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Fat extraction from fleshings - optimization of operating conditions

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Abstract

Fat from fleshings is an excellent source of energy since it is composed of glycerides, fatty acids and glycerol. The present work aims to optimize the operating conditions of fat extraction from fleshings. Animal fat was obtained after thermal treatment of fleshings in a stainless steel batch reactor using different operating conditions ($100 \leq T \leq 155$ $^{\circ}$C; $1 \leq P \leq 5.5$ bar, t= 2 h, r= 1000 rpm). The fat containing phases were extracted in a heated Soxhlet using n-hexane and ethanol to recover the fat from the solid residue. In addition, 6 M HCl acid was added to the remaining solid and heated to boiling conditions for 2 h to further extract fat that is encapsulated in the tissues of the fleshing. The best conditions of fat extraction were obtained at 155 $^{\circ}$C during 2 h and 1000 rpm that generated a pressure of 5.5 bar inside the batch reactor. It was also found that significantly higher amount of fat could be obtained at the operating conditions mentioned ($\sim$50 wt. % on dry basis), sparring chemical treatments by using a 6 M HCl acid for additional fat extraction. Finally, this approach allowed to significantly reduce the environmental impact of solid waste, decreasing also the costs associated with disposal, while contributing to the circular economy of the tanning sector.

1. Introduction

Limed and green fleshings are by-products/solid waste obtained during the traditional tanning process [1]. These fleshings are obtained from cows after removal of the flesh from the animal skin [2], and they can be composted, or they can be an excellent source for the production of fats [3] and proteins [4]. Economic aspects, especially increasing fuel prices, make fat recovery from hides (which has an average content of ca. 10 wt. % on wet basis) increasingly interesting. The fat obtained is therefore a potential excellent source of energy since it is composed of glycerides, fatty acids and glycerol. It can be used as feedstock for biofuels to be used in vehicles [5–7] or to obtain producer gas [8].

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The first steps used for the treatment of raw hide and skin are the soaking and unhairing [9], the last step commonly known as liming. Keratinous substances such as hair, wool or epidermis, or interfibrillary proteins such as albumins or globulins are removed [10]. Once the hides and skins are taken out of the processing vessels, they are slippery, alkali-swollen and translucent. The pelt obtained, lime treated hide/skin, has now to pass the so-called "Fleshing", a mechanical operation in leather processing where substantial amounts of solid waste are produced. This part of the process involves cutting or removing unwanted flesh from the pelt so that the diffusion of tanning agents is assured. Thus, fleshings can be called as green fleshings (or pre-fleshings) and limed fleshings, depending on being obtained before or after the liming treatment, respectively [3,11].

As mentioned before, tanning industry is an important supplier of leather; however, the amounts of fleshings obtained represent a serious impact on environment due to large amounts of solid waste produced and liquid effluents released. Currently, most of the fleshings are disposed of in a landfill, which generates very high costs. It is also known that fleshings represent around 50 to 60% of the total amount of solid waste generated in the tanning industry [10]. It has been estimated that around 30 to 40 tons of fleshings are produced each day in Portugal, 30% of which corresponds to green fleshings and the remaining 70% to lime fleshings. The procedures used during tanning are mainly performed in aqueous medium and the water consumption is high [12] due to its properties as solvent for many chemical compounds such as $Ca(OH)_2$ and Na_2S that increase the pH above 7. In addition, other solids such as fibres, hair, rock salt, etc. are present. Unfortunately, these problems have never been overcome. As a result, procedures must be outlined to minimize the environmental impact, and to valorize the fleshings obtained. It is here that the concept of circular economy comes into play [13]. Thus, in this work, the treatment of fleshings through thermal hydrolysis is the preferred way to obtain fats and proteins. Therefore, the ideal operating conditions must be determined for optimal process run, considering also the tanning process and compounds present in the fleshings. After thermal treatment three phases are obtained; a solid upper phase composed of fat plus solid residue, an intermediate liquid phase composed of hydrolysed proteins and a bottom solid phase composed of solid residues.

2. Experimental

2.1. Materials

Cowhide fleshings were collected from *CTIC — Centro Tecnológico das Indústrias do Couro*, at *Demoscore*, a Portuguese tannery, just after fleshing operation, in a polyethylene box and brought to the laboratory immediately for experimentation or stored in a freezer. Two types of cowhide fleshings were used, green fleshing prominent from Argentina and limed fleshing from Azores islands (Portugal) (Fig. 1).
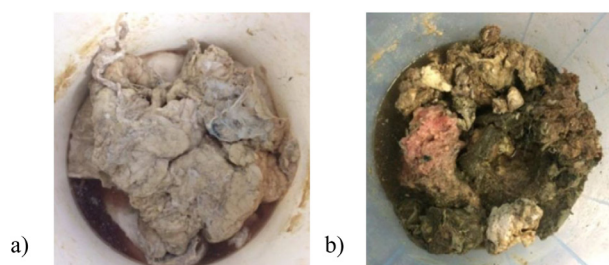

Fig. 1. Physical aspect of a typical green (a) and limed (b) fleshing.

Chemicals which are generally used in the tannery were used for the experiments: n-Hexane (commercial grade), hydrochloric acid (commercial grade) and ethanol (96% v/v commercial grade). Reagents used to determine total fat, iodine value, saponification value, and acid value, were purchased from VWR Limited (Portugal) and are P.A. grade.

2.2. Characterization of the materials

Moisture content (wt %) and total fat (wt %) was determined using standard methods as described in literature [14]. Moisture (m_{water}), fat (m_{fat}) and remaining solid (m_{solid}) content of the fleshings were obtained directly for

each experimental run. The fleshings obtained after thermal treatment (TT) in the batch reactor were placed in a glass balloon. This balloon was placed in a rotary evaporator to obtain the mass of water. Quantitative sulphur content, as well as qualitative element detection of the fat samples obtained after thermal treatment and extraction, were analysed with an energy-dispersive X-ray fluorescence spectrometer (Oxford Instruments X-Suprime 8000).

The glycerides obtained by thermal treatment were identified and quantified after esterification/transesterification by gas Chromatographs (DANI GC-1000) equipped with flame ionization detector using autosampler, according to EN 14103 Standard.

Hereafter the remaining dry solid in the glass balloon was placed in a heated Soxhlet with a total volume of 2 L. Several extraction cycles with fresh hot n-hexane (∼60 °C) were performed so that fat could be extracted from the solid. The liquid extract obtained in the glass balloon was placed in the rotary evaporator for n-hexane distillation. The amount of crude fat obtained was determined using gravimetry. In a next step, fresh ethanol (96 vol. %) was used to perform further extractions at a temperature close to boiling point. The same procedure and methodology for fat quantification was used as previously mentioned for the n-hexane extraction.

The remaining solid, mostly composed by proteins, minerals and "unreachable fat", was treated with a solution of 6M HCl acid during 2 h, at boiling conditions, to destroy the remaining tissues that encapsulate fat, since it is known that fat is still retained inside the fibres of the fleshing (Fig. 2).

Fig. 2. Treatment of a fleshing with 6M HCl under boiling conditions during 2 h.

The obtained suspension was cooled down and 24 h after an extraction procedure with n-hexane and ethanol (96%) was done as previously mentioned. This is essential to assure that all fat could be extracted.

Finally, the remaining solid, mostly composed of proteins and minerals, was also weighted.

The saponification value was determined according to the standard method NP 940:1985. It is based on the addition of alkali ethanolate to esterify glycerides and free fatty acids. The iodine value (IV) is an index for the characterization of fats and oils. It is expressed as the amount of iodine in grams per gram of fat and it is a measure of unsaturated carbon–carbon bonds in the compounds or unsaturated fatty acid residues in the glycerides. The iodine value was determined according to Wijs method (EN 14111: 2003). The procedure is based on the addition of iodine monochloride to the olefinic double bonds present in fat molecules. The higher the amount of olefinic double bonds in a fat, the higher is the amount of iodine that can be added. As a result, the iodine value increases. The acid value was determined using the standard method EN 14104:2003. The acid value is used to quantify the acidity of oils and fats (important measurement e.g. for biodiesel production). The amount of nitrogen in the solids after crude fat extraction and treatment with 6M HCl during 2 h was determined by the method of Kjeldahl.

2.3. Reaction

The reaction studies were carried out in a stainless steel batch reactor (Fig. 3a), Parr series 5100 low pressure reactor (max. 225 °C and 69 bar) with a total vessel volume of 1600 cm^3 from Parr Instrument Company, Illinois, USA. Experimental runs were performed during 2 h at temperatures between 100 and 155 °C, as well as associated pressures between 1 and 5.5 bar. During the start-up procedure the temperature was increased at a heating rate of 1 °C/min until reaching the desired reaction temperature. The temperature inside the reactor (centre of the reaction
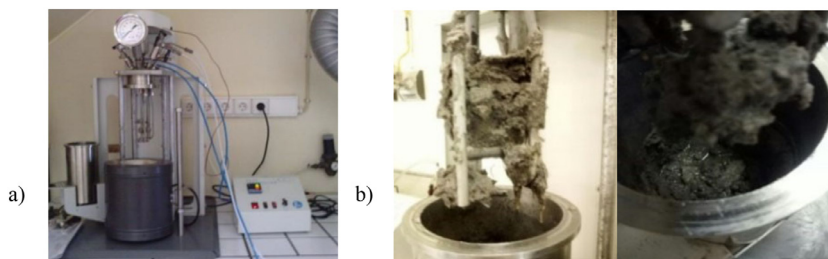
Fig. 3. Reaction unit (a) with hydrolysed fleshings (b).

zone) was monitored with a thermocouple and regulated with an internal cooling system. The total initial mass input of fleshing was 1000 g. For all the measurements an average reproducibility error of ± 2% was considered.

3. Results and discussion

3.1. Characterization of the materials (fleshings) before treatment and extraction

The results of moisture content (wt %) and total fat (wt %) of the fleshings are shown in Table 1, as a range of values due to their heterogeneity; the fleshings sampled were a mixture of different bovines and part of bovines, frozen and saved for fat extraction experiments.

Table 1. Characteristics of unreacted cowhide fleshings.

Parameter	Green fleshing	Limed fleshing
Moisture [wt. %]	48.5 – 76.5	79.4 – 59.2
Fat [wt. %]	13.7 – 24.8	33.9 – 6.5
Proteins [wt. %]	15.9 – 26.7	6.8 – 14.5
Ash [wt. %]	2.41	4.95
Sulphur [wt. %]	76 ppm	3.5 – 2.5
$Ca(OH)_2$ (slaked lime) [wt. %]	0	2.5 – 1.5

It can be observed that in general the fleshings show a very high content of water, most probably due to the adsorption capacity of the fleshings for water. The content of crude fat represents an average value of 20 wt. % (wet base), which is quite good in terms of yield. This yield is a pre-requisite for fat processing, since it is the objective to use this animal fat as raw material for several applications such as biofuel and bio lubricant production, heating source, soap synthesis for cosmetics, and agent for leather treatment. Finally, the fleshings represent an ideal source of proteins that can be used for amino acids synthesis by hydrolysis or as a source of nitrogen fertilizer.

3.2. Characterization of the proteins

The nitrogen content was obtained by the Kjeldahl method. The results obtained are shown in Table 2. It can be seen that the nitrogen content represents little more than 1/3 of the total mass of the protein part analysed. Conventional fertilizer that are commercially available show in general a nitrogen content of around 15 wt. %. If for example pure urea would be used as fertilizer, then a nitrogen content of 45 wt. % would be available. Considering this finding, the protein rich part obtained after thermal treatment and fat extraction of the fleshings used represents a good source for nitrogen. Since this protein rich part is water soluble it is most probably an excellent candidate for its application as fertilizer. Another aspect to be considered is the fact that the protein rich part may have amino acids in abundance due to the treatments made in this work (hydrolysis). It is expected that the proteins have been partially or totally decomposed into amino acids. Furthermore, it is also expected that the amino acids obtained are mostly of polar nature, since they show a good water solubility.

Table 2. Nitrogen content in the solid material after fat extraction.

Material	[g N/100 g solid]
Green fleshings	36.2
Extracted HCl-treated green fleshings	36.2
Limed fleshings	36.6
Extracted HCl-treated limed fleshings	36.6

3.3. Fat treatment and extraction

In Fig. 4 the effect of temperature and pressure on hydrolysis of fleshings for fat extraction, using solvents and 6N HCl are depicted.
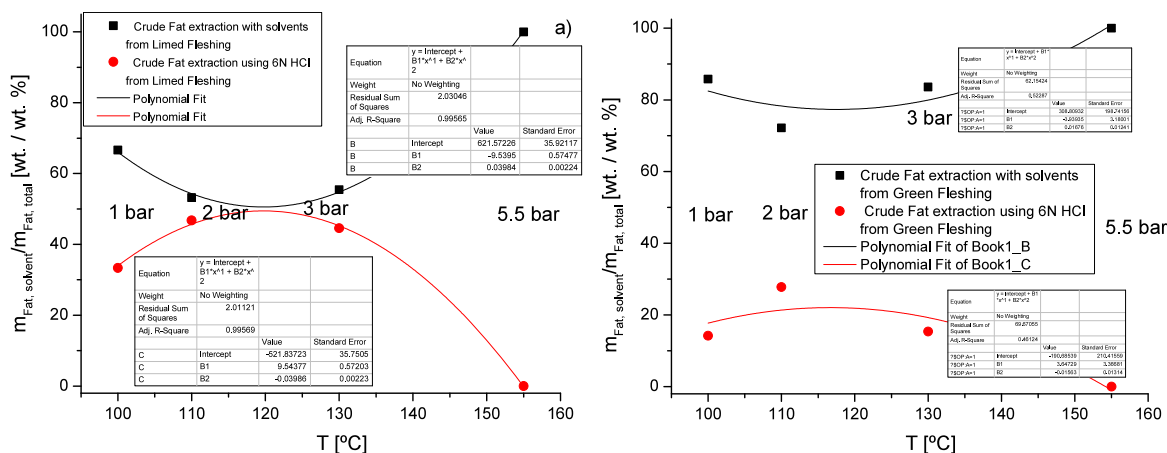
Fig. 4. Effect of temperature and pressure on fleshings for fat extraction using solvents and 6N HCl.

In addition, a polynomial regression was made to fit the experimental points. It has been found that with increasing temperatures and associated pressure the amounts of extracted crude fat, using n-hexane and ethanol, increase. While the amounts of fat extracted after thermal treatment with 6N HCl decreased. However, at 100 °C and 1 bar it is observed that the crude fat extracted does not follow the general tendency. Here it must be said that at this operating conditions indeed the extraction was made under atmosphere, thus no "additional" pressure was present as it would be in a closed reactor system. Most probably, this is the explanation for the deviation observed. The percentage of fat extracted from lime fleshings was lower than the obtained from green fleshings suggesting that $Ca(OH)_2$ present in limed fleshings acts like a cement, reducing the effect of the thermal treatment and/or the extraction by the solvent.

3.4. Characterization of the fats treated and extracted

Physicochemical properties for the fat extracted are shown in Table 3. The results obtained have also been compared with reports in the literature [15]. At room temperature, the crude fat extracted was almost solid. However, in some cases it could be observed small amounts of fat also in liquid state, as for example for the crude fat from green fleshings extracted at 100 °C and 1 bar during 2 h (Fig. 5). The solid fat extracted is mostly of white colour, while the liquid fat shows a brown colour, due to the pigmentation of the green fleshings dragged after extraction or due to the existence of conjugated double bonds in the hydrocarbon chains that may be responsible for the colour in the fat.

The observed liquid state for fat is a consequence of many unsaturated carbon chains with double bonds in cis configuration present in liquid fat. On the other hand, solid fat, at room temperature, is due to a high content of saturated carbon chains and eventually to some unsaturated chains with double bonds in trans configuration. Another explanation for the liquid state of the crude fat can also be the presence of free fatty acids. This happens mostly

Table 3. Properties of the extracted fat and comparison with reports in literature.

Parameter	Green fleshing [This work]	[a]	Limed fleshing [This work]	[a]	[b]	[c]	[d]
Physical state at 25 °C	Solid or liquid		Solid or liquid		Solid	/	/
Iodine value [mg I_2/g fat]	10 – 2	59.4	18 – 2	20.8	52.1	40.9	43.7
Saponification value [mg KOH/g fat]	∼200 or ∼160	n.a.	∼200 or ∼130	n.a.	188.5	198.6	175.0
Acid value [mg KOH/g fat]	0 – 15	5.3	0 – 20	0.5	32.0	73.9	2.5
Ash [wt. %]	2.41	0.02	4.95	0.64			
Sulphur [wt. %]	n.a.	76 ppm	0.379 – 0.094	665 ppm			

[a] [16]; [b] [10]; [c] [17]; [d] [4].

Fig. 5. Fat extraction with n-hexane for green fleshings thermally treated at 100 °C, 1 bar and 2 h.

when the fleshings have been treated with 6M HCl for further crude fat extraction. Here, the crude fat or glycerides are hydrolysed by the acid that means the esters are converted into carboxylic acids (free fatty acids) and alcohols (glycerol).

The results of this work are reported in Table 3. Saponification and acid value clearly match with results reported previously. Since the saponification value is around 180 mg$_{KOH}$/g of fat, it is clear that the fat extracted is excellent for the synthesis of soap. Therefore, additional characterizations were made for synthesized soaps using the fats extracted at different operating conditions. The respective soaps were prepared from the extracted fat by saponification using potassium hydroxide. The soap obtained was in general of white colour, soluble in water together with good lathering and cleansing features, and with an average pH of 8.5. This pH value obtained is an excellent aim to keep human skin healthy, it might be recalled that the ability of cleaning using a soap, is dependent on its alkalinity. Since human healthy skin has a pH of about 5.7 [18], the soap synthesized must be below a pH of 9, otherwise a higher pH can damage the protecting film of epidermis (antibacterial barrier and lipid lamellae) resulting in skin dryness, entering of irritants and allergens [19]. In agreement with these results, a basis is provided for a suitable soap production on a large scale, if the process is properly and commercially optimized.

Methyl esters (biodiesel) were also synthesized from the fats obtained by acid esterification pre-treatment of the free fatty acids followed by basic transesterification with methanol.

Iodine value for fats extracted

The results represented in Fig. 6 show that in general the iodine value decreases with increasing temperature and pressure. The reason for this is most probably the thermal treatment performed on the fleshings. It is known that when unsaturated fats are heated (e.g. when frying), the iodine value decreases, and their viscosity increases due to polymerization. However, also other organic compounds with olefinic double bond character such as sterols can also react with iodine, thus mislead to improper values. Lipids are classified based on their degree of saturation, since this is crucial for ageing ("drying") during storage; fats and oils with higher iodine values age faster.

It could also be observed that with increasing temperature and pressure the values drop from around 20 g I_2 /g fat to around 2 g I_2 /g fat when limed fleshing was used for fat extraction, while the use of green fleshing results in even lower values. Probably this is related with the nature of the fleshings since the green fleshings have another
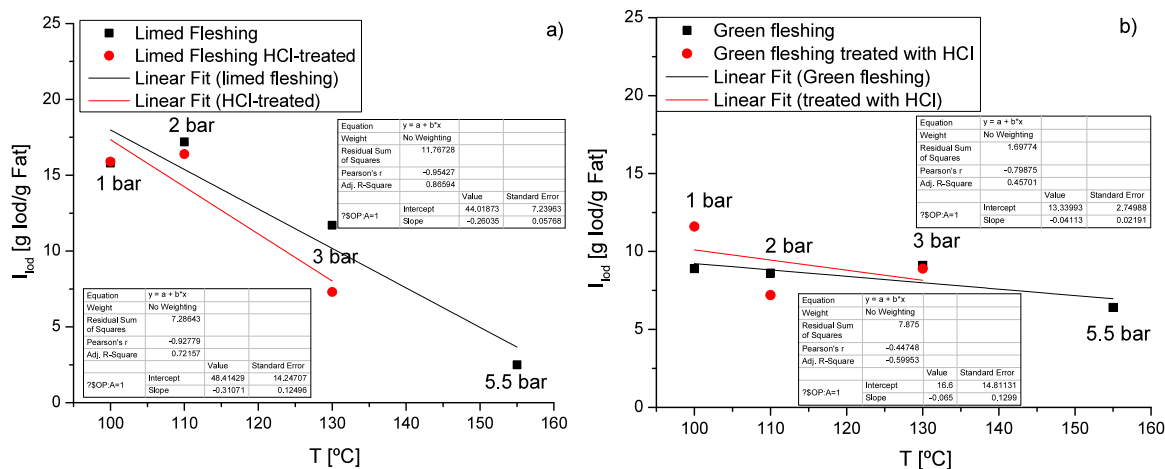
Fig. 6. Iodine values for crude fats extracted at different operating conditions.

origin than the limed fleshing, thus the metabolism of the respective cows was different or simply the processing of limed fleshings is the cause. Finally, the fat from the hydrochloric acid treated fleshings and from untreated fleshings show similar iodine values.

3.4.1. Saponification value

Fig. 7 depicts the saponification value of the crude fats extracted from fleshings as a function of the operating conditions used. It can be observed that the crude fats extracted from the fleshings after thermal treatment with 6 N HCl exposition show saponification values in the range reported in the literature, around 200 mg KOH/g fat, while the fats extracted without any acid treatment show lower values of around 160 or 130 mg KOH/g fat, respectively for green or limed fleshings fat. Typical values for animal fats are between 190 and 205. One can say that in general the saponification value slightly decreases with increasing temperature and pressure. A possible explanation for the observations reported above may be the fact that during acid treatment at boiling conditions the mineral content in the fat material is removed, while a simple thermal treatment (hydrolysis) does not show the same effect. The inorganic material present is unsaponifiable, so if no inorganic material would stick on the crude fat, the saponification value would easily be higher. Another factor that may contribute to lower saponification values is high average molar mass of fat. Long chain fatty acids would have a low saponification value because
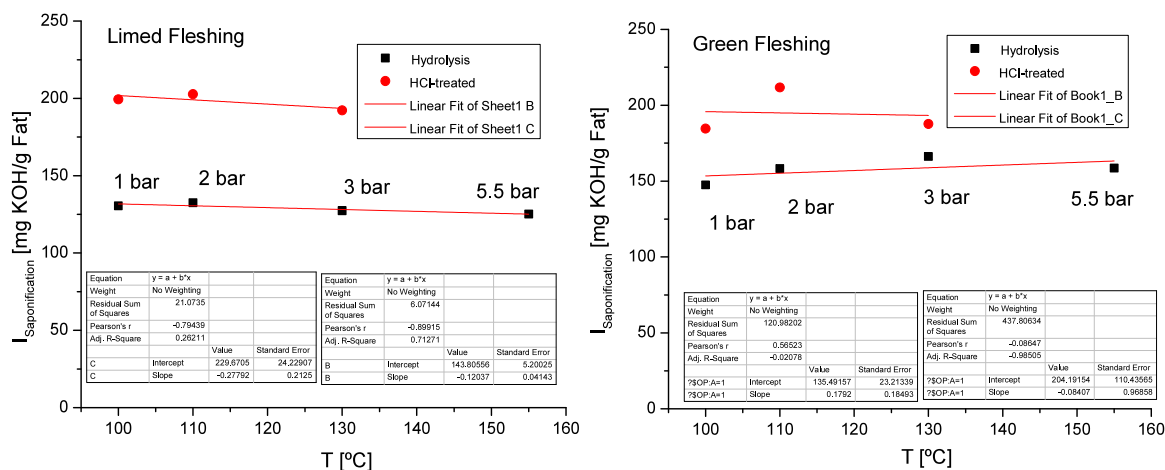

Fig. 7. Saponification value ($I_{Saponification}$) for fats obtained at different temperatures and pressures without and with 6N HCl treatment.

they have a relatively lower number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. Moisture, inorganic material as well as little organic material such as hydrocarbons, sterols and aliphatic alcohols of high molecular mass are to be considered as the unsaponifiable fraction.

The saponification value is a key figure for the chemical characterization of fats and oils; it is used for their purity check and quality control. It indicates how much potassium hydroxide (in mg) is necessary to cleave the existing ester bonds (saponification) and to neutralize the free fatty acids contained in 1 g of fat.

3.4.2. Acid value

The acid value of the fats obtained as function of the hydrolysis temperature and pressure was measured and is depicted in Fig. 8. It is observed that in general the acidity of the crude fats obtained after thermal treatment and hydrochloric acid treatment increase with temperature and pressure. In the latter case, the acidity is much higher. This phenomenon is a consequence of the severe hydrolysis process that attacks the triglycerides in a way that carboxylic acids are released together with glycerol. The higher the temperature and pressure the higher is the amount of the products mentioned before. The amounts of FFA give feedback about decomposition processes or pre-treatments made that reduce its quality. The oxidation stability of the oils decreases with increasing acid value.
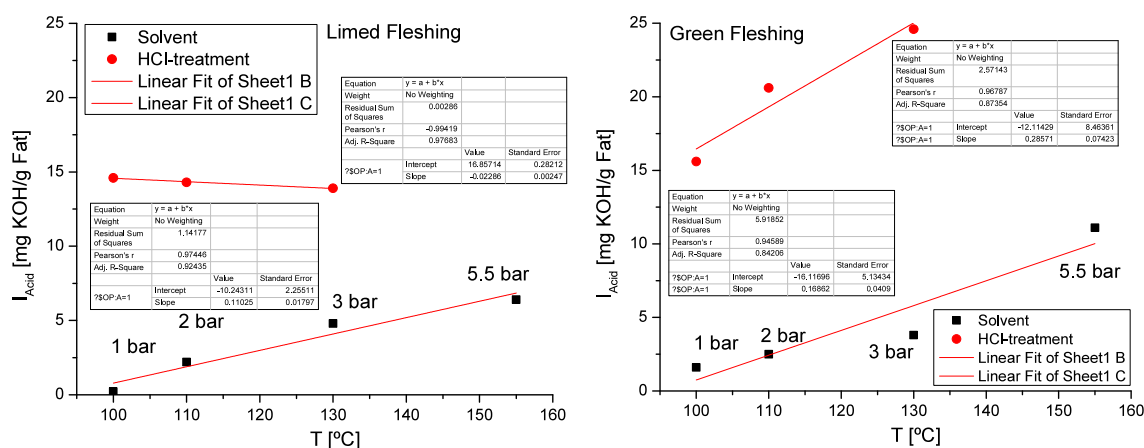

Fig. 8. Acid value for fats obtained at different temperatures and pressures with and without 6N HCl treatment.

The values obtained for acidity also suggest the need of an additional esterification treatment of the crude fat due to the presence of FFA in the feedstock if biodiesel production is performed under conventional alkali transesterification. FFA may react with alkaline catalyst to form soap and emulsions during transesterification, which hinders biodiesel purification and decreases FAME yield [20].

Another possibility to valorize this high acid value fat would be to convert it into a biolubricant, by using enzymes [21,22].

4. Conclusions

The main conclusion of this work is that a considerable amount of fat is encapsulated inside the tissues of the fleshing that unavoidably hinders the extraction process. Therefore, severe operating conditions must be employed to destroy the tissues, and further extract the entangled fat. In an optimal case, small particle sizes in micrometre range shall be obtained after thermal treatment. The best fat extraction was obtained at 155 °C and 5.5 bar where 100% of the fat could be collected by a simple decantation since both phases, the lipophilic fat phase and the hydrophilic protein rich phase, are not soluble. The disadvantage of this severe operating condition is the low iodine value obtained ($\sim$5 g I_2/g fat) and the high acid number, 5 and 10 mg$_{KOH}$/g, for fat from lime fleshings and from green fleshings respectively. Finally, the saponification values are low, probably due to the lack of purity of the extracted fat.

Concerning the limed fleshings, $Ca(OH)_2$ present acts like a cement, reducing the effect of the thermal treatment and/or the extraction by the solvent.

The results obtained show also that the crude fat must be further processed for biofuel or biolubricant synthesis. After removal of moisture, minerals and Sulphur, an additional step for the removal of free fatty acids is required (esterification), before a conventional biodiesel synthesis (transesterification) can be done.

CRediT authorship contribution statement

A.F. Cunha: Investigation, Formal analysis, Validation, Writing - original draft. **N.S. Caetano:** Writing - review & editing. **E. Ramalho:** Conceptualization, Supervision, Data curation, Writing - review & editing. **A. Crispim:** Conceptualization, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Declaration of competing interest

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

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