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Valorisation of waste olive pomace: Laboratory and pilot scale processing to extract dietary fibre

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ABSTRACT

The olive oil industry generates large quantities of waste pomace which has the potential to be used in a range of applications, including as a source of dietary fibre in the food ingredients sector. This study determined that hexane extracted olive pomace still retained 10.6% soluble dietary fibre (SDF) although the total sugar content of 27% was low. The lower and upper yields from single trials on freeze dried olive pomace (10 g) for hexane extracted IDF (insoluble dietary fibre) and SDF were 41–53% and 0.5–2.5%, respectively. These results tentatively indicated that pH and homogenization (high shear mixing) were important factors that affect IDF and SDF yields. The pilot scale processing of 36 kg (wet weight) frozen olive pomace, equivalent to 5.65 kg (dry weight), focused on recovery yield of SDF using an alkaline treatment approach combined with wet milling (2 h). There was no increase in microbial contamination during the trial. While a relatively high yield of SDF (5.6%) was obtained, the monosaccharide content was low, and this fraction did not exhibit gelation properties which is one of the key indicators for functionality in the food ingredients sector. A lower-than-expected IDF yield (13.6%) was obtained during the pilot trial compared with initial laboratory results (41.3–53.0%). However, this process enriched the fibre content from 40% to more than 70% in the majority of the IDF samples collected during the pilot trial. It was determined that the highest water and oil holding capacities in these samples were 6.9 and 4.1, respectively, which were associated with IDF extracted at pH 4.5. This study revealed that SDF could be recovered from olive pomace and the recovery of IDF could be scaled up, where physical disruption and pH conditions caused apparent changes in the yields, and the water and oil holding capacities of dietary fibre fractions.

1. Introduction

The most recent data reported by the International Olive Council (2019) highlighted that the majority of European production occurred in Spain (73%), Greece (11%), Italy (10%) and Portugal (4%), producing 1.7 million tonnes in 2016–17. The total quantities of olive oil production varied substantially in the prior years of 2013–2015 but the countries involved in most of its production remained unchanged. It was estimated that 8.4 million tonnes of olive pomace were generated during the period 2012–2015, which once dried amounted to ~ 2 million tonnes that was used in limited applications including energy production and as an additive in animal feed (Berbel and Posadillo, 2018). However, an evaluation of farm data from Italy revealed that farms using three phase decanting to extract higher oil quantities resulted in olive pomace with a considerably higher water content. This required a higher energy input to dry material, thereby making the process economically unsuitable for use in thermal biomass energy production (Salomone and Ioppolo, 2012). Consequently, most olive pomace derived from the three decanting process was mixed with manure and composted on-site or transported to an industrial composting facility. At present, there is no European Union legislation regulating how olive mill waste is disposed and it is left to individual countries to determine end of life options, which does include dispersal into water courses and on-land (Di Giacomo and Romano, 2022).

The major components present in olive pomace include dietary fibre, protein, and phenolic compounds, which were determined to be present in one study at 620, 118 and 4 g per kg dry weight of a sieved fraction of the olive pomace (Ribeiro et al., 2020a). Consequently, olive pomace is a

Abbreviations: IDF, insoluble dietary fibre; SDF, soluble dietary fibre; WHC, water holding capacity; OHC, oil holding capacity.

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rich source of dietary fibre (Lin et al., 2017), with insoluble dietary fibre (IDF) being the predominant component compared with soluble dietary fibre (SDF) which is present in most vegetables (O'Shea et al., 2012). Compositional analysis of IDF indicated the presence of larger molecular weight carbohydrates, cellulose and hemicellulose, whereas SDF is composed of small hemicellulose molecules and pectin (Dhingra et al., 2012). The health benefits associated with incorporating IDF into people's diets is in reducing blood pressure (Aljuraiban et al., 2015) and improving gastrointestinal function (Lin et al., 2017), while SDF can lower postprandial glucose absorption (Cassidy et al., 2018) and cholesterol (Gunness and Gidley, 2010). Therefore, there is potential to utilise olive pomace without any downstream processing or modification, in a range of food products including biscuits, bread and fish burgers, but there have been technical challenges in doing so, that include the presence of unwanted colour, flavours and aromas associated with olives (Difonzo et al., 2021). Yoghurt is an example of one of these foods which when supplemented with 5% (w/v) olive pomace powder, sieved to remove residual stone material, provided the required dietary fibre intake of a standard yoghurt size of 120 g (Ribeiro et al., 2021).

The recovery of IDF can be achieved by centrifuging olive pomace to obtain a solid fraction which can then be freeze-dried (Ribeiro et al., 2020b). The traditional method for SDF recovery from fruits and vegetables involves starch gelatization by boiling, then treating with amyloglucosidase at pH 4.75 and finally ethanolic precipitation of water soluble compounds (Asp et al., 1983). Another method that was used for SDF extraction involved shaking flax seed under alkaline conditions (pH 7–9.5), followed by treatment with a thermostable amylase (pH 3.5) to remove residual solids and finally ethanolic precipitation of the soluble fraction (Moczkowska et al., 2019). Another similar method for SDF extraction from sugarcane bagasse involved stirring the material with increasing concentrations of alkali, neutralizing the soluble fraction to pH 5.5 and using ethanolic precipitation to recover hemicelluloses which contained high levels of xylose (Peng et al., 2010).

The quantities of IDF and SDF in fresh olives, determined in a previous study on two varieties were ~14% and ~3.5%, respectively, following defatting (Guillén et al., 1992). Another study which also utilised defatted olives reported total dietary fibre values from 10 to 15%, based on fresh weight (Jiménez et al., 2000). Another report highlighted that a significant proportion of SDF can be extracted into the olive mill wastewater during the decanting step to recover olive oil (Galanakis et al., 2010), resulting in lower quantities of SDF in the olive pomace. However, the commercial processing of olive pomace reported IDF and SDF levels to be 73% and 6%, respectively (Lin et al., 2017) although only limited information on the process steps was described.

The aim of this study was to investigate different methods, including mechanical processing, to recover of dietary fibre (IDF and SDF) from olive pomace. In addition, we report here the first protocols, to the best of our knowledge, involving the pilot scale processing of olive pomace to obtain dietary fibre. Fractions of dietary fibre collected at each stage in the process were evaluated in terms of yields, moisture and fibre contents, and water/ oil holding capacities. Furthermore, the monosaccharide contents of SDF samples recovered from hexane extracted olive pomace and during pilot processing were determined, in order to establish whether these materials possessed gelation properties.

2. Methods

2.1. Materials

Olives originating from mixed varieties of olive trees (ˇIstrska belica', ˇLeccino', ˇMaurino', ˇBuga'; Slovenia) were processed in November 2018 using a two-phase decanter at the Lisajak mill and a three-phase decanter at the Marzi mill, Slovenia. The crushed stone fragments were removed from the olive pomace using a destoning machine and samples were then freeze dried and milled. Initial analysis of the freeze-dried samples from the three-phase decanter indicated that a sedimentation step was required to remove the fragmented stones (comprising 33% of the olive pomace), that would otherwise be carried over into the extracted IDF. Therefore, samples were used from the two-phase decanter which did not require sedimentation to remove the fragmented stones, thereby facilitating further analysis of the IDF fraction to determine the quantities of residual SDF. Fresh olive pomace (36 kg) was collected in November 2019 from a three-phase decanter at the Marzi mill (Slovenia), which was frozen immediately and shipped to the UK for processing in the pilot scale facility at Bangor University.

2.2. Experimental protocols to recover dietary fibres from olive pomace

2.2.1. Standard extraction method from hexane extracted olive pomace

Freeze-dried olive pomace (5 g) was defatted with hexane (250 mL) at 70 °C (1 h) in a flask with an attached condenser, then the solvent was decanted, and the remaining olive pomace was dried by rotary evaporation, which was ground with pestle and mortar. The SDF composition was determined using an assay kit (Megazyme, Ireland) and the following method: 50 ml 0.08 M phosphate buffer, pH 6, was added to 1 g of the defatted olive pomace and was readjusted to pH 6 with 2 M sodium hydroxide. Then thermostable α- amylase (50 μL) was added and the suspension was incubated at 100 °C (20 min) with periodic mixing. During the next step, the suspension was adjusted to pH 7.5 with 0.275 N sodium hydroxide (10 mL), purified protease (100 μL) was added, and the suspension was incubated at 60 °C (30 min). In the next step, the suspension was adjusted to pH 4.5 with 0.325 N hydrochloric acid (10 mL), purified amyloglucosidase (200 μL) added and then incubated at 60 °C (30 min). The suspension was filtered through pre-weighed glass microfibre filters (Whatman). The filtrate was mixed with ethanol (280 mL), incubated at 4 °C (1 h) and then filtered to collect the SDF on a pre-weighed glass microfibre filter. The solid on the surface of the filter was washed with 78% ethanol (3 × 20 mL), 95% ethanol (2 × 10 mL) and acetone (2 × 10 mL).

2.2.2. Laboratory experiments using olive pomace

The experiments described in Sections 2.2.3 to 2.2.5 were performed using olive pomace without prior defatting in order to examine protocols that could be used during the pilot scale trial, which avoided using large volumes of hexane. The hexane extraction was performed after recovery of the dried dietary fibres.

2.2.3. Standard extraction method without amylase and protease

Freeze-dried olive pomace (10 g) was resuspended in deionized water (200 ml) with stirring at ambient temperature (15 min) in duplicate. Only one suspension was homogenized using high shear mixing (7000 rpm, 5 min) with an attached an emulsor screen. Both suspensions were adjusted from pH 5.1 to 6.5 with 2 M sodium hydroxide and the suspensions were incubated (40 °C, 80 rpm) on a bottle roller (1 h). The suspensions were cooled and adjusted to pH 4.5 with 2 M HCl, then centrifuged (6000 rpm, 5 min) and the supernatants were filtered through pre-weighed glass microfibre filter membranes. The pellet collected by centrifugation and the residue collected by filtration were oven dried (103 °C) and weighed. The filtrates were mixed with four volumes of ethanol, incubated (4 °C, overnight), filtered through pre-weighed filters and weighed after oven drying at 103 °C for 2 h. The remaining oils associated with IDF and SDF undergoing high shear mixing were extracted in hexane (50 mL) at 70 °C (1 hr), then the solvent was decanted, and the pellets were dried *in vacuo* and re-weighed.

2.2.4. Lipase assisted extraction

A similar procedure was followed to that described in Section 2.1 where only one suspension was homogenized. To 200 ml suspensions containing freeze-dried olive pomace (10 g), 5% (v/w) Tail 127 {lipase (1,3 specific) – involved in hydrolysis of triglycerides into glycerol and fatty acids at the first and third positions} was added with an optimum pH and temperature range of 6.5 and 30–40 °C

respectively. The suspensions were incubated (40 °C, 80 rpm) on a bottle roller (1 h) and then with occasional shaking (20 min.) and heated to 80 °C in a water bath to deactivate the enzyme, before cooling to ambient temperature.

2.2.5. Alkaline extraction

Freeze-dried olive pomace (10 g) was resuspended in deionized water (200 ml) with stirring at ambient temperature (15 min) in duplicate. Only one suspension was homogenized using high shear mixing (7000 rpm, 5 min) using an emulsor screen. The suspensions (pH 5.0) were adjusted to pH 9.5 with 2 M sodium hydroxide and incubated (50 °C) on a bottle roller (80 rpm, 1 h). The suspension that had been shear mixed previously was mixed again (7000 rpm, 5 min.) and after incubation the pH had increased (pH 7.8). Both suspensions were centrifuged (6000 rpm, 5 min.) and then filtered through pre-weighed filters. The solids, collected by centrifugation and filtering, were combined, oven dried (103 °C, 4 h) and weighed to determine the IDF content. Four volumes of ethanol were added to the filtrates, mixed, and incubated (4 °C, overnight). The newly formed suspensions were filtered through pre-weighed filters and weighed after oven drying at 103 °C for 2 h. The remaining oils associated with IDF and SDF undergoing high shear mixing were extracted in hexane (50 mL) at 70 °C (1 hr), then the solvent was decanted, and the pellets were dried *in vacuo* and re-weighed.

2.2.6. Pilot scale extraction

The process for the pilot scale extraction of dietary fibre (Fig. 2) involved resuspending olive pomace (36 kg wet weight; 5.65 ± 0.1 kg dry weight equivalent) in water (80 L) in a continuously stirred tank reactor (CSTR-150 L capacity, 160 rpm) and adjusting to pH 9.5 with 2 M sodium hydroxide. The suspension was continuously cycled between two CSTRs (150 L) and passing through a colloidal wet mill (FrymaKoruma, model no. MZ50), which was placed between the two reactors. The setting of the gap between the stator and rotator of the colloidal wet mill was 0.1 mm. The suspension was stirred at ambient temperature (2 h) and then pumped into a decanting centrifuge (Model UCD 205–00–32, GEA Westfalia Separator) operated at 3000 rpm. The decanter centrifuge separated the solid fraction from the centrate containing mostly soluble compounds, and this was returned to the tank reactor. The volume of the centrate was determined using a scale within the tank reactor and a sample of 1 L of centrate was collected to determine SDF content. The material collected after decanting was weighed, and the moisture content determined in triplicate from three different locations of the solid material. This fraction was then resuspended in water (50 L) and the pH was adjusted to 4.5 with 20% phosphoric acid with stirring (160 rpm, 1 h). This suspension was pumped back into the decanting centrifuge and the solid and liquid fractions collected. The solid fraction was weighed, and the moisture content determined. A 1 L sample of the centrate was collected and the remaining centrate was combined with the fraction collected after the first centrifugation. The combined centrate fractions were passed through a disc stack centrifuge (GEA Westfalia, model no. SB 7–06–576) operated at 9120 rpm, yielding a fraction containing resuspended solids and the centrate. The centrate was filtered through a 100 kDa filter to remove any remaining solid particles, then concentrated using ultrafiltration (10 kDa filter membrane). A 1 L sample was collected from the centrate at the start of ultrafiltration. During ultrafiltration any oils and fats collecting on the surface were skimmed off using a 0.1 mm sieve.

The samples collected after extractions at pH 9.5 and at pH 4.5 were filtered through glass microfibre filters to remove any remaining suspended solids. These samples and the retentate collected at the end of the ultrafiltration step were concentrated *in vacuo,* four volumes of ethanol were added to each of the concentrated solutions, incubated (4 °C, 1 h) and then filtered through pre-weighed glass microfibre filter membranes. These were freeze dried and then weighed to determine the weight of SDF at each stage of the extraction. Ordinarily, the concentration *in vacuo* step could be achieved by spray drying.

2.3. Analysis of samples

2.3.1. Sugars analysis

The total sugar content in olive SDF (35 mg) was determined (Li et al., 2013) by hydrolysing in 2 M HCl (800 μL) for 1 h and then diluting with dimethyl sulfoxide to 5 mL volume. The hydrolysis step was omitted when determining soluble sugars. The sample (100 μL) was mixed with acetic anhydride (150 μL) and 1-methylimidazole (30 μL) and stirred (10 min) in glass vials. Deionized water (500 μL) was added to dissolve the excess acetic anhydride. The acetylated derivatives were extracted twice in dichloromethane (200 μL) and 1 μL of the extract was used for GC–MS analysis. Sugar concentrations were quantified with the addition of a known standard sugar mix (5 μg). A quadrupole GC–MS was used in the analysis of acetyl derivatives on a Restek rxi-17 Sil MS fused capillary column (30 m x 0.25 mm x 0.25 μm film thickness). The oven temperature program was initiated at 100 °C (1 min. hold), then gradually ramped to 190 °C (10 °C/min.), held for 6 min.; 250 °C (10 °C/min.), held for another 6 min.; ramped to 280 °C (10 °C/min.) and held for 10 min. The temperature of the injector was 250 °C and the transfer line was 260 °C. Helium was used as the carrier gas (flow 1.2 mL/min.), splitless injection. Sugars were identified when the retention times and fragmentation patterns matched those that were determined with the reference sugars: rhamnose, xylose, arabinose, ribose, galactose, glucose, fructose and mannose.

2.3.2. Fibre analysis of olive samples

Olive samples (5 g) were extracted with hexane (250 mL) at 70 °C (1 h) to remove residual oils and fats and then dried *in vacuo*. Fibre analysis was performed sequentially as previously described (Baker et al., 2015) on samples (0.5 ± 0.025 g) placed into Ankom bags in duplicate. Briefly, the neutral detergent fibre (NDF) was determined in an Ankom 200 Fibre Analyser (Ankom, USA) using NDF reagents containing sodium sulphite (20 g) and amylase (5 ml). Then the acid detergent fibre (ADF) was determined using ADF reagent containing sulphuric acid (2.6% (v/v). Finally, the lignin contents were determined using 70% sulphuric acid in a Daisy D200 Incubator (Ankom, USA). The hemicellulose content was calculated by subtracting ADF from NDF, while the cellulose content was calculated by subtracting the lignin content from ADF. The ash content was determined on 0.5 g of the original sample by combustion in an oven furnace (600 °C, 4 h) and the proportions of ash in each sample were calculated and subtracted.

2.3.3. Determination of bacterial populations in the processed olive pomace samples

The bacterial populations were determined aseptically by serially diluting the samples ten-fold in 0.85% (w/v) sodium chloride, which had been autoclaved (15 mins, 121 °C) and cooled to ambient temperature. The samples were mixed for 20 s on a multi-tube Vortex mixer (Grant V-32) after each serial dilution and 100 μl of each dilution was plated onto spread plates containing R2A medium. The plates were incubated at 20 °C for one week. The whole process from sampling to plating was completed within one day at ambient temperature (~ 20 °C).

2.3.4. Measurement of gelation properties

Duplicate samples of the olive SDF recovered after pilot scale extraction (0.5 g) were resuspended in 5 ml of 30 mM potassium dihydrogen phosphate (pH 4.3) and was adjusted to pH 1 with 2 M hydrochloric acid. The suspension was incubated at 25 °C for 1 h with occasional mixing and then adjusted to pH 8 with 2 M sodium hydroxide. These conditions were used to approximately mimic digestion through the human intestinal system.

Table 1
Percentage composition of olive pomace.

Component	unextracted	hexane extracted
Oil	21.1 ± 5.9	none
Soluble compounds	17.7 ± 1.1	22.4 ± 1.4
IDF	52.9 ± 1.1	67.0 ± 1.4
SDF	8.4 ± 2.2	10.6 ± 2.8

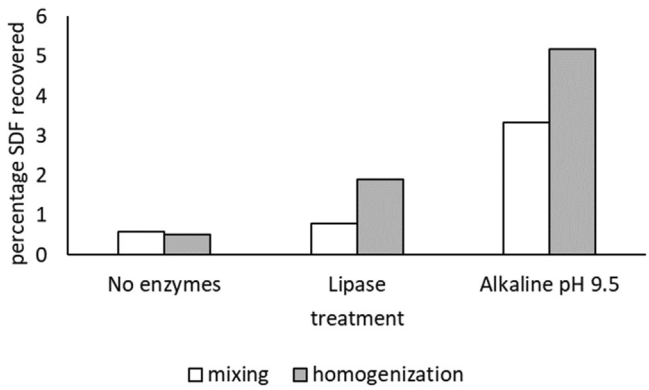

Fig. 1. The recovery of SDF from olive pomace using three different procedures with end-over-end rotation at 40 rpm and with homogenization involving end-over-end rotation at 40 rpm, 1 h and high shear mixing at 7000 rpm, 5 min. It was presumed that these extracts still contained oils.

2.3.5. Measurement of water and oil holding capacities of dietary fibres

These measurements were performed using a previously described method (Chau and Haung, 2003). Briefly, deionized water or oil was added to 50 mg of each sample in triplicate at a ratio of 10:1 and incubated at room temperature for 24 h. The samples were centrifuged at 3000 g for 5 min and the supernatants were collected. The hydrated or oil saturated samples were weighed. Moisture contents were calculated which accounted for the water associated with each of the samples.

3. Results

3.1. Determination of dietary fibre in hexane extracted olive pomace

It was determined that olive pomace contained higher than expected levels (21%, dry weight basis) of oils and fats. The defatted olive pomace used in this study contained 10.6% SDF and when back calculated to account for the oil content before defatting, the actual amount was 8.4% (Table 1). GC–MS analysis of the SDF isolated from the defatted olive pomace indicated that the soluble and total monosaccharide contents were 6.0% and 27.9%, respectively (Table 2). However, only 3.7% of soluble monosaccharides and 8.1% of total monosaccharides matched the sugar standards. It was determined that 77% of the total monosaccharides could not be identified, although 56% of these comprised pentose sugars, possibly arabinose derivatives. The data from another previous study on SDF extracted from olive orojo (Valiente et al., 1995a) showed higher concentrations of arabinose compared with other monosaccharides (Table 2).

3.2. Laboratory experiments to extract dietary fibre from olive pomace

Initial laboratory trials on olive pomace samples indicated that a limited quantity of SDF could be recovered using a standard approach without enzymes (Fig. 1). The use of highly purified amylase and protease to selectively target non-dietary fibre was avoided because olive pomace does not contain starch and the use of industrial proteases would contain side activities that could compromise dietary fibre yields.

To increase SDF yield, further work focused on the use of a high shear mixing process to reduce the particle size in the olive pomace and increase the surface area. The range in yields of SDF obtained during laboratory trials, after oils were removed by hexane extraction, were comparatively low using different methods (0.5–2.4%% per g dry biomass) and it is likely that the presence of oils and fats reduced the efficiency of the extraction process (Table 3). The range in yields of IDFs were much higher (41.3–53.0%) once the oils and fats had been removed by hexane extraction. The combined use of lipase and high shear mixing appeared to result in a small increase in the recovery of SDF compared with the control experiment where no enzymes was used. The yield of SDF obtained by alkaline hydrolysis along with high shear mixing was 48.9%, which was higher compared to the same process without high shear mixing. However, despite the apparent effect of high shear mixing on olive pomace, replication would be required along with statistical analysis to determine whether this homogenization process is significantly different compared to controls without high shear mixing.

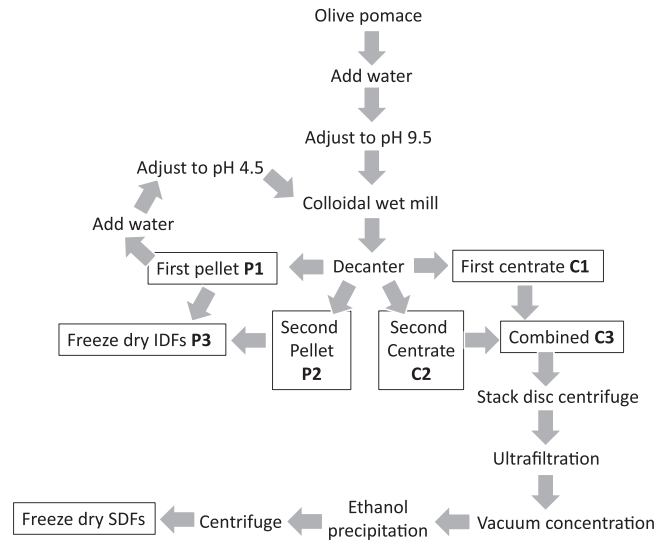

Fig. 2. Flow diagram of process steps to isolate dietary fibres, IDF and SDF from olive pomace at pilot scale (sub- sampling at various stages of the process is highlighted in the text boxes, in order to determine IDF and SDF content).

The composition of the oil-free IDFs were evaluated to determine the actual proportions of IDF and whether any SDF was remaining (Table 4). The actual proportions of IDFs were high when extracted without enzymes and under alkaline conditions. It was evident that some SDF was retained in the oil-free IDFs with higher levels (Table 4) that were inversely related to the lowest SDF yields (Table 3). An analysis of the fibre content of IDF samples indicated that there was a higher fibre content compared with the original olive pomace and that lignin was the predominant component (>40%) in most of the samples processed in laboratory trials (Table 4, Fig. 3). Only, the IDF recovered from olive pomace and treated with lipase contained <40% lignin, with a content similar to the non-fibre component (Fig. 3).

3.3. Pilot scale extraction of dietary fibre from olive pomace

The olive pomace used in the pilot scale trial was suspended in water and the initial pH (4.7) was adjusted to 9.5. Following colloidal wet milling of the suspension for 2 h, the pH had decreased to 8.5 and the insoluble material was decanted. The moisture content was determined from subsamples to calculate the remaining dry weight (P1; Fig. 2, Table 5). The percentage of original biomass recovered as IDF was considerably lower (26% at pH 9.5) than the levels obtained under laboratory conditions, perhaps reflecting the effect of continuous processing by wet milling which may cause a reduction in lignin content (Fig. 3). The yield of IDF was reduced further with additional processing at pH 4.5 (P2), with only a small quantity of SDF being recovered

Table 2
Percentages of sugars associated with extracted SDFs.

Sugar	Retention time (min)	Standard method			Alkaline method	
		soluble	total	total[1]	soluble	total
rhamnose	13.66	0.04	0.58	1.62	0.06	0.05
xylose	14.333	0.09	5.3	2.95	0.01	0.01
arabinose	14.758	2.09	0.42	9.01	0.01	0.02
ribose	15.278	<LOD	<LOD	–	0.02	0.02
galactose	19.971	0.05	0.42	2.66	0.01	0.02
glucose	20.219	1.19	1.21	3.99	0.42	0.54
fructose	20.337	0.17	0.12	1.77	0.71	0.76
mannose	20.384	0.08	0.02	2.95	0.03	0.02
total		3.71 (6.04)	8.07 (27.9)	24.96	1.27 (3.39)	1.44 (3.78)

[1] data from Valiente et al., 1995b which were precipitated in ethanol but would normally be soluble in water. <LOD below level of detection. Values of totals in brackets include unidentified sugars *e.g.* ribose, sorbitol, myo-inisitol, where reference sugars were not used.

Table 3
Percentages of IDF and SDF extracted from olive pomace.

Treatment	insoluble pomace			ethanolic precipitate		
	total	IDF	oil	total	SDF	oil
No enzymes	60.94	53.02	7.92	0.53	0.53	0.00
Lipase	70.80	51.40	19.40	1.90	1.11	0.79
Alkaline pH 9.5	57.19	41.29	15.90	5.18	2.37	2.82
defatted	–	–	–	9.87	9.87	0.00

Table 4
Percentage composition of oil free IDF recovered from olive pomace after extraction of SDF.

	soluble	IDF	SDF
No enzymes	6.61	88.60	4.79
Lipase	32.07	64.51	3.42
Alkali pH 9.5	8.32	89.30	2.37

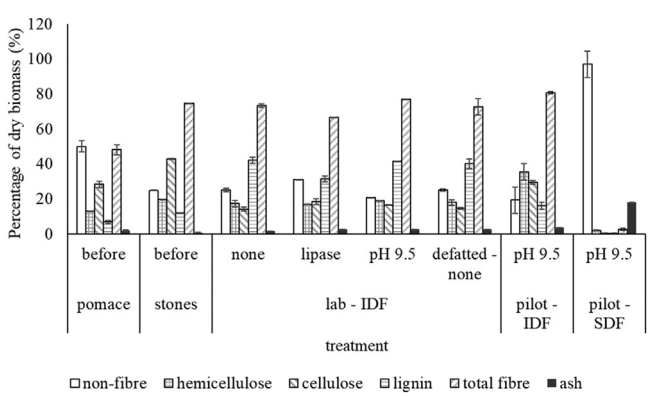

Fig. 3. Fibre analysis of IDFs after hexane extraction of samples that were not defatted. Error bars represent within sample variation.

(1.2%) (C2), while the majority of SDF was recovered during alkaline extraction (98.8%) (C1). The centrates contained insoluble biomass which were filtered prior to concentration and precipitation steps to remove these solids prior to precipitation of the SDFs. However, it is also possible that concentration by microfiltration may have enabled soluble compounds to precipitate when the concentration of these compounds had exceeded their maximum solubility concentrations. In this case, these solids would be a significant portion of the SDFs that were discarded. Sedimentation of samples collected from the extracted IDF indicated no fragmented stones were present which were presumably disintegrated during colloidal wet milling. Compositional analysis of the extracted IDF indicated that the lignin content was considerably lower compared with hemicellulose and cellulose (Fig. 3), which contrasted with the laboratory studies showing lignin to be the major component. It would have been expected that the lignin content would have increased considering the slightly higher lignin content associated with olive stones compared with olive pomace, but this was not the case. However, it was evident that there was an increase in cellulose content which was possibly derived from the fragmented stones present in the original samples collected from the mill. The fibre content showed a considerable increase from 40% in the original olive pomace to 70% in the processed IDF, with hemicellulose becoming a major component (Fig. 4). In addition, the processed samples appeared as a finer material and lighter in colour compared with the original starting material.

The centrate containing the SDF recovered under alkaline conditions at pilot scale was combined with the centrate containing the SDF recovered under acidic conditions to create a suspension at pH 7.54 (C3; Fig. 2). The centrates were combined in order to provide sufficient volume to conduct ultrafiltration. The majority of the monosaccharides were mostly soluble because there was little difference between soluble and total monosaccharides (Table 2). Furthermore, monosaccharide concentrations were lower than those recovered using the standard method from hexane extracted olive pomace.

Bacterial contamination during the pilot trial was a concern but it was determined that the population of aerobic microorganisms in the

Table 5
Properties of pellets collected during each step in the pilot scale trial.

Sample	Dry biomass (g)	Percentage recovery (%)	Moisture content (%)	WHC	OHC
P1 (IDF pH 9.5)	1490 ± 100	26	85.9 ± 0.1	4.81 ± 0.26	3.04 ± 0.36
P2 (IDF pH 4.5)	780 ± 100	13.8	90.6 ± 1.0	6.85 ± 0.26	4.09 ± 0.20
P3 (IDF pH 7.5)	780 ± 100	13.8	79.9 ± 0.4	3.73 ± 0.08	2.05 ± 0.05
C1 (SDF pH 9.5)	609*	10.8*	ND	ND	ND
C2 (SDF pH 4.5)	7.2*	0.1*	ND	ND	ND
C3 (SDF pH 7.5)	316	5.6	60.6 ± 0.20	1.43 ± 0.05	1.29 ± 0.01

* Contained solids that were removed by filtration prior to concentration and ethanolic precipitation.Errors present within sample variation. Moisture contents are when fully saturated. WHC and OHC represent water and oil holding capacities, respectively.

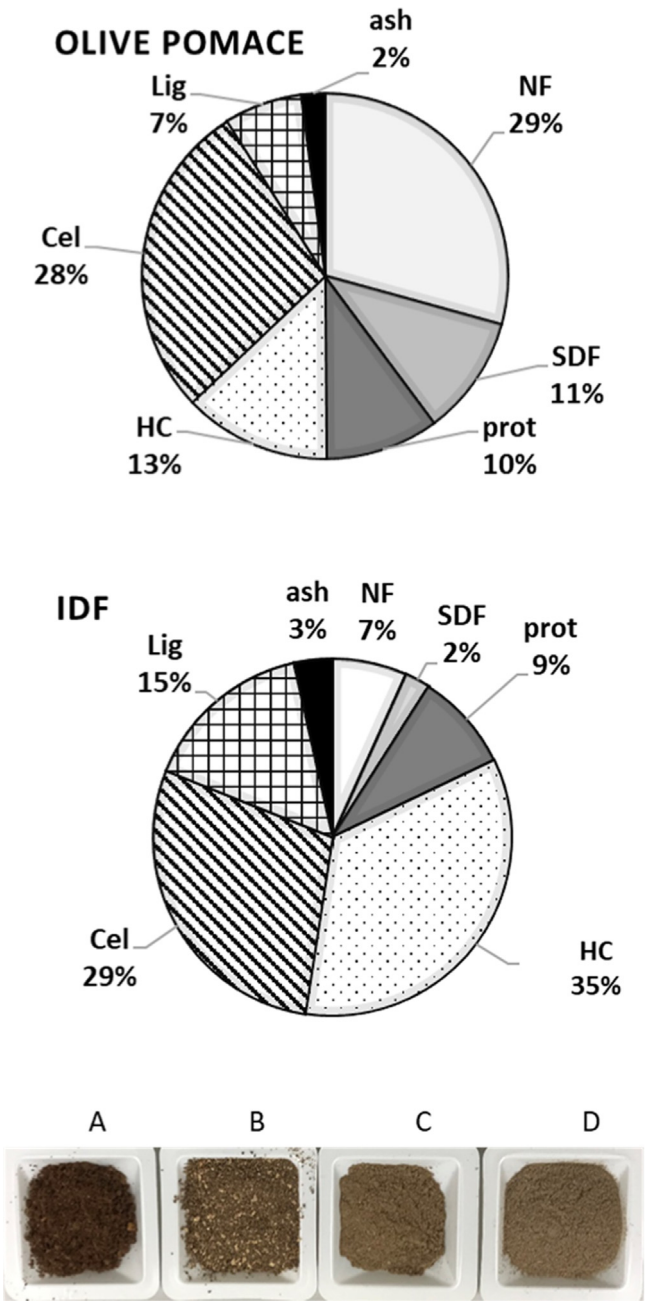

Fig. 4. Compositional estimations of olive pomace and IDF from pilot scale extraction with soluble material shown by solid filled pies, fibre shown by patterned pies and ash as a black pie. Abbreviations are: NF non-fibre, HC hemicellulose, Cel cellulose, Lig lignin, and Prot protein. Samples of olive pomace (A) dried (B) hexane extracted containing olive stone fragments (C) IDF and (D) hexane extracted IDF.

reactor was 1.43×10^6 cells per g original dry biomass, which decreased to 5.39×10^5 in the centrate. The populations remained similar in each of the centrates after each decanting stage and showed a minor decrease during ultrafiltration to 2.88×10^5 in the retentate that contained the SDF, and to 8.29×10^4 in the permeate that was discarded. Therefore, no apparent microbial growth occurred, which was possibly due to the inhibitory properties of the phenolic compounds extracted from the olive pomace.

The WHC and OHC properties of the IDFs and SDF samples isolated from the olive pomace were highest for the IDF recovered at pH 4.5 (Table 5). The results from our study appear to show that WHC and OHC were affected by pH although replication of pilot scale trials would be required to confirm this observation.

4. Discussion

4.1. Determination of dietary fibres in hexane extracted olive pomace

The higher-than-expected oil content associated with the olive pomace after oil extraction by decanting was perhaps not surprising given that the reported oil content of fresh olives can range from 69.7% to 78.9% and moisture contents ranging from 9.6% to 13.9% (Jiménez et al., 2020). However, the actual oil content of the olives prior to oil extraction by decanting was not determined and whether it would fall into this range is uncertain.

A previous study on olive mill cake reported that monosaccharides were present as a minor component (1.6%) along with uronic acids (1.51%), which together can act to form a gel (Valiente et al., 1995a). In our study, a higher monosaccharide content was obtained (27%) but no galacturonic acids were detected and thus could be attributed to that fact that different olive varieties that were investigated, rather than any significant variations in the experimental methods used. SDF was recovered in distilled water and concentrated by dialysis in the former study whereas extraction was achieved at pH 7.5 and then at pH 4.5 as described in the method by Asp (1983) followed by ethanol precipitation. The extraction of olive oil results in two waste products, olive pomace and oil mill wastewater. However, an examination of olive mill wastewater revealed a higher concentration of galacturonic acids (3.3%) in the alcohol insoluble fibres recovered from although pectin (7.2%) was the predominant component contributing to its gelation properties (Galanakis et al., 2010).

4.2. Laboratory experiments to extract dietary fibres from olive pomace

The use of lipase showed an increased recovery of SDF compared with the same protocol without using enzymes. An increased effectiveness of lipase may have been achieved if the reaction had been performed in warm ethanol resulting in the solubilization of triglycerides (Blasi et al., 2007), instead of using water where triglycerides remain insoluble. While the alkaline protocol showed higher SDF recovery yields from flaxseed, it was determined that some IDF had carried over into the SDF fraction (Moczkowska et al., 2019). The composition of SDF was not analysed in our study although analysis of the IDF revealed a high fibre content. In addition, alkaline hydrolysis was also shown to lead to the breakdown of some IDF into SDF (Zhou et al., 2020). The highest yields were obtained with high shear mixing, which correlated well with a previous study using high pressure micronisation, and which reported an increase in SDF yields from 10% to 25% using carrots as the process feedstock (Chau et al., 2007).

The protein content of milled olive pomace samples (6.78%) was determined, which was within the range of previously reported data (Baker and Charlton, 2020; Wen et al., 2020). However, it is difficult to solubilize this protein with low yields without the use of enzymes (0.1–1.2%) and a higher yield (4%) when using a protease (Alcalase) (Baker and Charlton, 2020), while the breakdown of protein into small molecular weight peptides, resulted in only a low yield of recovered peptides (1.2 mg peptides per g dry biomass) (Fathi et al., 2022). Therefore, the majority of the protein would be expected to remain with IDF.

The extracted IDFs and SDFs were further purified to remove oils using hexane extraction, although whether this process would be necessary at commercial scale would be dependant on the final food application for the dietary fibre. The use of hexane does present problems in the food industry because hexane does have neurotoxicological effects (Cravotto et al., 2009), even though hexane extraction in food applications is acceptable provided the residual level of this solvent is <5 mg per kg (Union, 2009). Nevertheless, there is a potential risk of cumulative hexane intake and there is perhaps a requirement to provide consumers

with clear labelling describing the use of hexane. Furthermore, there is a growing body of international organizations that are prohibiting the use of hexane, which is stimulating the development of alternatives such as mixtures of water, ethanol and ethyl acetate (Mwaurah et al., 2020).

The significant lignin content in the majority of extracted IDF obtained during this study shows a similar trend with a previous study which reported that the oil-free, alcohol insoluble fibre extracted from olive mill wastewater contained a high (84%) Klason lignin content (Galanakis et al., 2010). However, it was concluded that the lignin content was overestimated due to the additional presence of tannins, associated proteins and Mallard reaction by-products. In contrast, lignin extracted in our study would represent the lower lignin estimates due to the sequential extraction of fibres with strong detergents and acids compared with direct extraction of lignin using the Klason method which is a single extraction step.

4.3. Pilot scale extraction

While the total fibre content in the IDF recovered from the pilot trial was similar to those recovered during the laboratory trials, the lignin content in samples from the pilot trial was considerably lower than those obtained from the smaller scale studies. The differences between the laboratory experiments and the pilot trials were primarily in the choice of the available homogenization equipment and duration of its use. Homogenization was achieved using high shear mixing during the laboratory trials for 5 min in contrast to the use of wet milling for 2 h during the pilot trial. The IDF yield was lower in the pilot trial along with lignin content compared with the laboratory experiments and it has previously been reported that knife milling reduced the lignin content in olive pomace due to solubilization of smaller lignin fragments (Speroni et al., 2020). That study also showed an increase in hemicellulose content from 18% to 36% and increasing cellulose content from 1% to 13%, while similar increasing trends for hemicellulose and cellulose were also observed in our study. The high fibre content associated with the recovered IDFs would support the passage of food through the intestine although other factors such as the roughness of the material are also important in stimulating the release of water from the intestine (McRorie and McKeown, 2017).

The recovery of SDF during the pilot trial was slow and this was perhaps impeded by oils being extracted under alkaline conditions. SDFs are usually composed of pectins or gums that leads to the characteristic gelation property (Cardoso et al., 2003; Dhingra et al., 2012), yet there was a low monosaccharide content and no gelling activity of the SDF recovered from our pilot trial. The protein content was at the lower end of previous reported studies which were described as ranging from 8 to 10% from other agricultural sources (Wen et al., 2020; Peng et al., 2010). It is likely that these soluble proteins along with attached compounds precipitated with the addition of ethanol and formed a major component of SDF.

The moisture contents of the IDFs collected during the pilot trial indicated that a large volume of water was retained. The WHC and OHC showed similar trends to the moisture contents, although moisture contents were considerably higher than WHC, reflecting the extent of the highly saturated material and the difficulty in using the decanter to remove most of the water. A previous study reported that the knife milling of olive pomace particles >2 mm showed an increasein WHC from 1.3 to 2.7 and in OHC from 0.6 to 1.3, whereas these properties remained unchanged for smaller sized particles (Speroni et al., 2020). The moisture contents associated with the original olive pomace at the start of the trial (84%) and with IDFs (80–90%) were similar, yet the concentration of fibres in IDF could make the drying process slightly more economically favourable due to the lower quantity of bulk material.

One concern during any pilot process is the appearance of microbial contamination but no apparent microbial growth occurred, which was possibly due to the strong alkaline and acidic conditions throughout most of the trial. In addition, the phenolic compounds extracted from the olive pomace may also have some inhibitory effects on bacterial growth. The necessity in using such strong alkaline and acidic conditions during the pilot scale extraction would need to be evaluated in further trials.

5. Conclusions

It was expected that olive pomace would contain a minimal quantity of SDF because most of the SDF would be lost in olive mill wastewater (Galanakis et al., 2010). However, filtration to remove all solids and the addition of two volumes of ethanol to the filtrate resulted in the formation of an insoluble material described as SDF, which comprised 10.6% (dry matter equivalent), which was composed of 27.9% monosaccharides. Further experiments conducted on olive pomace without defatting beforehand indicated that the alkaline procedure combined with high shearing mixing resulted in the highest apparent yield of SDF. We report here, to the best of our knowledge, details of the first pilot scale protocol to extract dietary fibres from olive pomace. A pilot scale trial was performed using a slightly modified laboratory protocol, resulting in a high SDF yield, although the monosaccharide content of this SDF was very low, and this material possessed no gelation property unlike SDF recovered from olive mill wastewater (Galanakis et al., 2010). One significant problem identified in the pilot trial, which was not evident during the laboratory trials, was that SDF recovery was impeded by the high oil content resulting in a lengthy ultrafiltration time. Nevertheless, the recovery of SDF during a pilot trial would provide sufficient material for further studies to determine whether this SDF has human health benefits. The outcome of this study has shown that the pilot scale processing could generate olive pomace IDF with an increased fibre content, compared with the starting pomace. Currently, small quantities of olive pomace are used as dietary supplements which requires energy intensive drying and defatting using large quantities of solvent. Both drying requirements and solvent use could be reduced by increasing the yield of the fibre in the recovery of olive pomace IDF. However, the limitation of this process is the requirement for a large volume of water that during processing would become discoloured wastewater. These results provide an insight into the potential for the recovery of olive pomace IDF. It should be emphasized that these results are based on a single trial that would require replication and using data obtained from the processing of different olive varieties and at a variety of commercial mills. Further work is therefore required to optimise the operating conditions during pilot processing, in order to maximise the IDF yield.

Author contributions

AC, MS funding; PB; AC, MS conceptualization; PB, AMV, KP methodology: PB, AMV, KP AC, MS writing – original draft; PB, AC, MS review and editing.

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.

Data availability

Data will be made available on request.

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References

Aljuraiban, G.S., Griep, L.M., Chan, Q., Daviglus, M.L., Stamler, J., Van Horn L., Elliot, Frost, G.S., 2015. Total, insoluble and soluble dietary fibre intake in relation to blood pressure: the INTERMAP Study. Br. J. Nutr. 114 (9), 1480–1486. doi:10.1017/S0007114515003098.

Asp, N.G., Johansson, C.G., Hallmer, H., Siljestroem, M., 1983. Rapid enzymic assay of insoluble and soluble dietary fiber. J. Agric. Food Chem. 31 (3), 476–482. doi:10.1021/jf00117a003.

Baker, P.W., Charlton, A., 2020. A comparison in protein extraction from four major crop residues in Europe using chemical and enzymatic processes-a review. Innov. Food Sci. Emerg. Technol. 59, 102239. doi:10.1016/j.ifset.2019.102239.

Baker, P.W., Charlton, A., Hale, M.D.C., 2015. Increased delignification by white rot fungi after pressure refining Miscanthus. Bioresour. Technol. 189, 81–86. doi:10.1016/j.biortech.2015.03.056.

Blasi, F., Cossignani, L., Simonetti, M.S., Damiani, P., 2007. Biocatalysed synthesis of sn-1, 3-diacylglycerol oil from extra virgin olive oil. Enz. Microb. Technol. 41 (6–7), 727–732. doi:10.1016/j.enzmictec.2007.06.005.

Berbel, J., Posadillo, A., 2018. Review and analysis of alternatives for the valorisation of agro-industrial olive oil by-products. Sustainability 10 (1), 237. doi:10.3390/su10010237.

Cardoso, S.M., Coimbra, M.A., Da Silva, J.L., 2003. Calcium-mediated gelation of an olive pomace pectic extract. Carbohydr. Polym. 52 (2), 125–133. doi:10.1016/S0144-8617(02)00299-0.

Cassidy, Y.M., McSorley, E.M., Allsopp, P.J., 2018. Effect of soluble dietary fibre on postprandial blood glucose response and its potential as a functional food ingredient. J. Funct. Foods 46, 423–439. doi:10.1016/j.jff.2018.05.019.

Chau, H.F., Huang, Y.L., 2003. Comparison of the chemical composition and physicochemical properties of different fibers prepared from the peel of *Citrus sinensis* L. Cv. Liucheng. J. Agric. Food Chem. 51, 2615–2618. doi:10.1021/jf025919b, 2003.

Chau, C.F., Wang, Y.T., Wen, Y.L., 2007. Different micronization methods significantly improve the functionality of carrot insoluble fibre. Food Chem 100 (4), 1402–1408. doi:10.1016/j.foodchem.2005.11.034.

Dhingra, D., Michael, M., Rajput, H., Patil, R.T., 2012. Dietary fibre in foods: a review. J. Food Sci. Technol. 49 (3), 255–266. doi:10.1007/s13197-011-0365-5.

Difonzo, G., Troilo, M., Squeo, G., Pasqualone, A., Caponio, F., 2021. Functional compounds from olive pomace to obtain high-added value foods–a review. J. Sci. Food Agric. 101 (1), 15–26. https://doi//10.1002/jsfa.10478.

Di Giacomo, G., Romano, P., 2022. Evolution of the Olive Oil Industry along the Entire Production Chain and Related Waste Management. Energies 15 (2), 465. doi:10.3390/en15020465.

Fathi, M., Hosseini, F.S., Ramezani, R., Rashidi, L., 2022. Optimized Enzymatic Hydrolysis of Olive Pomace Proteins Using Response Surface Methodology. Appl. Food Biotech. 9 (2), 79–90. doi:10.22037/afb.v9i2.36192.

Galanakis, C.M., Tornberg, E., Gekas, V., 2010. A study of the recovery of the dietary fibres from olive mill wastewater and the gelling ability of the soluble fibre fraction. LWT-Food Sci. Technol. 43 (7), 1009–1017. doi:10.1016/j.lwt.2010.01.005.

Guillén, R., Heredia, A., Felizón, B., Jiménez, A., Montaño, A., Fernández-Bolaños, J., 1992. Fibre fraction carbohydrates in *Olea europaea* (Gordal and Manzanilla var. Food Chem 44 (3), 173–178. doi:10.1016/0308-8146(92)90183-3.

Gunness, P., Gidley, M.J., 2010. Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. Food Funct 1 (2), 149–155. doi:10.1039/C0FO00080A.

International Olive Council, 2019. Olivae 126., 2019. Official Journal of the International Olive Council. https://www.internationaloliveoil.org/

Jiménez, A., Rodríguez, R., Fernández-Caro, I., Guillén, R., Fernández-Bolaños, J., Heredia, A., 2000. Dietary fibre content of table olives processed under different European styles: study of physico-chemical characteristics. J. Sci. Food Agric. 80 (13), 1903–1908. doi:10.1002/1097-0010(200010)80:13≤1903::AID-JSFA720≥3.0.CO;2-N.

Li, K, Liu, S., Tan, Y., Chao, N., Tian, X., Qi, L., Powell, W.A., Jiang, X., Gai, Y., 2013. Optimized GC-MS Method To Simultaneously Quantify Acetylated Aldose, Ketose, and Alditol for Plant Tissues Based on Derivatization in a Methyl Sulfoxide/1-Methylimidazole System. J. Agric. Food Chem. 61, 4011–4018. doi:10.1021/jf3053862.

Lin, S., Chi, W., Hu, J., Pan, Q., Zheng, B., Zeng, S., 2017. Sensory and nutritional properties of chinese olive pomace based high fibre biscuit. Emir. J. Food Agric. 29, 495–501. doi:10.9755/ejfa.2016-12-1908.

McRorie, J.W., McKeown, N.M., 2017. Understanding the physics of functional fibers in the gastrointestinal tract: an evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. J. Acad. Nutr. Diet. 117, 251–264. doi:10.1016/j.jand.2016.09.021.

Moczkowska, M., Karp, S., Niu, Y., Kurek, M.A., 2019. Enzymatic, enzymatic-ultrasonic and alkaline extraction of soluble dietary fibre from flaxseed–A physicochemical approach. Food Hydrocoll 90, 105–112. doi:10.1016/j.foodhyd.2018.12.018.

Mwaurah, P.W., Kumar, S., Kumar, N., Attkan, A.K., Panghal, A., Singh, V.K., Garg, M.K., 2020. Novel oil extraction technologies: process conditions, quality parameters, and optimization. Comprehens. Rev. Food Sci. Food Saf. 19 (1), 3–20.

O'Shea, N., Arendt, E.K., Gallagher, E., 2012. Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. Innov. Food Sci. Emerg. Technolog. 16, 1–10. doi:10.1016/j.ifset.2012.06.002.

Peng, F., Ren, J.L., Xu, F., Bian, J., Peng, P., Sun, R.C., 2010. Fractional study of alkali-soluble hemicelluloses obtained by graded ethanol precipitation from sugar cane bagasse. J. Agric.Food Chem. 58 (3), 1768–1776. doi:10.1021/jf9033255.

Ribeiro, T.B., Bonifacio-Lopes, T., Morais, P., Miranda, A., Nunes, J., Vicente, A.A., Pintado, M., 2021. Incorporation of olive pomace ingredients into yoghurts as a source of fibre and hydroxytyrosol: antioxidant activity and stability throughout gastrointestinal digestion. J. Food. Eng. 297, 110476. doi:10.1016/j.jfoodeng.2021.110476.

Ribeiro, T.B., Oliveira, A., Coelho, M., Veiga, M., Costa, E.M., Silva, S., João Nunes, J., Vicentec, A.A., Pintado, M., 2020a. Are olive pomace powders a safe source of bioactives and nutrients? J. Sci. Food Agric. 101 (5), 1963–1978. doi:10.1002/jsfa.10812.

Ribeiro, T.B., Oliveira, A.L., Costa, C., Nunes, J., Vicente, A.A., Pintado, M., 2020b. Total and sustainable valorisation of olive pomace using a fractionation approach. Appl. Sci. 10 (19), 6785. doi:10.3390/app10196785.

Salomone, R., Ioppolo, G., 2012. Environmental impacts of olive oil production: a Life Cycle Assessment case study in the province of Messina (Sicily). J. Clean. Prod. 28, 88–100. doi:10.1016/j.jclepro.2011.10.004.

Speroni, C.S., Bender, A.B.B., Stiebe, J., Ballus, C.A., Ávila, P.F., Goldbeck, R., Morisso, F.D.P., da Silva, L.P., Emanuelli, T., 2020. Granulometric fractionation and micronization: a process for increasing soluble dietary fiber content and improving technological and functional properties of olive pomace. LWT – Food Sci. Technol 130, 109526. doi:10.1016/j.lwt.2020.109526.

Union, E., 2009. Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. OJEU 5, 2009. http://data.europa.eu/eli/dir/2009/32/2010-09-16.

Valiente, C., Arrigoni, E., Esteban, R.M., Amadò, R., 1995a. Chemical composition of olive by-product and modifications through enzymatic treatments. J. Sci. Food Agric. 69 (1), 27–32. doi:10.1002/jsfa.2740690106.

Valiente, C., Arrigoni, E., Esteban, R.M., Amadò, R., 1995b. Composición de la fibra alimentaria en el orujo de aceituna. Aminoácidos asociados a la fibra insoluble, soluble y total. Grasas y Aceites doi:10.3989/gya.1995.v46.i2.910.

Zhou, D., Liu, J., Liu, S., Liu, X., Tang, X., Lv, X., 2020. Characterisation of alkaline and enzymatic modified insoluble dietary fibre from *Undaria pinnatifida*. Int. J. Foof Sci. Technol. 55 (12), 3533–3541. doi:10.1111/ijfs.14686.

Wen, L., Zhang, Z., Zhao, M., Senthamaraikannan, R., Padamati, R.B., Sun, D.W., Tiwari, B K, 2020. Green extraction of soluble dietary fibre from coffee silverskin: impact of ultrasound/microwave-assisted extraction. Int. J. Food Sci. Technol. 55 (5), 2242–2250. doi:10.1111/ijfs.14477.