Innovative Food Science and Emerging Technologies 71 (2021) 102712

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Supercritical CO_2 extraction of oil from Arctic charr side streams from filleting processing

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ARTICLE INFO

Keywords:
Supercritical carbon dioxide extraction
Arctic charr
Fatty acids composition
Astaxanthin
Antioxidant activity

ABSTRACT

Although Arctic charr side streams contain limited amounts of fish flesh, they are a rich fish oil source (46.3 ± 0.6%). The aim of the study was to investigate the potential for valorization of Arctic charr filleting side streams through the extraction of oil by supercritical CO_2 technology. The effect of temperature (40 °C and 80 °C) and pressure (20, 35 and 45 MPa) on the final extract after supercritical fluid extraction (SFE) was evaluated. Temperature increase enhanced the yield but decreased the antioxidant activity at 45 MPa, did not affect the yield and the antioxidant activity at 35 MPa, whereas yield was limited at 20 MPa and 80 °C. Extracts were rich in monounsaturated fatty acids (56.7–58.3%, especially oleic acid 37.2–38.0%), and polyunsaturated fatty acids (20.2–26.1%, especially DHA 7.3–11.4%). The presence of astaxanthin significantly preserved the extracts from oxidation.

Industrial relevance: Supercritical carbon dioxide extraction is a green technology appropriate for the recovery of non-polar and heat sensitive compounds. The extracted Arctic charr oils were rich in polyunsaturated fatty acids and astaxanthin which inhibited oxidation in combination with the absence of oxygen and light during the process. This technology could be an excellent alternative for more sustainable valorization of fish processing side streams.

1. Introduction

The increasing global consumption of fish and fish products and the expansion of fish processing are resulting in high quantities of offal and other by-products, which can be up to 70% of the initial catch weight (FAO, 2020). Annual fish production reached 178 million tons in 2019 and approximately 90% was intended for human consumption. According to FAO (2018), food loss and wastage until the consumption was estimated at 27% of landed fish. Presently fish industry by-products are mainly used as animal feed or as organic fertilizers. As these discards are rich in exploitable valuable compounds such as polyunsaturated fatty acids, proteins, minerals, vitamins and pigments, improved and environmentally friendly processes have been investigated during the last decades for the optimization of the recovery of these components, in a framework of a more sustainable utilization of fish processing side streams (FAO, 2018, 2020).

Arctic charr (*Salvelinus alpinus*) is a member of the trout and salmon family, showing similar characteristics such as high concentrations of omega-3 fatty acids in flesh. It is a cold-water fish which is mainly found in inshore marine waters, lakes and rivers and is known for its desirable texture and taste (Dalsgaard et al., 2010; Heasman & Black, 1998). Due to the increased growth rates at lower temperatures, Arctic charr is one of the prominent aquaculture species in Iceland, Sweden, Norway and Canada, which are the leading producers worldwide, and its global production is 6000–10,000 metric tonnes. Farming of Arctic charr in the Nordic countries constitutes more than 90% of the European production (Sæther, Siikavuopio, Thorarensen, & Brännäs, 2013). The annual production in 2016 has been estimated 4200 and 300 t in Iceland and Norway, respectively. The production in Sweden shows a constantly increasing trend and the respective value was 1760 t for 2016 which was about 2.5 times higher compared to annual production in 2008 (FEAP, 2017; Statista, 2019). Arctic charr is sold as whole or more often in the form of fully trimmed fillets which in turn leads to side stream products. Therefore, the valorization of its side streams is a crucial problem for

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https://doi.org/10.1016/j.ifset.2021.102712
Received 11 March 2021; Received in revised form 20 April 2021; Accepted 17 May 2021
Available online 19 May 2021

Arctic charr industries (Dalsgaard et al., 2010; Gunnarsson et al., 2010).

Omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA / C20:5 n-3) and docosahexaenoic acid (DHA / C22:6 n-3) are important bioactive compounds for human nutrition and health that are present in fish and especially in fatty species like Arctic charr (Ackman, 2007). Valorization of fish discards such as skins, flesh, heads and viscera, is a promising goal for oil production and therefore different types of fish have been examined as raw materials. Fish oil represents 2% of total fats and oils consumed worldwide and is basically used in food and pharmaceutical industries, agriculture and aquaculture as feed additive (Ivanovs & Blumberga, 2017; Rubio-Rodríguez et al., 2012). Moreover, fish oil derived from salmonoids contains natural antioxidants such as astaxanthin, a lipid-soluble carotenoid pigment belonging to xanthophylls group. This pigment is responsible for the pinkish color of Arctic charr and it has a strong antioxidant activity and several health benefits (Miki, 1991).

Solvent extraction and hydraulic pressing are conventionally used to extract oil from fish tissues. The main disadvantage of these extraction techniques is the toxicity of the solvents for humans and their environmental impact. In general, solvents cannot be completely removed from the extract and the presence of impurities in the recovered components may raise limitations regarding their applicability, e.g. as food or feed ingredients (Haq & Chun, 2018; Ivanovs & Blumberga, 2017). In addition, conventional extraction methods require processing at high temperatures to enhance the low yields and remove solvents, leading to thermal degradation of heat-sensitive compounds (Gustinelli, Eliasson, Svelander, Alminger, & Ahrné, 2018; Kuvendziev, Lisichkov, Zeković, Marinkovski, & Musliu, 2018). In this framework, the last two decades, new environmentally friendly technologies have been introduced as alternatives to the organic solvents, in order to recover high quality bioactive compounds (Gustinelli et al., 2018; Ivanovs & Blumberga, 2017).

Supercritical fluid extraction (SFE) with CO_2 as solvent is one of the most promising methods for valuable compounds and especially for non-polar compounds. Carbon dioxide is a non-toxic, non-flammable, "green" solvent appropriate for food industry and relatively to other solvents, it is characterized by a critical point at mild conditions (Tc = 31 °C and Pc = 7.38 MPa). The low temperature and the absence of oxygen during the extraction preserve the bioactive compounds and make this technology appropriate for thermally sensitive components, such as polyunsaturated fatty acids. Moreover, CO_2 can be easily separated from the extract by changing the operating conditions below the critical point. In addition, supercritical extraction is a flexible method for the fractionation of solutes through the variation of temperature and pressure or the addition of co-solvent (Gustinelli et al., 2018; Ivanovs & Blumberga, 2017; Kuvendziev et al., 2018; Seader & Henley, 2006).

Although supercritical CO_2 has extraction limitations for polar compounds, it is highly suitable solvent for lipophilic compounds such as fats, oils, and other non-polar compounds. The use of co-solvents even in small quantities may modify solvents polarity, which is necessary to extract more polar molecules (Ivanovs & Blumberga, 2017; Rubio-Rodríguez et al., 2012).

The aim of the study was to investigate the potential for valorization of Arctic charr filleting side streams through the extraction of oil by supercritical CO_2 technology. With respect to that concept, the effect of pressure (20, 35 and 45 MPa) and temperature (40 and 80 °C) during the supercritical CO_2 extraction on the extraction yield, fatty acids composition, astaxanthin content, antioxidant activity and peroxide value of extracted oil from Arctic charr residues were evaluated.

2. Materials and methods

2.1. Raw materials and pretreatment

2.1.1. Arctic charr side streams

Arctic charr (*Salvelinus alpinus*) filleting side streams were provided by a Swedish fish producer (Umlax AB, Lycksele, Sweden) in May 2019 and consisted of heads, skin, bones, frames and tails. The raw material was transferred to the laboratory and stored at -40 °C. The offcuts were milled for 15 s in a knife mill (Tecator 1094 Homogenizer, Tecator, Höganäs, Sweden), freeze dried for 48 h (Alpha 1–2 LDplus, Christ, Osterode am Harz, Germany) and milled again for 10 s in order to reduce the internal mass transfer resistance during the extraction. Samples were stored at -40 °C until the oil extraction.

2.1.2. Solvents and reagents

Carbon dioxide (>99.99% purity), used for the extraction process, was obtained from Air Liquide (Nässjö, Sweden). The materials used for the analysis of the extracts were 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Alfa Aesar (Steinheim, Germany) and the standard of astaxanthin (>97% purity) from Sigma–Aldrich (Steinheim, Germany). Acetyl chloride (≥99.0%) was obtained from Fluka, Sigma-Aldrich (Stockholm, Sweden). Methanol (≥99.9%), ethanol (≥99.9%), toluene (≥99.0%), *n*-hexane for HPLC (≥95%), acetone (≥99.5%), acetic acid glacial (100%), chloroform (≥98.0%) were obtained from Sigma–Aldrich (Steinheim, Germany). For the GC–MS analyses a FAME standard mixture (Supelco 37 Component fatty acids methyl esters mix, Sigma–Aldrich, Steinheim, Germany) was used to quantify fatty acids of the samples. In addition, C7–C30 alkanes mixture (Sigma–Aldrich, Steinheim, Germany) was analysed for the determination of retention indices, while the extracted oil was diluted in 2,2,4-trimethylpentane (Sigma–Aldrich, Steinheim, Germany) prior to GC–MS analysis.

2.2. Methods

2.2.1. Supercritical CO_2 extraction (SFE)

SFE was carried out in a laboratory-scale SFE system WATERS SFE-500 M1–2-C50 (Waters Inc., Pittsburgh, PA, USA). This unit consisted of a CO_2 pump connected to a cooling bath (CF32-HD Julabo GmbH, Seelbach, Germany) at 1 °C, a 500 mL stainless steel extractor equipped with a heating jacket and a 500 mL cyclone to separate the extracts from the solvent. The cyclone was kept at a pressure of 1 MPa and a temperature of 25 °C.

Glass wool (2 g) was used in the bottom of the extraction basket to protect its filters and then 50 g of dried by-products were added. The remaining empty space was filled up with 2 g of glass wool, too. The solvent was pure CO_2 and its flow rate was constant at 30 g min^{-1}. Six different operating conditions were examined consisted of 3 different pressures (at 20, 35 and 45 MPa) and 2 temperatures, at 40 °C (close to the critical temperature of CO_2 which is 31 °C) and one higher at 80 °C. The total time of extraction was 2 h and extract was collected every 20 min in Falcon tubes to create the extraction curve. The samples were weighted a few minutes after the collection in order to remove the remaining CO_2 in the tubes. Extractions at each condition were performed in duplicate. The extraction yield was expressed as percentage (%) of extracted oil per 100 g of dry material. The extracted oils were stored at -40 °C for further analysis.

The total oil content of Arctic charr by-products was defined using Soxhlet extraction for 8 h and *n*-hexane as solvent, according to performed preliminary trials. Then, the solvent was evaporated in a rotary vacuum evaporator (Heidolph G1, Schwabach, Germany) at 45 °C. Hexane was reported as a common solvent for determination of total lipids in fish and seafood products (Mathew et al., 2019). The extraction recovery by the SFE process was expressed as percentage (%) of the total yield extracted with Soxhlet. Since the extracts by means of Soxhlet extraction were exposed to oxygen and high temperature due to the boiling of the solvent for several hours, no further analysis was undertaken in terms of oil characterization. Fatty acids degradation with Soxhlet extraction has been reported in other studies (Joo-Hee et al., 2012; Rubio-Rodríguez et al., 2008).

2.2.2. Fatty acid analysis

The direct transesterification of fatty acids (FA) extracted from Arctic charr by-products based on the method described by Lepage and Roy (1984) with modifications was followed. 10 mg of extract were dissolved in 5 mL of methanol:toluene 3:2 (*v/v*) and mixed with 5 mL of acetyl chloride:methanol mixture 1:20 (*v/v*). The tubes were heated at 100 °C for 1 h and after cooling at room temperature, 5 mL of distilled water and 5 mL of *n*-hexane were added. Samples were centrifuged at 3000 rpm for 5 min (Thermo Scientific Heraeus Megafuge 16R Thermo Fisher Scientific, Waltham, MA, USA) and the upper organic phase was collected and subjected to evaporation of solvent in a rotary vacuum evaporator (Heidolph G1, Schwabach, Germany). The obtained methyl esters of fatty acids (FAME) were diluted with isooctane and analysed on an HP 7890 GC system (plus +) coupled to an HP 5975 mass selective detector (Hewlett Packard, Palo Alto, CA, USA), and equipped with an HP-5 MS column (30 m × 250 μm, 0.25 μm, Hewlett Packard, Palo Alto, CA, USA). The split ratio was set at 50:1. Oven temperature was started at 125 °C, raised to 240 °C at 5 °C min^{-1} rate and hold at 240 °C for 12 min. Helium was used as the carrier gas with a fixed flow rate of 1 mL min^{-1}, inlet temperature at 220 °C and split 20:1. The mass range was 40–400 *m/z* and compounds were identified by comparison of their mass spectra with the data of NIST and Wiley mass spectral libraries. The determined retention indices (RIs) of the compounds were compared with the ones reported in the literature in order to verify the identifications. The results were expressed as percentage (%) of the total amount of FA.

2.2.3. Astaxanthin content measurement

The concentration of astaxanthin was determined with a spectrophotometric method as proposed by Dave, Liu, Pohling, Trenholm, and Murphy (2020) with modifications. Each oil sample was diluted in acetone to a final concentration of 0.25 mg oil mL^{-1} and filtered with membrane filter (0.22 μm pore size) to remove impurities. The absorbance of the extracts was measured at 477 nm using a UV–Vis spectrophotometer (Heλios α, Spectronic Unicam EMEA, Cambridge, UK) and the results were expressed as mg astaxanthin per g of oil through a standard curve using astaxanthin in a concentration range of 0.5–10 mg mL^{-1}. Pure acetone was used as a blank. The standard curve of astaxanthin concentration in acetone is expressed by the following equation:

$$C_{astaxanthin} = 0.240 \cdot A \left(R^2 = 0.995\right) \quad (1)$$

where $C_{astaxanthin}$ is the concentration of astaxanthin expressed as mg per mL of acetone and A the absorbance at 477 nm.

2.2.4. Free radical scavenging activity (DPPH)

Since the Arctic charr contains lipophilic antioxidants, such as astaxanthin, which are extracted together with the fish oil, the impact of the operating conditions on the antioxidant activity was evaluated. The antioxidant activity of the extracts was measured using the 2,2-diphenyl-1-picrylhydrazil (DPPH) method according to Brand-Williams, Cuvelier, and Berset (1995) with modifications. The same protocol for DPPH measurement has been also reported by Gustinelli et al. (2018). Preliminary trials were performed to select the optimal conditions for this analysis. The DPPH stock solution was prepared every day after dilution in ethanol at a concentration of 75 μM. Extracted oil was also diluted in ethanol at concentrations of 5, 10, 15, 20 and 25 mg mL^{-1}. 1 mL of each dilution was mixed with 1 mL of the stock DPPH solution in tubes and the mixture was vortexed for 10 s. The tubes were incubated at room temperature in darkness. After 120 min, the absorbance was measured at 517 nm in a spectrophotometer. The results were expressed through the efficient concentration (EC_{50}) which is the concentration of extract that gives rise to a 50% reduction in DPPH absorbance. EC_{50} value was calculated using linear regression analysis.

2.2.5. Peroxide value (PV)

Oil oxidation was monitored through the determination of PV method according to the AOCS method Cd 8–5 (AOCS, 1998) with modifications. 2 g of sample were transferred into a 250 mL flask and mixed with 20 mL of the acetic acid:chloroform 3:2 (*v/v*) solvent mixture and 0.5 mL of freshly prepared potassium iodide (KI). The mixture was shaken for 1 min and allowed to stand in darkness for 5 min. Then, 20 mL of distilled water and the starch indicator were added in the flask. The samples were titrated with 0.01 N sodium thiosulfate solution ($Na_2S_2O_3$) until the blue color disappears. A blank sample was determined under the same conditions. The PV was calculated with Eq. (2) and was expressed as milli-equivalents peroxide per 1 kg of oil ($meq_{O2}\ kg_{oil}^{-1}$).

$$PV = \frac{(S - B) \cdot N \cdot 1000}{m} \quad (2)$$

where S and B is the consumption of $Na_2S_2O_3$ in the sample and blank test in mL respectively, N the normality of $Na_2S_2O_3$ solution and m the sample mass in g.

2.3. Statistical analysis

All experiments were carried out in duplicate and analyses were carried out on all samples. One or two-way analysis of variance (ANOVA) were carried out at a significant level of 95% to determine statistically significant differences using temperature and/or pressure as factors. Differences were determined according to Tukey post hoc test (a = 0.05) with STATISTICA 7.0 (StatSoft Inc., Tulsa, USA). Values were consisted significant at $p < 0.05$.

3. Results and discussion

3.1. Extraction yield

The moisture content of dried Arctic charr side streams was 0.9 ± 0.4% on dry basis, and the oil content as determined by Soxhlet extraction method with *n*-hexane was 46.3 ± 0.6%. Based on preliminary experiments it was concluded that *n*-hexane was the appropriate solvent for Soxhlet and the total time of extraction was 8 h, while the average time for each extraction cycle was approximately 8 min.

SFE oil extraction was limited the first 20 min as shown on Fig. 1a and b, due to the time required to reach the desired pressure, ranging from 5 to 11 min, depending on the conditions. At higher times, the yield was increased intensively within the first hour and finally approached an equilibrium (plateau) after 120 min equal to the total extraction yield of each experiment. The total yields of recovered oils after 2 h of extraction are presented in Fig. 2 for all the examined conditions and expressed on dry basis. The total yield of Arctic charr oil ranged from 15.4 to 35.4% (*w/w*) depending on the applied conditions, except for 20 MPa and 80 °C in which the fish oil yield was very low (0.6%). The yields at 35 MPa ranged between 27 and 28% ($p > 0.05$), while at 45 MPa there were significant differences ($p < 0.05$) between the tested temperatures, i.e. 28.2 ± 3.0% and 35.4 ± 0.5% at 40 °C and 80 °C, respectively. According to the results, the effect of operating temperature on oil extraction depended on the applied pressure because temperature's increase could enhance, decrease or not significantly affect this parameter. This could be explained by the complex influence of temperature and pressure on the viscosity and density of supercritical CO_2 and oil volatility. Increasing temperature under constant pressure leads to lower solvent density and higher solute volatility which are competitive effects on total oil solubility. As the density of supercritical fluid is decreased, the distance between molecules of solvent and solute is increased and consequently, the mass transfer rates are decreased. On the other hand, increasing solutes vapor pressure improves oil solubility to supercritical CO_2. Therefore, the result of temperature under constant pressure

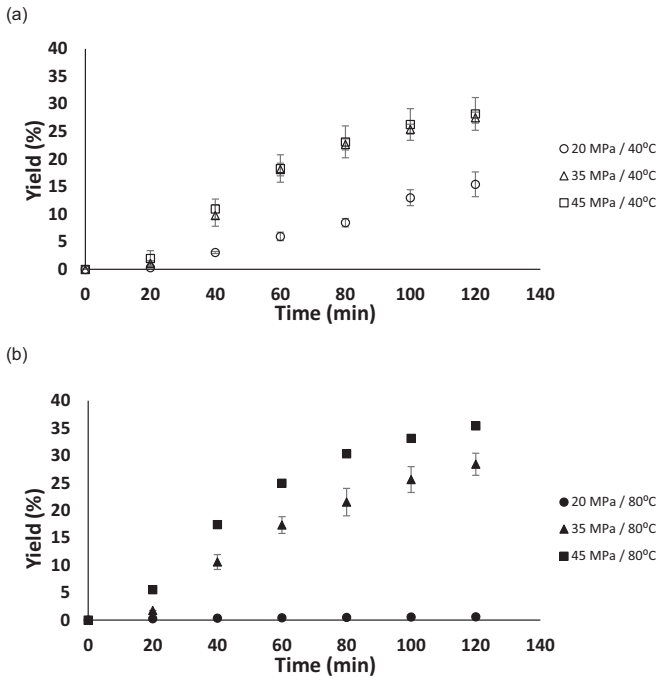

Fig. 1. Extraction yield of Arctic charr oil using SFE at (a) 40 °C and (b) 80 °C (Mean ± standard deviation).

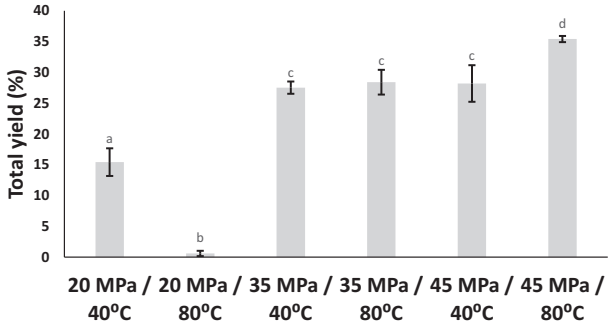

Fig. 2. Extraction yield (*w*/w) of Arctic charr oils extracted at different conditions after 120 min of extraction (Mean ± standard deviation). Different letters above error bars denote significant difference according to ANOVA followed by Tukey's post hoc test.

depends on the predominant effect. The pressure at which the temperature influence on the oil yield is reversed, is called 'crossover pressure' and this phenomenon has been reported in relevant studies (Gustinelli et al., 2018; IPCC, 2005; Kuvendziev et al., 2018; Orellana, Smith, & Kitchens, 2013). The results of the present study indicated a crossover pressure close to 35 MPa since the temperature change did not affected the oil yield at this pressure. At 20 MPa, an increased temperature hindered the oil solubilization which could be explained by a predominant effect from a reduced solvent density. The remarkably reduced solubility of oil at 20 MPa and 80 °C has been reported by Fattori, Bulley, and Meisen (1988) for oil extracted from canola seeds. This could explain the low yield (0.6%) of extracted oil from Arctic charr side streams. On the other hand, at 45 MPa, the oil yield was enhanced with increasing temperature thus the increased vapor pressure from the solute was the dominant factor. The influence of operating pressure on the extracted oil at 40 °C was in accordance with Kuvendziev et al. (2018) for common carp flesh at the same range of pressure.

Estimation of oil recovery by means of SFE by comparison to the total oil of raw material based on Soxhlet method was carried out. Supercritical CO_2 extraction achieved relatively high recovery of the total oil from Arctic charr side streams under specific conditions. The recovery of oil ranged from 59.7 to 76.8% at 35 MPa and 45 MPa and it was lower than 35% at 20 MPa. This study indicated that although the side streams of Arctic charr contain limited amounts of fish flesh, they are a rich fish oil source suitable for further valorization. The extractability of oil with SFE compared with Soxhlet may be attributed to the fact that the oil was obtained mainly from the particle surface and was limited from the sample interior, due to the restricted oil diffusion within the solid matrix. Similar extractability levels of SFE have been reported by Sahena et al. (2010) for extracts from Indian mackerel skin without the use of co-solvent or pretreatment and by Haq, Ahmed, Cho, and Chun (2017) for oil extracted from Atlantic salmon by-products in which the extractability ranged from 76 to 86% at 20 MPa and 45 °C after 3 h of extraction.

3.2. Fatty acids composition

The extracted oil from Arctic charr side streams were rich in both saturated (SFA) and unsaturated fatty acids (UFA). FA composition and the total concentration of SFA, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) expressed as percentage (%) of total FA are presented in Table 1. The total amount of MUFA represented about 55% of the total FA, while SFA (17.2–22.9%) and PUFA (20.2–26.1%) are significantly lower than MUFA. According to the results, SFA were relatively lower at 45 MPa compared to the other applied pressures showing that pressure increase resulted in lower selectivity for UFA. These results were in agreement with the results reported by Sánchez-Camargo, Martinez-Correa, Paviani, and Cabral (2011) for oil acquired from shrimp waste. The major FA of Arctic charr oil was the oleic acid (C18:1) which represented up to 38% of total FA depending on the conditions (37.2–38.0%), followed by the palmitic acid (C16:0) representing up to 13% (11.5–12.5%), DHA ranged from 7.3 to 11.4%, linoleic acid (C18:2 n-6) ranged from 8.1 to 8.9% and palmitoleic acid (C16:1) ranged from 6.7 to 7.7%. The results were in agreement with the results of Haq and Chun (2018), who reported that oil from other salmonoids, such as Atlantic salmon by-products, had similar FA profile apart from erucic acid (C22:1, less than 0.5% of total FA recovered) and

Table 1
Fatty acids composition (% of total fatty acids) of Arctic charr oil extracted at different conditions.

Fatty acids	20 MPa 40 °C	20 MPa 80 °C	35 MPa 40 °C	35 MPa 80 °C	45 MPa 40 °C	45 MPa 80 °C
C12:0	2.29 ± 0.74	2.59 ± 0.04	3.16 ± 0.02	3.25 ± 0.65	0.05 ± 0.02	0.03 ± 0.00
C14:0	4.07 ± 0.53	3.36 ± 0.07	4.20 ± 0.42	4.07 ± 0.23	3.59 ± 0.09	3.20 ± 0.08
C16:0	12.09 ± 0.64	11.64 ± 0.29	12.38 ± 0.76	12.53 ± 0.17	11.85 ± 0.50	11.50 ± 0.11
C16:1	7.68 ± 0.56	6.72 ± 0.16	7.67 ± 0.43	7.71 ± 0.05	7.33 ± 0.30	7.11 ± 0.09
C18:0	1.78 ± 0.01	1.88 ± 0.02	1.81 ± 0.05	1.84 ± 0.09	1.88 ± 0.09	1.92 ± 0.01
C18:1 n9	37.59 ± 0.12	37.25 ± 0.54	38.05 ± 0.51	37.71 ± 0.85	37.23 ± 1.33	37.45 ± 0.39
C18:2 n6	8.57 ± 0.31	8.07 ± 0.14	8.18 ± 0.16	8.30 ± 0.03	8.37 ± 0.34	8.88 ± 0.09
C20:1 n9	4.46 ± 0.19	5.70 ± 0.20	4.33 ± 0.53	4.84 ± 0.18	5.92 ± 0.69	5.11 ± 0.06
C20:5 n3	4.59 ± 0.08	4.26 ± 0.08	4.11 ± 0.18	4.18 ± 0.17	4.65 ± 0.01	5.19 ± 0.09
C22:1 n9	6.77 ± 1.21	8.26 ± 0.81	6.55 ± 0.46	6.25 ± 0.30	7.21 ± 1.03	6.72 ± 0.30
C22:6 n3	8.71 ± 1.23	8.96 ± 0.53	7.32 ± 0.76	7.59 ± 0.80	10.63 ± 0.81	11.36 ± 0.79
SFA	20.8	20.0	22.9	22.3	17.9	17.2
MUFA	56.9	58.3	56.9	56.9	58.0	56.7
PUFA	22.3	21.7	20.2	20.8	24.1	26.1

lauric acid (C12:0, not detected). Several studies in the literature reported that FA composition of Arctic charr flesh presents significant variation depending on fish diet and seasonality. The major FA were myristic acid (C14:0), C16:0, C16:1, C18:1 n-9, eicosenoic acid (C20:1 n-9), EPA, C22:1 and C22:6 n-3 (Olsen & Henderson, 1997). Moreover, at 45 MPa there was absence of lauric acid (C12:0) while at all the other conditions lauric acid recovery ranged from 2.3 to 3.3% of total FA. Based on those results, it can be assumed that the fish oil obtained from Arctic charr side streams had similar FA profile with the respective fish oil extracted from fish flesh as well as from other salmonoids.

In terms of the FA concentration of the extracted Arctic charr oil expressed as μg per mg of oil, pressure played a crucial role at higher temperature (80 °C) in which pressure increase, raised the concentration of extracted FA ($p < 0.05$) as shown on Fig. 3. On the other hand, there were no significant differences between the total UFA of samples at 40 °C ($p > 0.05$) in which the total concentration was 519.0 ± 24.8, 530.9 ± 75.6 and 501.4 ± 30.9 μg mg^{-1} at 20, 35 and 45 MPa, respectively. Total SFA concentration was similar at 20 and 35 MPa (136.4 ± 14.5 and 158.0 ± 21.7 μg mg^{-1}, respectively) at 40 °C, while at 45 MPa it was significantly lower (109.1 ± 5.3 μg mg^{-1}) because of the lack of lauric acid. Pressure has also been reported to be the dominant parameter on the UFA yields on *Pistacia terebinthus* berries (Senyay-Oncel, Ertas, & Yesil-Celiktas, 2011) due to higher solvent density at higher pressure. Apart from extracted oil at 45 MPa and 80 °C in which almost the entire extract was related with FA, FA content in the other oils represented up to 70% of the extract. The remaining percentage was not related with FA and could comprise other lipid-soluble compounds like astaxanthin and impurities since the extract is crude fish oil. Crude fish oil could contain impurities such as moisture, volatile compounds, proteins, minerals and oxidation products depending on the extraction method and conditions, for that reason after the extraction there is commonly a refining treatment for removing the impurities and increase the content of MUFA and PUFA (Bonilla-Mendez & Hoyos-Concha, 2018; EFSA, 2010).

3.3. Astaxanthin concentration

It has been reported that astaxanthin is the major carotenoid contained in Arctic charr and other salmonoid fish and therefore, its concentration was determined in this study and presented on Fig. 4. At intermediate pressure (35 MPa), temperature had a negative effect on astaxanthin concentration ($p < 0.05$) which was equal to 11.7 ± 0.7 μg g_{oil}^{-1} and 7.5 ± 0.1 μg g_{oil}^{-1} at 40 °C and 80 °C, respectively. In general, astaxanthin is an unstable component and prone to oxidation and

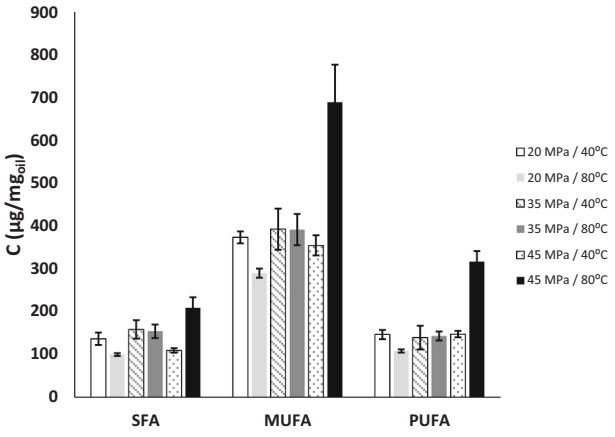

Fig. 3. Total SFA, MUFA and PUFA concentration (μg mg^{-1}) Arctic charr oil extracted at different conditions (Mean ± standard deviation). Different letters above error bars denote significant difference between the same FA group according to ANOVA followed by Tukey's post hoc test.

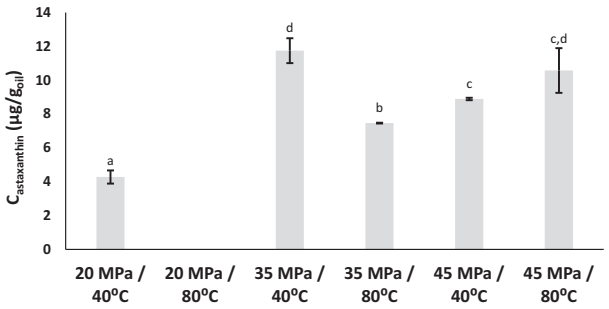

Fig. 4. Astaxanthin content of Arctic charr oils extracted at different conditions after 120 min of extraction (Mean ± standard deviation). The condition 20 MPa and 80 °C could not be analysed due to a very low oil yield. Different letters above error bars denote significant difference according to ANOVA followed by Tukey's post hoc test.

therefore, extraction at high temperatures should be avoided as it may accelerate degradation and isomerization of carotenoids (Ahmadkelayeh & Hawboldt, 2020). At high pressure (45 MPa), temperature increase resulted in higher concentration of astaxanthin ($p < 0.05$) because of the higher solubility of astaxanthin at 80 °C similar to the higher FA solubility. This could explain the high astaxanthin concentration at 45 MPa and 80 °C despite the high temperature which was responsible for degradation. Sánchez-Camargo et al. (2011) showed that the highest yields of astaxanthin from shrimp waste were achieved in the range from 40 to 50 °C and 30 to 37 MPa, whereas at lower pressures, astaxanthin was significantly lower, in agreement with the results in the present study. According to the results, it seems that the crossover pressure for astaxanthin was higher compared to the crossover pressure of oil. Haq and Chun (2018) reported that astaxanthin concentration in oils extracted from Atlantic salmon by-products ranged from 25 to 28 μg g^{-1} at 45 °C with the use of ethanol as co-solvent which increase the extractability of carotenoids compared to pure CO_2 as solvent.

3.4. Antioxidant activity

On Fig. 5, the EC_{50} values of the extracted oils are shown. This is the concentration of extract that reduces 50% the DPPH absorbance and hence, the lower the EC_{50} value the more robust the antioxidant activity. The results were expressed in mg oil per mL of ethanol. At 20 MPa and 80 °C the extract was not sufficient to measure its antioxidant activity. According to the results, at highest operating temperature (80 °C) and pressure (45 MPa) the EC_{50} value was significantly higher than any other condition and equal to 25.3 ± 2.2 mg mL^{-1}. The other extracted oils did not show significant differences ($p > 0.05$) in their antioxidant activity

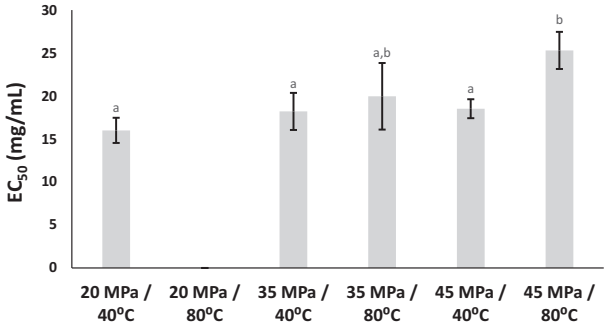

Fig. 5. Values of EC_{50} of Arctic charr oils extracted at different conditions after 120 min of extraction (Mean ± standard deviation). The condition 20 MPa and 80 °C could not be analysed due to a very low oil yield. Different letters above error bars denote significant difference according to ANOVA followed by Tukey's post hoc test.

and EC_{50} ranged from 16 to 20 mg mL^{-1}. It is noteworthy that despite the differences in astaxanthin concentration, the extracts had similar antioxidant activity. The fish oil extracts are often complex mixtures of compounds and their synergistic effect can contribute to the free radical scavenging activity. Apart from astaxanthin, it has been reported that omega-3 PUFA (especially DHA and EPA) had antioxidant potential (Anderson et al., 2014). Moreover, tocopherols, astaxanthin, phospholipids and phenolic compounds, which were present in salmon oil, where characterized by radical scavenging activity (Haq et al., 2017; Wu & Bechtel, 2008). The free radical DPPH solution has a deep purple color and the reaction with hydrogen donating or electron transferring molecules results in the yellowish reduction product ($DPPH_2$). Astaxanthin molecular structure contains conjugated double bonds and hydroxyl and keto groups on the ionone ring which are responsible for the free radical scavenging activity of astaxanthin. These compounds react with DPPH molecules through electron transfer and/or hydrogen atom transfer (Dose et al., 2016). In addition, tocopherols molecules have phenolic hydrogens which can be transferred to a free radical molecule and the resulting tocopheroxyl radical can further react with DPPH (Di Mambro, Azzolini, Valim, & Fonseca, 2003). Phospholipids can act either as antioxidants or as prooxidant depending on the other components. The synergistic effect of phospholipids with tocopherols can enhance radical scavenging ability (Cui & Decker, 2015). Moreover, Tsimogiannis, Bimpilas, and Oreopoulou (2017) have reported that a mixture of phenolic antioxidants could result in synergistic, antagonistic or no effect in terms of antioxidant activity.

Comparing the FA concentrations in the extracts, it is evident that SFE showed high selectivity for FA under the highest temperature (80 °C) and pressure (45 MPa), since almost the entire extract was related with FA content. Therefore, the lower antioxidant activity under the tested extraction conditions may be related to the absence of additional antioxidant compounds, which may be present and act synergistically in other extracts.

3.5. Peroxide value

Fish oils are prone to oxidation, therefore PV was measured in order to estimate the oxidation level between the different samples. The rate of oxidation increases with increasing UFA content in oil, while antioxidant components such as astaxanthin could preserve fatty acids from oxidation. In addition, temperature may affect the rate of oxidation of the oil which was in accordance with the results in this study. According to the results presented on Fig. 6, increasing temperature resulted in higher oxidation levels of oil (i.e. 72% increase). In addition, higher pressure raised the PV at both temperatures except for the extracted sample at 20 MPa and 40 °C in which the peroxide value (1.83 ± 0.68 meq kg^{-1}) was higher than the respective samples at the same temperature. This may be attributed to the limited concentration of astaxanthin. Moreover, the results of PV on the examined conditions related quite well with astaxanthin content apart from oil extracted at 45 MPa and 80 °C. The concentration of UFA at these conditions is about two times higher than the total UFA at any tested extraction conditions which made the oil highly susceptible to oxidation in combination with the higher temperature despite the high concentration of astaxanthin. Moreover, PV in all oil samples was below the limit of 5 meq kg^{-1}, which is the maximum value recommended by the Codex Alimentarius Commission for fish oils (FAO/WHO, 2017). The low values could be explained because of the absence of oxygen and light during the extraction, which are crucial parameters for oil oxidation. These parameters can accelerate the initiation step of lipid autoxidation which includes free radical formation (Choe & Min, 2006). Haq et al. (2017) reported PV values between 1.10 and 1.25 meq kg^{-1} for supercritical extracted oil from salmon side streams.

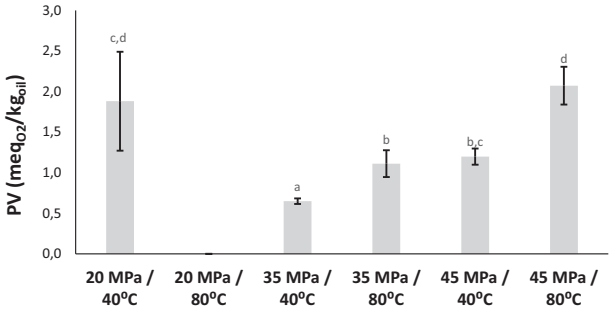

Fig. 6. Peroxide value of Arctic charr oils extracted at different conditions after 120 min of extraction (Mean ± standard deviation). The condition 20 MPa and 80 °C could not be analysed due to a very low oil yield. Different letters above error bars denote significant difference according to ANOVA followed by Tukey's post hoc test.

4. Conclusion

This study indicated that the side streams of Arctic charr filleting are a rich fish oil source (46.3 ± 0.6%) suitable for further valorization. To the best of our knowledge, this is the first study regarding the valorization of Arctic charr side streams by means of supercritical CO_2 extraction and the characterization of the acquired extracts. The present study investigated the effects of pressure and temperature on the recovery, the composition and stability of oils extracted from Arctic charr processing side streams by supercritical CO_2 extraction at 20–45 MPa and 40–80 °C. Total yield depended on the applied conditions since they affected both the solvent's density and compound solubility. The extract at higher temperature and pressure was characterized by the highest oil yield and FA concentration but was found to be more oxidized and with lower antioxidant activity compared to extracts extracted using conventional methods. Furthermore, the higher concentration of astaxanthin, achieved in the extract at 35 MPa and 40 °C, and in combination with the mild temperature conditions, resulted in the significantly lower oxidation of the oil. The results indicated that SFE can effectively be applied as an alternative method for fatty acids extraction and may contribute to a more sustainable utilization of Arctic charr processing side streams.

Declaration of Competing Interest

The authors declare that they have no financial or non-financial competing interest.

Acknowledgments

This research was supported by a mobility grant from the Erasmus+ Key Action 2 Strategic Partnership Project on Sustainable Intervention Technologies for controlling Food Safety and Stability (SUIT4FOOD) of the European Union. We would also like to thank Umlax AB (Lycksele, Sweden) for the kind collaboration to provide the Arctic charr side streams.

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