in the graph indicate a statistically significant difference (p<0.05).

Fish side streams contain a large amount of protein and other bioactive compounds with high-added value, which can be used as a source of high-quality amino acids. In recent years, in order to reduce the waste of fish side streams, a variety of technologies have been used for the recovery of protein from fish side streams. In this line, Álvarez et al. [24] used ultrasound-assisted acid/alkaline isoelectric solubilization precipitation to recover the protein in mackerel. The results showed that compared with traditional methods, ultrasound-assisted can significantly increase the protein recovery rate from 50~64% to 94%. Similarly, Al-Khawli et al. [25] also used ultrasound to assist the extraction of protein from the sea bass side stream. Under ultrasound-assisted treatment, the protein content of the fish side stream extracts was significantly increased, observing the highest modifications for viscera samples. ASE and PEF have been also recently used as green processing technologies to assist the recovery of nutrients and bioactive compounds from side streams of different species. For instance, De la Fuente et al. [26] used pressurized liquid-extraction (PLE) assisted technology to obtain a protein with antioxidant activity from sea bream side stream, showing that the protein recovery rate can reach 1.2~4.5 times that of the control group. During PLE processing, high pressures and temperature increase the solubility and diffusion rate of high-added-value compounds, thereby improving their extraction efficiency.

3.2. Total antioxidant capacity

Figure 2 shows the antioxidant capacity of different extracts, oxygen-free radical absorbance capacity (ORAC) and trolox equivalent antioxidant capacity (TEAC) were used to evaluate the antioxidant capacity of the extracts. For rainbow trout side streams extracts, both ASE and PEF treatments improved the ORAC values of the extracts, showing significant ($p<0.05$) differences, except for the PEF-assisted skin sample. Among the different side streams extracts, the skin extract obtained under ASE-assisted showed the most obvious difference, which was about three times higher than that observed for the control group. The TEAC values also showed the same trend after applying ASE, observing a significant increase for all the extracts independently of the target side streams evaluated. For instance, compared with the control group, the TEAC values were increased by about 1.4~3.3 times. On the contrary, after PEF-assisted treatment, the TEAC values of PEF fish heads extracts were slightly lower than those of control samples. Moreover, no significant effect on TEAC values was observed after applying PEF to the skin compared to control samples ($p>0.05$). However, interestingly, PEF enhanced the TEAC values of viscera extracts to a certain extent.

ASE also significantly increased the TEAC values of the extracts, which was about 1.7~2.0 times higher than that of the control group (without ASE). Previously, the antioxidant capacity of extracts without sterile membrane filtration was also measured [19]. Comparing the results before and after filtration, it was found that filtration had relatively little effect on the antioxidant capacity of the extracts, meaning the nutrients and antioxidant compounds in the extracts are well retained, which provides a basis for further experiments.

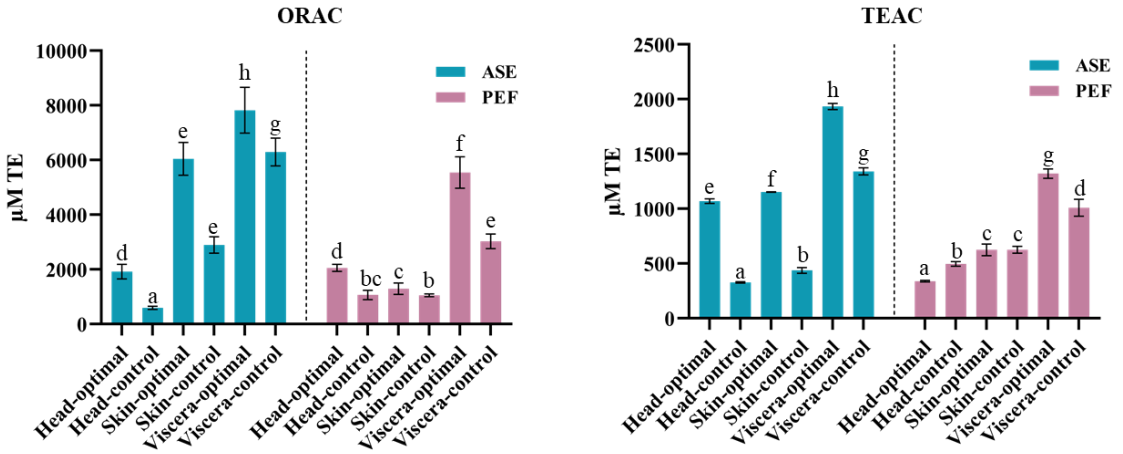

Figure 2. Total antioxidant capacity (ORAC and TEAC) in control and optimal PEF/ASE-assisted extracts from fish by-products. PEF: pulsed electric fields; ASE: accelerated solvent extraction; Different letters in the graph indicate a statistically significant difference ($p<0.05$). * ORAC: oxygen radical absorbance capacity; ABTS: $ABTS^{+}$ scavenging ability.

3.3. Impact of fish side streams extracts on bacterial growth

3.3.1. Anti-bacterial activity against pathogenic bacteria

The effects of different extracts on the growth of four common pathogens are presented in **Tables 3**. The growth rate of different bacteria in 20 h and the optical density of bacterial strains were obtained by fitting the Gompertz equation [27]. As can be depicted from **Table 3**, the extracts obtained from rainbow trout side streams after applying PEF and ASE can induce the growth of *Listeria* and *E. coli*, being the viscera extracts that show the most obvious effect. In addition, viscera extracts also significantly increased the optical density of bacteria. When the effect of PEF was evaluated, it was observed that the extracts obtained from the head and skin had a significant effect on the growth of *E. coli* ($p<0.05$), but there was not a significant effect on the optical density ($p>0.05$), independently of the PEF treatment. Compared to the control group, the ASE-assisted extracts reduced the optimal density of the viscera extract, not observing significant differences with the blank group (without ASE extract).

Moreover, for PEF extracts, no significant effect was found on the growth of *S. aureus*. For instance, the extracts increased the optical density of bacterial growth, showing a significant difference for the viscera extracts ($p<0.05$). Unlike PEF, the head and skin extracts obtained by ASE reduced the growth rate of *S. aureus* ($p<0.05$), but the viscera extract showed an inducing effect on the growth of *S. aureus.* The addition of the extracts did not show any inhibitory effect on the growth of *Salmonella* and had no significant effect on the optical density of the bacteria. In summary, among the four pathogens, rainbow trout head and skin extracts had an inhibitory impact on *S. aureus*, being significant ($p<0.05$) when ASE was used.

Since fish side streams contain a large amount of high-added-value compounds, many people have explored their antibacterial properties over the last years to further expand their applications in food and health. For instance, Robert et al. [28] evaluated the *in vitro* antibacterial activity of tilapia by-products hydrolysate, the peptides produced by hydrolysis showed an important antibacterial activity against *Yersinia ruckeri*. Moreover, they also observed the resistance of these hydrolysates against *Edwardsiella tarda* and *Bacillus megaterium*, thus indicating that tilapia by-products have an important antimicrobial activity. In another study, Ennaas et al. [29] used different proteases to hydrolyse the by-products of Atlantic mackerel skin and the antibacterial properties of hydrolyzed collagen were evaluated. The results showed that the crude hydrolysates of mackerel had an inhibitory effect on *Listeria* and *E. coli*, while the inhibition rate

varied according to the different hydrolysates used. In addition to the properties of the pathogens themselves, the different types of fish also had an impact on the antibacterial properties. Previous studies have shown that fish by-products with a large number of low-molecular-weight peptides have higher activity [30]. In this study, the two kinds of fish showed inhibitory effects on *S. aureus* and could be considered as one of the potential sources of new antibacterial products.

Table 3. Effect of rainbow trout extracts on the growth rate and maximal optical density of four pathogenic bacteria strain

Sample	PEF[1]		ASE[2]	
	Growth rate (μmax, h^{-1})	MOD *	Growth rate (μmax, h^{-1})	MOD*
Listeria				
Bacteria-control	0.442±0.027[a]	1.558±0.039[a]	0.435±0.017[a]	1.524±0.015[a]
Head	0.472±0.004[a]	1.538±0.046[a]	0.479±0.025[a]	1.511±0.019[a]
Head-control	0.461±0.001[a]	1.543±0.022[a]	0.454±0.016[a]	1.525±0.031[a]
Skin	0.464±0.001[a]	1.503±0.001[a]	0.467±0.014[a]	1.588±0.017[b]
Skin-control	0.475±0.006[a]	1.498±0.026[a]	0.435±0.011[a]	1.486±0.020[a]
Viscera	0.599±0.007[c]	1.692±0.023[b]	0.576±0.007[b]	1.666±0.026[c]
Viscera-control	0.526±0.031[b]	1.686±0.015[b]	0.614±0.037[b]	1.635±0.028b[c]
E. coli				
Bacteria-control	0.176±0.009[a]	2.346±0.009[a]	0.176±0.009[ab]	2.346±0.009[ab]
Head	0.208±0.002[b]	2.300±0.044[a]	0.180±0.002[b]	2.307±0.061[ab]
Head-control	0.201±0.001[b]	2.284±0.030[a]	0.193±0.003[c]	2.203±0.034[a]
Skin	0.194±0.009[b]	2.318±0.033[a]	0.185±0.002[b]	2.472±0.103[b]
Skin-control	0.195±0.011[b]	2.292±0.006[a]	0.168±0.003[a]	2.338±0.120[ab]
Viscera	0.172±0.000[a]	2.644±0.046[b]	0.275±0.002[d]	2.188±0.038[a]
Viscera-control	0.178±0.001[a]	2.730±0.064[b]	0.167±0.008[a]	2.757±0.135[c]
S. aureus				

Bacteria-control	0.591±0.039^{b}	2.216±0.215^{a}	0.524±0.056^{cd}	2.401±0.047^{a}
Head	0.560±0.054^{ab}	2.309±0.142^{a}	0.441±0.041^{ab}	2.559±0.018^{ab}
Head-control	0.559±0.003^{ab}	2.309±0.124^{a}	0.448±0.026^{b}	2.455±0.053^{a}
Skin	0.505±0.043^{ab}	2.545±0.126^{ab}	0.404±0.008^{a}	2.796±0.034^{c}
Skin-control	0.496±0.036^{a}	2.533±0.135^{ab}	0.482±0.003^{bc}	2.492±0.048^{a}
Viscera	0.550±0.026^{ab}	2.751±0.067^{b}	0.579±0.037^{d}	2.724±0.034^{bc}
Viscera-control	0.596±0.041^{b}	2.579±0.077^{ab}	0.578±0.049^{d}	2.642±0.053^{bc}
Salmonella				
Bacteria-control	0.335±0.026^{a}	1.838±0.065	0.335±0.026^{a}	1.838±0.065
Head	0.353±0.030^{ab}	1.831±0.164	0.308±0.002^{a}	1.714±0.151
Head-control	0.361±0.025^{ab}	1.756±0.151	0.308±0.005^{a}	1.655±0.055
Skin	0.323±0.007^{a}	1.859±0.043	0.315±0.024^{a}	1.766±0.157
Skin-control	0.346±0.022^{ab}	1.810±0.171	0.302±0.025^{a}	1.798±0.049
Viscera	0.308±0.021^{a}	1.863±0.214	0.418±0.021^{b}	1.687±0.049
Viscera-contro	0.390±0.005^{b}	1.678±0.132	0.447±0.031^{b}	1.675±0.004

[1] PEF: pulsed electric fields. [2] ASE: accelerated solvent extraction. Results are expressed as mean ± standard deviation. Different superscripts in the same column indicate a statistically significant difference ($p<0.05$) and mean values without any superscript indicate statistically non-significant difference. *MOD: maximal optical density measured at 595 nm (difference between initial and final optical density); Bacteria-control: bacterial growth without fish by-products extracts; Head-control/Skin-control/Viscera-control: Head/Skin/Viscera extracts without PEF/ASE-assisted treatment.

3.3.2. Effect on the growth of probiotic bacteria

Table 4 shows the effect of different extracts on the growth of two probiotics. By analysing the effect of rainbow trout on the growth of probiotics, it can be seen that the addition of head and skin extracts in the PEF group promoted the growth of *Lactobacillus casei* with significant differences ($p<0.05$) while no significant differences were observed between PEF and control group ($p>0.05$). Moreover, these extracts also increased the optical density of *Lactobacillus casei*. Compared to the control group, the optical density of PEF extracts was lower than that of the control group, not observing significant differences between the two skin extracts ($p>0.05$). In addition, the viscera extracts did not have any significant effect on the growth rate and optical density of the *Lactobacillus casei*. Studying the effect of PEF extracts on *Bifidobacterium lactis* it was observed that the addition of extracts did not have any significant effect on the growth rate of *Bifidobacterium lactis*.

On the other hand, the addition of ASE extracts reduced the growth rate of *Lactobacillus casei*. Compared with the control group, the head and viscera extracts obtained with the ASE-assisted treatment did not show any significant difference on the *Lactobacillus casei* growth rate. The ASE-assisted skin extract had a weaker effect on the growth rate of *Lactobacillus casei* than the control group (skin-control), being this difference significant ($p<0.05$). At the same time, it was also seen that the head and skin extracts increased the optical density of the *Lactobacillus casei*, showing a significant difference compared to the control group, but there was not a significant effect of ASE-assisted extracts ($p>0.05$). Compared to the control group, the ASE-assisted extracts increased the growth rate of *Bifidobacterium lactis*, but no significant differences were observed. For example, the addition of ASE-assisted viscera extracts did not have any effect on the growth rate of *Bifidobacterium lactis*. On the other hand, head and skin extracts increased the optical density of *Bifidobacterium lactis*, being the optical density of ASE-assisted extracts significantly higher than that of the control group.

Table 4. Effect of rainbow trout extracts on the growth rate and maximal optical density of two probiotic bacteria strain

	Sample	PEF [1]		ASE [2]	
		Growth rate (μmax, h^{-1})	MOD *	Growth rate (μmax, h^{-1})	MOD *
Rainbow trout	*Lactobacillus casei*				
	Bacteria-control	0.349±0.008^{ab}	3.597±0.011^{ab}	0.360±0.012^{c}	1.524±0.015^{a}
	Head	0.382±0.011^{cd}	3.681±0.038^{b}	0.349±0.007^{bc}	1.603±0.008^{bc}
	Head-control	0.374±0.004^{cd}	3.836±0.053^{c}	0.337±0.009^{b}	1.554±0.041^{ab}
	Skin	0.369±0.002b^{cd}	3.719±0.062^{bc}	0.334±0.007^{b}	1.503±0.037^{a}
	Skin-control	0.390±0.017^{d}	3.683±0.069^{b}	0.288±0.011^{a}	1.512±0.016^{a}
	Viscera	0.360±0.000^{bc}	3.533±0.036^{a}	0.283±0.006^{a}	1.613±0.015^{cd}
	Viscera-control	0.336±0.012^{a}	3.595±0.071^{ab}	0.271±0.005^{a}	1.616±0.005^{d}
	Bifidobacterium lactis				
	Bacteria-control	0.536±0.027	3.597±0.011^{b}	0.536±0.027^{ab}	2.346±0.009^{abc}
	Head	0.542±0.035	3.681±0.006^{c}	0.557±0.019^{b}	2.274±0.039^{ab}
	Head-control	0.544±0.027	3.836±0.006^{d}	0.536±0.024^{ab}	2.256±0.029^{ab}
	Skin	0.508±0.028	3.719±0.000^{d}	0.547±0.014^{b}	2.202±0.106^{ab}
	Skin-control	0.561±0.014	3.683±0.010^{c}	0.498±0.008^{a}	2.159±0.059^{a}

Viscera	0.550±0.021	3.533±0.007[a]	0.529±0.010[ab]	2.299±0.003[abc]
Viscera-control	0.532±0.023	3.595±0.009[a]	0.530±0.007[ab]	2.243±0.022[ab]

[1] PEF: pulsed electric fields. [2] ASE: accelerated solvent extraction. Results are expressed as mean ± standard deviation. Different superscripts in the same column indicate a statistically significant difference ($p<0.05$) and mean values without any superscript indicate statistically non-significant difference. *MOD: maximal optical density measured at 595 nm (difference between initial and final optical density). Bacteria-control: bacterial growth without fish by-products extracts; Head-control/Skin-control/Viscera-control: Head/Skin/Viscera extracts without PEF/ASE-assisted treatment.

Exploring the effect of ASE, it was observed that skin extract significantly ($p<0.05$) reduced the growth rate of *Lactobacillus casei*, thus having ASE an obvious impact on the results. The ASE-assisted viscera and head extracts did not have a significant effect on the growth rate of *Lactobacillus casei*, while the viscera extract without ASE-assisted treatment reduced the growth rate of *Lactobacillus casei*. In addition, all the extracts increased the optical density, but there was not a significant difference between each other ($p>0.05$). At the same time, the addition of head extract decreases the growth rate of *Bifidobacterium lactis*, but ASE did not have any significant effect on the results. Moreover, skin and viscera extracts did not show any significant effect on the growth rate of *Bifidobacterium lactis*. Compared to the control group, the ASE-assisted head and skin extracts increased the optical density of *Bifidobacterium lactis* and the viscera extract also increased the optical density, but no impact of ASE was observed ($p>0.05$).

In recent years, many studies have shown that bioactive compounds and hydrolysates derived from fish side streams have antibacterial activity, which can inhibit the growth of pathogenic bacteria to a certain extent, although there are only a few studies on the growth of probiotics. Probiotics are used in the fermentation and preservation of food to help maintain food quality and improve nutrition. In the study of Safari et al. [31], two different peptones were obtained from the yellowfin tuna (*Thunnus albacares*) head by enzymatic hydrolysis, their effects on the growth of a variety of bacteria including pathogens and probiotics were explored. The results obtained by these authors showed that the proteins obtained by hydrolysis promoted the growth of *Lactobacillus plantarum* and *Lactobacillus bulgaricus*, making their growth rate higher than that of *Lactobacillus sakei* and others. It is speculated that the main reason may be that the growth of *Lactobacillus* from different sources has different requirements for the types of amino acids, while the matching degree of the peptides produced by enzymatic hydrolysis to different types of *Lactobacillus* is also different. Combining with the effect of the extract on the pathogenic bacteria, it can be known that the type of peptide in the extract will affect the growth of bacteria, while peptides of appropriate molecular weight can make probiotics show greater growth activity. In addition, the difference in the form and concentration of the samples during PEF and ASE-assisted extraction is also one of the reasons for the different results.

3.5. Anti-inflammatory activity

The anti-inflammatory potential of rainbow trout fish side streams are shown in **Figure 3**. For rainbow trout, PEF extracts did not show any significant anti-inflammatory potential, while ASE extracts had a significant inhibitory effect on NF-κB activity, which could inhibit ≈40~45% of TNF-α-induced NF-κB activity in viscera extracts. Interestingly, in the PEF group of rainbow trout extracts, the extracts from skin and viscera (without PEF and ASE treatments) enhanced the TNF-induced NF- κB activity to levels of 150 and 126%, suggesting that PEF treatment could alter some components of these extracts and reduce their intrinsic pro-inflammatory potential.

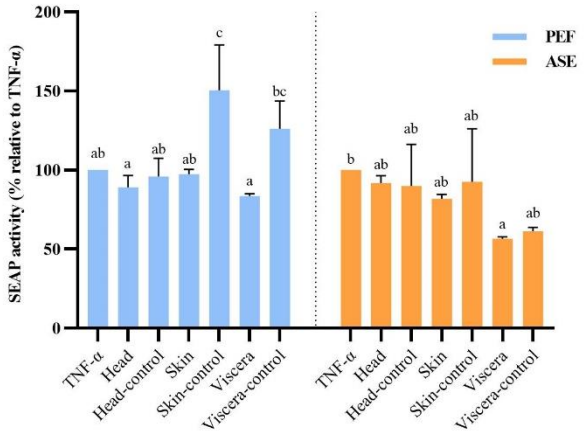

Figure 3. The NF-κB activation induced by TNF-α and the effect of fish by-products extracts using different treatment technology (PEF/ASE) were determined. The SEAP activity induced by TNF-α was considered 100%. *PEF: pulsed electric fields; ASE: accelerated solvent extraction; Different letters in the graph indicate a statistically significant difference ($p<0.05$).

Inflammation is the immune system's response that can effectively protect our body from injury and infection, however, excessive release of inflammatory mediators can become chronic and lead to many inflammatory diseases. In the intestinal tract, proinflammatory stimulants activate in intestinal epithelial cells NF-κB, a master regulator of inflammatory processes among several others, which upregulates cytokines and chemokines. [32,33]. Studies have shown that protein polypeptides are anti-inflammatory, anti-hypertensive, *etc.* For instance, Gao et al. [34] obtained synthetic peptides from sturgeon muscle and found that they can effectively reduce the release of inflammatory mediators and cytokines. It can be speculated that the anti-inflammatory potential of some extracts may be related to the bioactive peptide in extracts.

4. Conclusions

From the results obtained in this study, it may be concluded that PEF and ASE may be used as useful alternative approaches in recovering extracts with antimicrobial, prebiotic, and anti-inflammatory properties. Some extracts showed antibacterial and anti-inflammatory effects, including those obtained by ASE rainbow trout extracts that promoted inhibitory effects on the growth of *S. aureus* and *Salmonella*. When PEF was studied, PEF rainbow trout head and skin extracts also showed an inhibitory effect on the growth of *S. aureus*. In addition, they also enhanced the growth of *Lactobacillus casei*. It was also found that some extracts showed anti-inflammatory potential, including those obtained from ASE and non-ASE rainbow trout viscera. This may be because the bioactive peptides in them play a vital role. In general, these extracts can be considered as potentially valuable functional substances to further study their beneficial effects on humans.

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