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# Evaluation of the interrelated effects of slaughtering, drying, and defatting methods on the composition and properties of black soldier fly (*Hermetia illucens*) larvae fat

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ABSTRACT

The interrelated effect of different slaughtering, drying and defatting methods of black soldier fly larvae (BSFL) on the lipid composition and properties of the fat was studied. Blanching and freezing were compared as slaughtering methods, oven or freeze-drying as drying methods, and mechanical pressing or supercritical fluid extraction (SFE) as defatting methods.

The different modes of slaughtering, drying, and defatting, along with both binary and ternary interactions caused significant effects on processes yields, lipid composition, moisture content and thermal properties. Thus, considering the defatting degree and the yield in total valued products (defatted meal plus fat), the combination of blanching, freeze-drying plus mechanical pressing was the worst option (51.2% and 87.5%, respectively). In contrast, the other combinations demonstrated better and comparable efficiency, although SFE is preferable for defatting (83.2% and 96.9%, respectively). The content of major fatty acids (lauric, palmitic and myristic acids) was significantly affected by the BSFL treatments, although with unsignificant impact on the total saturated fatty acids content. To preserve the integrity of the fat, the combination of blanching and oven-drying was preferred, as non-thermal methods of slaughtering and drying caused intense lipolysis, releasing free fatty acids (FFA) in the range of 18.6–23.5%. To achieve the lowest moisture content in the fats ($\leq$0.1%), oven-drying with mechanical pressing were desired, regardless of the slaughtering method; while values $>$ 1% were reached for freezing, freeze-drying and SFE. Both differences in FFA and moisture contents caused different thermal behaviors in the samples. Specially, the melting temperature was lower for samples with higher FFA and moisture contents, with a notable difference when freezing, freeze-drying and SFE were combined (14.5 °C vs 30.6 °C, as the mean value for the rest of samples). The different modes of processing did not affect the minor lipid compounds.

Therefore, the modes employed for slaughtering, drying, and defatting of BSFL determine, either individually or in combination, the process yields, composition, and properties of the fat.

## 1. Introduction

Due to the expected population growth in the next years and the consequent growing demand for food, the interest around edible insects has been widely recognized in recent years as a potential alternative source of proteins for food and feed which is environmentally respectful (van Huis et al., 2013; Arnold van Huis, 2015; Arnold Van Huis, 2016). Accordingly, since 2021, the European Commission has authorized

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*Tenebrio molitor*, *Locusta migratoria*, *Acheta domesticus* and *Alphitobius diaperinus* as edible insect species to be marketed within the framework of novel foods legislation (European Commission, 2021a, 2021b, 2022, 2023). Likewise, it is expected that other insect species will be authorized soon, such as *Gryllodes sigillatus*, *Apis mellifera* and *Hermetia illucens* (Mancini et al., 2022). Nevertheless, their use is already accepted for feed in case of aquaculture, poultry, and pigs (European Commission, 2017, 2021c).

Among the aforementioned insect species, the larvae of *Hermetia illucens* (black soldier fly larvae, BSFL) are becoming increasingly popular due to their remarkable ability to transform organic wastes into insect biomass (Bessa et al., 2020). The combination of their environmental advantages with their high-quality nutritional value makes BSFL a promising opportunity for use as food and feed. The nutritional composition of BSFL comprises approximately 50% of crude protein on dry matter, which contains certain essential amino acids (Shelomi, 2020; Zulkifli et al., 2022). Concerning the lipid fraction, it can reach values closer to 35–40% on dry matter (Almeida et al., 2020; Shumo et al., 2019), consisting of a highly saturated fraction of fatty acids, mainly represented by lauric acid (around 36–60%) and followed by palmitic or myristic acid. Besides, monounsaturated and polyunsaturated fatty acids are also present, such as oleic acid, linoleic and α-linolenic acid (Almeida et al., 2020). In addition, BFSL contains low amounts of carbohydrates, which are mainly represented by chitin, as seen in many other insects (Caligiani et al., 2018); and diverse minerals are also found (Romano et al., 2023).

In general, the processing of most edible insects typically involves an initial slaughtering step followed by a drying process. Regarding the killing method, freezing and blanching are currently the most frequently methods used, whereas concerning the drying process, different methodologies are available, as freeze-drying or oven-drying (Caligiani et al., 2019; Hurtado-Ribeira et al., 2023; Larouche et al., 2019; Leni et al., 2019). Additionally, considering the high fat content of BFSL, a final process of defatting is often necessary to obtain a high-protein product. The defatting process can be carried out using organic solvents, although it is most frequently done by mechanical pressing, or alternatively, by supercritical carbon dioxide extraction (SFE) and centrifugation (Cantero-Bahillo et al., 2022; Hurtado-Ribeira et al., 2023; Kim et al., 2022; Laurent et al., 2022).

It is important to note that the choice of methodology for slaughtering, drying, and defatting, as well as the specific conditions employed, could affect the quality and nutritional value of the products. In this sense, different studies have been conducted to evaluate the impact of the different processing steps on the quality of the obtained protein flours, concerning their stability, colour changes, techno-functional properties, nutritional value, or digestibility (Buβler et al., 2016; Mshayisa et al., 2022; Zhen et al., 2020). Additionally, oils and fats are also obtained in remarkable amounts as coproducts during the processing of protein flours, and this fraction is especially relevant in the case of high-lipid insects as BSFL after the defatting process. However, unlike protein flours, the impact of processing conditions on the lipid fraction has not been extensively investigated. Therefore, to reach a complete use of the products obtained after processing BSFL, including valuable fats, it becomes necessary to study of the relationship between processing parameters of BSFL and the yield, quality, composition, and properties of the lipid fraction. In this sense, the thermal or non-thermal processing of the larvae seems to play a crucial role in the composition and quality of BSFL lipids. This is because steps performed at high temperatures can lead to the deactivation of endogenous enzymes like lipases. The high lipase activity found in insects has been related to intense lipolysis of lipids and a subsequent increase in the acidity. This parameter is related to worse quality of edible fats due to the loss of the integrity of the fat to free fatty acids (FFAs) and partial glycerides (Caligiani et al., 2019; Leong et al., 2022; Santana et al., 2017; Terra and Ferreira, 2012). Furthermore, such modification of the lipid profile might have a direct impact on the technological properties of fat because the melting and crystallization temperatures of lipids vary depending on the lipid species composition. Thus, the use of thermal methods for either killing or drying of insects, such as blanching and oven-drying, seems to prevent the lipolysis of lipids, in contrast with non-thermal methods, as freezing or freeze-drying (Caligiani et al., 2019; Leong et al., 2022). However, these lipolytic effects have been mainly related to the slaughtering step, but conclusive results regarding the impact of the entire processing of BSFL on lipid composition have not been reached. Additionally, the limited studies on insect lipids have mainly explored the impact of each individual step of the process, i.e., slaughtering, drying, or defatting. However, the consideration of the three technological processes together, and the interrelation between them as successive operations in the final process of obtaining insect meal and lipids, should be approached to find the best combination of processes that most efficiently allows the production of the lipid coproduct with the highest yield and the best quality, composition, and properties.

Therefore, the main aim of this study was to assess the yield of processes and the lipid composition of BSFL fat, focusing on the fatty acid profile, lipid species, and minor lipid compounds, after following different combined processes of slaughtering (by freezing and blanching), drying (by freeze-drying and oven-drying) and defatting (by mechanical pressing and SFE). Thus, those commonly employed methods and conditions for edible insect processing (Hernández-Álvarez et al., 2021; Ojha et al., 2021; A. van Huis, 2019) were compared. Furthermore, other relevant parameters related to the physicochemical properties and quality of the lipids, which could be influenced by the processing, such as moisture content and thermal properties of the resulting fats, were also assessed.

## 2. Materials and methods

### 2.1. Raw materials and chemicals

BSFL were reared by a company specialized in *Hermetia illucens* production (Entomo AgroIndustrial, Murcia, Spain).

Hexane (95%) was acquired from Macron (Gliwice, Poland). N,O-Bis (trimethylsilyl)trifluoroacetamide (BSTFA), boron trifluoride-methanol solution (14%), and squalene 98%, dodecanoic acid, myristic acid, palmitic acid, linoleic acid, 1-lauroyl-rac-glycerol, dilaurin mix isomers and glyceryl tridodecanoate standards were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Oleic acid standard was purchased from Alfa Aesar (Kandel, Germany). β-sitosterol standard was purchased from Biopurify Phytochemicals Ltd (Sichuan, China). Sodium hydroxide and methyl tert-butyl ether were purchased from Merck KGaA (Darmstadt, Germany). Ethanol absolute was purchased from Panreac Quimica S.L.U (Barcelona, Spain). Diethyl ether was purchased from Carlo Erba Reagents GmbH (Emmendingen, Germany). Potassium hydroxide was purchased from Scharlab S.L. (Barcelona, Spain).

### 2.2. Slaughtering of larvae samples

BSFL of 12-day-old were slaughtered, either by blanching or freezing, in duplicate (8 kg per replicate). These processes were carried out at the facilities of Entomo AgroIndustrial (Cehegin, Spain). Prior to slaughtering, the BSFL were sieved and washed in cold water. Blanching slaughtering was performed by immersing the larvae in water at 90 °C, with a sample to water ratio of 1:10 (w/v) for 40 s. Thereafter, the larvae were immersed in cold water and then drained. Freezing slaughtering was performed at −20 °C for 24 h. After each slaughtering process, the resulting batches were divided in half to be dried, either by oven-drying or freeze-drying.

### 2.3. Drying of larvae samples

Oven drying without internal ventilation was carried out at 65 °C for 24 h in trays using a conduction oven. Freeze-drying was performed for

4 days using a 3-tray freeze drier (LyoBeta 15, Telstar, Terrassa, Spain) following a program that started at −20 °C for 2 h, followed by a gradual increase from −20 to 20 °C, and then maintained at 20 °C for the remaining duration of the process, with the condenser set at −81 °C. After each drying procedure, each batch was divided in half for defatting, which was accomplished using, either mechanical pressing or SFE. The dried samples were stored at room temperature and defatted as soon as possible.

### 2.4. Defatting of larvae samples

For mechanical defatting, 450 g of dried larvae were defatted in a screw-press expeller (InVIA, Vilafranca del Penedès, Spain) equipped with a heating jacket. Pre-heating was carried out at 136.0 ± 13.1 °C for 8 min, and the temperature of the press head was continuously monitored during the pressing process. It reached a mean maximum value of 140.4 ± 10 °C and mean minimum value of 126.6 ± 11.9 °C, varying depending on the samples, since the lowest the initial moisture content of the larvae, the highest the reached temperature of the press head. The collected crude fat was then centrifuged (Multifuge 3SR + centrifuge, Thermo Scientific, Waltham, MA, USA) at 3400×*g* for 10 min to remove co-extracted solids.

Supercritical $CO_2$ defatting was performed using a supercritical $CO_2$ extraction equipment (Model Thar SF 2000, Thar Technology, Pittsburgh, PA, USA), and the optimized conditions were previously described in our previous study (Cantero-Bahillo et al., 2022), which was scaled-up as described by Fornari et al. (2023). Before extraction, the dried larvae resulting from each drying procedure were ground in a knife mill (Grindomix GM 200, Retsch GmbH, Haan, Germany). The extraction cell (1350 $cm^3$) was loaded with 450 g of sample. The defatting process was performed at 450 bar, 60 °C, and $CO_2$ flow of 130 g/min for 4 h.

To determine the initial fat content of samples, hexane defatting was performed, following the procedure outlined by Cantero-Bahillo et al. (2022). Thus, 2 g of ground sample were homogenized (Ultra-turrax T18 basic, IKA, Staufen, Germany) at 11,000 rpm for 5 min with 95% hexane, at a sample to solvent ratio of 1:5 (w/v). Then, the mixture was centrifuged at 4500 rpm for 10 min at 20 °C. The supernatant was then removed, and the precipitate underwent defatting again following the same procedure. Hexane was removed using a vacuum rotary evaporator. This same procedure was also applied to determine the remaining fat content of the defatted meals after extraction.

For each treatment of combined methods of slaughtering, plus drying and defatting, duplicate samples of fat and defatted meals were obtained.

### 2.5. Estimation of the yields of the processes

Fat yield was estimated as expressed in the following equation:

$$Fat\ yield(\%) = \frac{Weo}{Ws} 100 \quad (1)$$

where "*Weo*" is the weight of the extracted oil either by mechanical pressing or SFE and "*Ws*" is the weight of the dry sample that was placed in the screw-press expeller or SFE extraction vessel.

Defatted meal yield was calculated according to the following equation:

$$Defatted\ meal\ yield(\%) = \frac{Wdf}{Ws} 100 \quad (2)$$

where "*Wdf*" is the weight of the obtained defatted meal after each process.

Defatting degree was calculated as follows:

$$Defatting\ degree(\%) = \frac{Wo - \left(Wdf\ \frac{\%rfdf}{100}\right)}{Wo} 100 \quad (3)$$

where "*Wo*" is the total initial fat content of the sample and " %*rfdf*" is the remanent fat in the defatted meal expressed in percentage.

Total products yield was also evaluated as shown below:

$$Total\ products\ yield\ (\%) = Defatted\ meal\ yield + Fat\ yield \quad (4)$$

### 2.6. Fatty acids profile

Fatty acids were transformed into their corresponding fatty acid methyl esters (FAMEs). The methylation of fatty acids was carried out according to the AOAC Official Method 996.01 (Section E), using NaOH-methanol solution (0.5 N) and $BF_3$-methanol solution (~14%, w/v) as catalysts (Satchithanandam et al., 2001). The FAMEs were analyzed by GC according to the method described by Vázquez et al. (2017). Identification and quantification of FAMEs was carried out in an Agilent 6850 Network GC System (Avondale, US), coupled to FID detector and Agilent 6850 autosampler. The capillary column was a HP-88 (30 m, 0.25 mm i. d.) (Avondale, US). An injection volume of 1 μL and a 20:1 split ratio were used. The injector and detector temperatures were 220 and 250 °C, respectively. The temperature program started at 50 °C, rising to 220 °C at 15 °C $min^{-1}$. The final temperature (220 °C) was held for 10 min. Identification of FAMEs was based on retention times and the relative area percentages as compared to the No.3 PUFA reference standard (47085-U), obtained from Supelco (Bellefonte, US). Quantification was expressed as percentage of area.

### 2.7. Characterization of lipid species and minor compounds

The analysis of triglycerides (TG) was carried out according to Vázquez et al. (2016). Samples were prepared at 15 mg/mL in methyl tert-butyl ether (MTBE). Separations were performed on a Hewlett-Packard 5890 series II gas chromatograph with on-column injection using a 7-m HP-5MS capillary column, 0.25 mm I.D. (Agilent Technologies, Santa Clara, CA, USA). An injector and detector temperatures of 40 and 340 °C, respectively, were utilized. The temperature program was as follows: starting at 40 °C and then heating to 250 °C at 42 °C $min^{-1}$ with 15 min hold, followed by heating from 250 to 325 °C at 15 °C $min^{-1}$ with 20 min hold.

FFAs and other minor lipid compounds were analyzed by GC-MS-FID according to Herrera et al. (2019) previous derivatization by N,O-Bis (trimethylsilyl)trifluoroacetamide (BSTFA). Briefly, fat was mixed with BSTFA at 20 mg/mL and heated at 75 °C for 1 h, with shaking every 15 min. After that, samples were cooled at room temperature for 5 min and hexane was added to reach a final concentration of 10 mg/mL. Subsequently, samples were analyzed by a GC-MS (Agilent 7890 A, Agilent Technologies, Santa Clara, CA, EE. UU.) equipped with an autosampler (G4513A) a split/splitless injector, a flame-ionization detector, and a triple-axis mass spectrometer detector (5975C). Lipid compounds were separated by using a HP-5MS column (30 m length x 0.25 mm internal diameter x 0.25 μm film thickness). Helium was used as carrier gas at a flow of 2 ml/min. Injector temperature was 260 °C while the mass spectrometer ion source and interface temperatures were 230 °C and 280 °C, respectively. Injection of samples (1 μL) were carried out in splitless mode. The analysis started holding the oven at 50 °C for 3 min and then increased at 15 °C/min until 310 °C, then held for 25 min. The mass spectra were obtained by electronic impact at 70 eV. The scan rate was 1.6 scans/s at a mass range of 30–700 amu. Identification of lipid compounds was performed by NIST MS Data library. For quantification, calibration curves were obtained using the standards of lauric acid, myristic acid, palmitic acid, linoleic acid, oleic acid, as well as β-sitosterol and squalene, which were also subjected to the same derivatization procedure as the samples.

### 2.8. Acidity determination

The acidity determination was performed by volumetric titration based on ISO 660:2020 (ISO, 2020). Fat samples were dissolved in 50 mL of ethanol:diethyl ether (1:1, v/v) and each solution was titrated with 0.1 N KOH. Phenolphthalein (1%, w/v) in ethanol was used as the indicator. Results were expressed as mass percentage.

### 2.9. Moisture content determination

The moisture content in the BSFL fat was determined using a gravimetric analysis based on ISO 662:2016 method (ISO, 2016). Before use, the crucibles were heated in an oven at 103 ± 2 °C for 24 h and then placed in a desiccator for 30 min. After that, the crucibles were weighed. Subsequently, 4 g of each sample were weighed into the crucibles and heated at 103 ± 2 °C for 45 min. After that, the crucibles containing the samples were placed in a desiccator for an additional 30 min. Following that, the crucibles were weighed once more, and the moisture content was expressed as a mass percentage.

### 2.10. Thermal properties of larvae fats

The thermal properties of BSFL fats were analyzed by using a differential scanning calorimeter (DSC) (SC-Q200 TA Instruments, USA), following the method described by Matthäus et al. (2019). Calibration was carried out using high-purity indium. The system was purged with nitrogen at 60 mL $min^{-1}$. The samples were melted at 55 °C, immediately weighted into an aluminium pan, and sealed with an aluminium cap. The following conditions were applied to obtain cooling and melting curves: heating to 80 °C at 5 °C $min^{-1}$, and held for 1 min. Then, cooling down to −50 °C at 5 °C $min^{-1}$, and held for 1 min. After that, the samples were heated up to 80 °C at a rate of 5 °C $min^{-1}$. The corresponding thermograms were obtained. The major melting and crystallization peak temperatures were measured. Moreover, the solid fat content (%) was estimated by the partial integration of the thermograms at different temperatures.

### 2.11. Statistical analysis

The effect of the factors of study (slaughter, drying and defatting procedures) and their respective interactions was evaluated by a three-way analysis of variance using the general linear model procedure of the SPSS 25.0 statistical package (SPSS Inc., Chicago, IL, USA). When the effect of any of the factors was significant ($p \leq 0.05$), differences between groups were analyzed by using the post-hoc Tukey. Pearson correlation tests were conducted for additional analyses.

## 3. Results and discussion

### 3.1. Effect of the larvae processing on the different yields

Initially, and in order to assess the different yields of defatted meal and fat after each BSFL processing, it was necessary to evaluate the initial fat content of the dried larvae, for which a conventional fat extraction procedure using hexane was applied (Cantero-Bahillo et al., 2022). As shown in Fig. 1, significant differences were observed due to slaughter and drying, and to the interaction between both factors. Thus, on average, and regardless of the drying method, the larvae slaughtered by freezing presented a higher fat content than those slaughtered by blanching (27% and 23%, respectively). This result may be partially due to a likely minor loss of lipids during the blanching in hot water. In fact, when we analyzed the employed water after the blanching process, a minor lipid fraction close to 1 mg/L was detected. Concerning the effect of the drying process, the larvae subjected to oven-dried contained, on average, more fat than those dried by freeze-drying (28% and 22%, respectively), probably due to the higher moisture that the freeze-dried samples contained (5% and 13%, respectively). Therefore, the combination of blanching and freeze-drying led to samples with a lower initial fat content (around 18%) compared to the other treatments, which exhibited values higher than 25%.

Fig. 2 show the efficiency of the different treatments applied to BSFL concerning fat yield (Fig. 2a), defatting degree attained (Fig. 2b), defatted meal yield (Fig. 2c), and total products yield (Fig. 2d). The different modes of slaughtering, drying, and defatting exhibited a significant effect on the fat yield, as individual factors, regardless of their combination ($p < 0.05$). Due to that, in general, freezing led to higher fat yields in comparison with blanching (18.7% and 16.9%, respectively). Similarly, fat yields of BSFL processed by oven drying were significantly higher than those samples dried by freeze-drying (19.6% and 16.0%, respectively). Moreover, SFE defatting resulted in significantly higher oil yields than mechanical pressing (20.4% and 15.2%, respectively). Furthermore, the binary and ternary interactions significantly affected fat yield (Fig. 2a). Consequently, the lowest fat yields were obtained with the combinations of drying by freeze-drying and defatting by pressing, particularly after slaughtering by blanching. This result could be related to the higher moisture content of these samples from freeze-drying, as previously explained, which might negatively affect the efficiency of mechanical pressing in terms of oil extraction and recovery. This effect was visually observed as a lower fat collection during the pressing of such freeze-dried samples. This was in agreement with the worst defatting degree observed in the meals from this treatment, which showed considerably lower values (around 50% defatting) compared to the other treatments, where the degree of defatting was closer to 80%

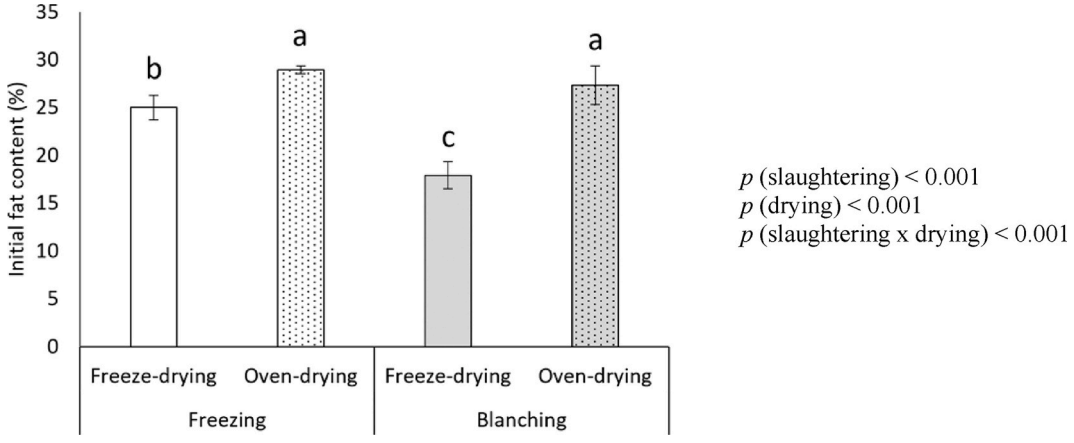



**Fig. 1.** Initial fat content of the fat (g/100 g sample) from BSFL processed by different methods of slaughtering, drying, and defatting. Different letters between the treatments mean significant differences.

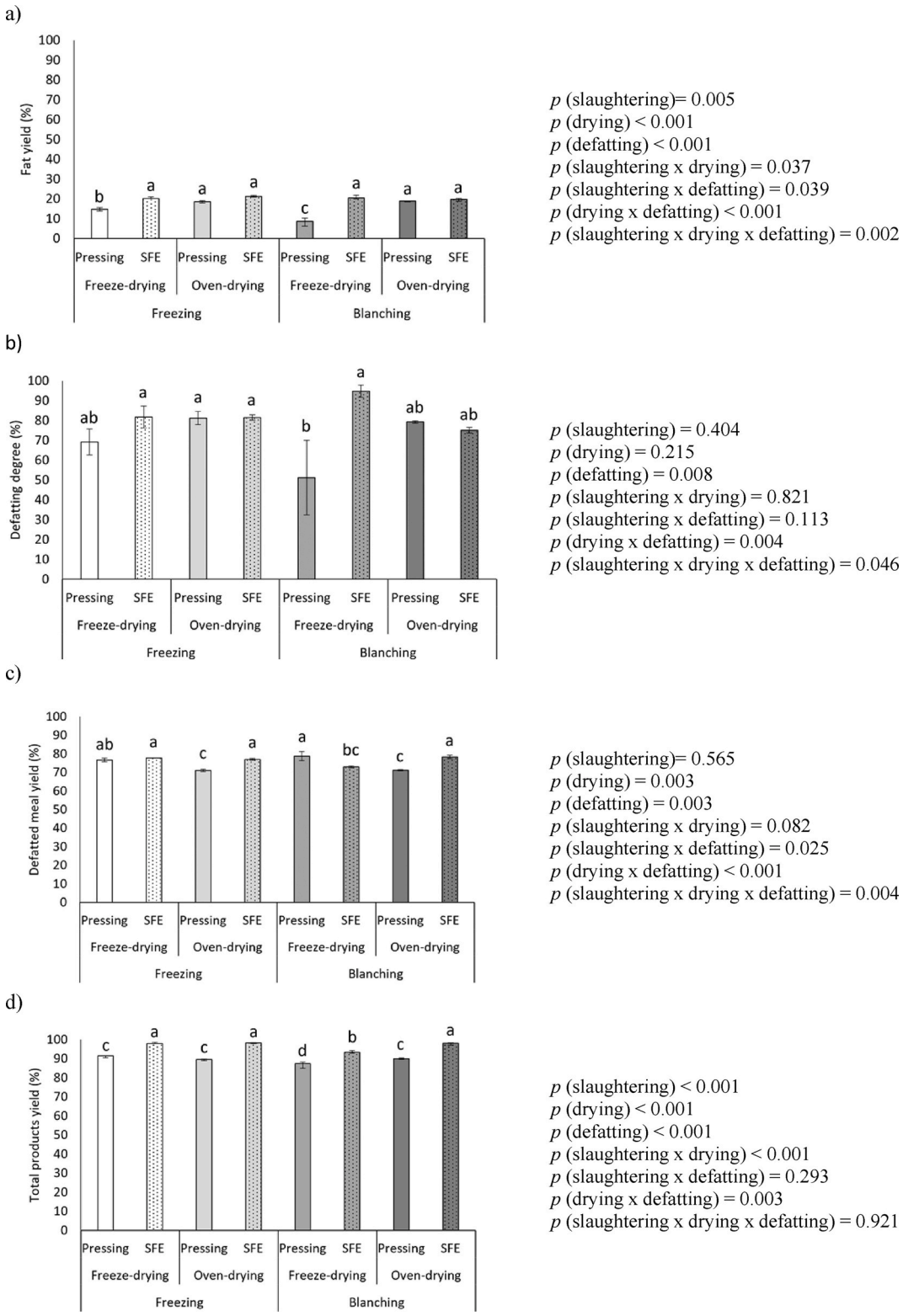



**Fig. 2.** Process yields of BSFL (a. fat yield, b. defatting degree, c. defatted meal yield, d. total products yield) obtained by different methods of slaughtering, drying, and defatting. Different letters between the treatments mean significant differences.

(Fig. 2b).

With respect to the meal yield (Fig. 2c), while drying and defatting treatments significantly affected the yield as individual factors of variability ($p < 0.05$), no significant effect was observed due to the slaughtering method applied ($p > 0.05$). Nevertheless, certain significant binary interactions, as well as ternary interactions, were observed between the variable factors studied, being oven-drying followed by pressing the treatments attaining the lower meals yields (c.a. 71%), regardless of the method of slaughtering. For the rest of combinations of treatments, meals yields were similar, with values close to 77%.

Finally, the yield of total valued products (i.e. defatted meal plus fat) was analyzed. This parameter reflects the generation of residues and,

thus, the efficacy and eco-environmental sustainability of the overall three-step larvae processing. Significant effects resulted for the slaughtering, drying and defatting treatments applied, with higher total yield of valued products in the case of freezing compared to blanching, oven-drying compared to freeze-drying, and SFE compared to mechanical pressing ($p < 0.05$ for each individual variability factor, Fig. 2d). In fact, one of the most remarkable results observed in Fig. 2d was that all samples defatted by SFE yielded a high percentage of total value product (c.a. 97%) compared to their counterparts subjected to defatting by pressing (yields around 90%). This was due to the fact that during mechanical pressing, a residue up to 10% w/w is generated as result of retained material within the screw-press and precipitated solid material after the necessary centrifugation of the extracted fat, which does not form part of the valued products. This reason also explains the lower yield of defatted meal obtained with mechanical pressing, particularly when combined with oven-drying (Fig. 2c).

As summary, considering the defatting degree as one of the most relevant parameters, together with the yield in total valued products, the combination of slaughtering by blanching, drying by freeze-drying and defatting by mechanical pressing is the least favorable option in the processing of BSFL, at least under the used conditions in the current study. The worst results obtained with these processes would be due to a sum of factors, such as the higher initial moisture content of the samples, which leads to worse efficiency in mechanical pressing, with the subsequent higher production of residual material and worse defatting of the meal. In contrast, the other combinations of slaughtering, drying, and defatting procedures for BSFL appear to be comparable to each other and adequate from the standpoint of process efficiency. Nevertheless, considering all efficiency parameters, any procedure of slaughtering and drying combined with SFE defatting yields both high meal and fat percentages, appropriate defatting degrees, and higher overall valued products, simultaneously, compared to mechanical pressing, mainly due to the fact that the typical waste stream generated during pressing is not produced by supercritical defatting.

### 3.2. Effect of the larvae processing on the fatty acid profile

The analysis of the fatty acids of the BSFL fats obtained after combining different treatments of slaughtering, drying, and defatting is shown in Table 1.

Although significant differences were observed in the content of specific fatty acids, there were no significant variations in the overall profile, considering the main families of saturated, monounsaturated, and polyunsaturated fatty acids (SFA, MUFA and PUFA, respectively). This mainly consisted of a mean value of 77% SFA, 12% MUFA and 11% of PUFA. This composition was due to a major content of the saturated lauric acid (mean value of 50%), along with palmitic acid (mean value of 13%) and myristic acid (mean value of 10%). The content of these major fatty acids was significantly affected by the BSFL treatments, although without an impact on the overall fatty acid profile.

Additionally, the most relevant MUFA was oleic acid (mean value of 9%), and the most representative PUFA was linoleic acid (7%). Minor levels of the n-3 linolenic acid (around 3%), palmitoleic acid (around 2%) and stearic acid (around 2%) were also present.

Therefore, although with minor variations due to the treatments, all the derived lipid samples presented, in general, the typical fatty acid composition described for BSFL (Barragan-Fonseca et al., 2017; Danieli et al., 2019; Spranghers et al., 2017), regardless of the mode of slaughtering, drying, and defatting of the larvae.

Based on the fatty acid profile, specific indices related to the nutritional quality of fats were estimated. These included the n6/n3 ratio, the PUFA/SFA ratio, as well as the thrombogenic (TI) and atherogenic (AI) indices. As shown in Table 1, all the obtained fats exhibited the same nutritional quality concerning the PUFA/SFA ratio, AI, and TI, regardless of the treatments applied. Nevertheless, significant effects due to the treatments of slaughtering, drying, and defatting, as well as their

**Table 1**
Fatty acid profile of the fat (%) from BSFL processed by different methods of slaughtering, drying, and defatting*.

| | Freezing | | | | Blanching | | | | *p* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Freeze-drying | | Oven-drying | | Freeze-drying | | Oven-drying | | | | | | | |
| | Pressing | SFE | Pressing | SFE | Pressing | SFE | Pressing | SFE | [S] | [D] | [Df] | [SxD] | [SxDf] | [DxDf] | [SxDxDf] |
| **C10:0** | 0.8 ± 0.20$^{abc}$ | 1.1 ± 0.03$^{ab}$ | 0.7 ± 0.04$^{bc}$ | 0.9 ± 0.02$^{abc}$ | 0.9 ± 0.09$^{abc}$ | 0.8 ± 0.04$^{abc}$ | 0.7 ± 0.17$^{c}$ | 1.2 ± 0.01$^{a}$ | 0.670 | 0.404 | 0.006 | 0.064 | 0.570 | 0.026 | 0.005 |
| **C12:0** | 49.0 ± 1.01$^{b}$ | 50.7 ± 0.48$^{ab}$ | 48.8 ± 0.24$^{b}$ | 49.9 ± 0.56$^{b}$ | 54.6 ± 2.37$^{a}$ | 49.3 ± 0.65$^{b}$ | 48.9 ± 1.70$^{b}$ | 51.4 ± 0.04$^{ab}$ | 0.038 | 0.081 | 0.975 | 0.302 | 0.041 | 0.013 | 0.006 |
| **C14:0** | 10.3 ± 0.29$^{ab}$ | 10.1 ± 0.00$^{ab}$ | 10.3 ± 0.07$^{ab}$ | 10.1 ± 0.11$^{ab}$ | 10.9 ± 0.42$^{a}$ | 10.6 ± 0.25$^{ab}$ | 10.4 ± 0.19$^{ab}$ | 9.9 ± 0.02$^{b}$ | 0.034 | 0.033 | 0.031 | 0.020 | 0.296 | 0.808 | 0.632 |
| **C14:1** | 0.3 ± 0.02 | 0.4 ± 0.01 | 0.4 ± 0.04 | 0.4 ± 0.01 | 0.3 ± 0.03 | 0.4 ± 0.02 | 0.4 ± 0.02 | 0.4 ± 0.00 | 0.380 | 0.088 | 0.044 | 0.337 | 0.953 | 0.726 | 0.685 |
| **C16:0** | 13.4 ± 0.48$^{b}$ | 12.9 ± 0.12$^{b}$ | 13.7 ± 0.07$^{ab}$ | 13.3 ± 0.19$^{b}$ | 11.4 ± 0.21$^{c}$ | 14.6 ± 0.22$^{a}$ | 13.7 ± 0.42$^{ab}$ | 12.7 ± 0.07$^{b}$ | 0.116 | 0.066 | 0.040 | 0.605 | <0.001 | <0.001 | <0.001 |
| **C16:1** | 2.4 ± 0.07$^{a}$ | 2.4 ± 0.03$^{ab}$ | 2.4 ± 0.02$^{a}$ | 2.5 ± 0.04$^{a}$ | 2.0 ± 0.23$^{b}$ | 2.3 ± 0.05$^{ab}$ | 2.5 ± 0.06$^{a}$ | 2.3 ± 0.04$^{ab}$ | 0.010 | 0.027 | 0.431 | 0.088 | 0.377 | 0.124 | 0.031 |
| **C17:0** | 0.5 ± 0.02$^{a}$ | 0.4 ± 0.02$^{ab}$ | 0.4 ± 0.01$^{ab}$ | 0.4 ± 0.02$^{ab}$ | 0.3 ± 0.06$^{b}$ | 0.4 ± 0.01$^{a}$ | 0.4 ± 0.01$^{ab}$ | 0.4 ± 0.01$^{ab}$ | 0.413 | 0.278 | 0.738 | 0.155 | 0.009 | 0.313 | 0.013 |
| **C17:1** | 0.3 ± 0.03 | 0.2 ± 0.02 | 0.3 ± 0.01 | 0.3 ± 0.01 | 0.2 ± 0.05 | 0.3 ± 0.01 | 0.3 ± 0.01 | 0.3 ± 0.14 | 0.659 | 0.526 | 0.581 | 0.410 | 0.188 | 0.360 | 0.951 |
| **C18:0** | 2.5 ± 0.04$^{bc}$ | 2.2 ± 0.06$^{d}$ | 2.5 ± 0.04$^{b}$ | 2.4 ± 0.08$^{bcd}$ | 1.9 ± 0.03$^{e}$ | 2.8 ± 0.09$^{a}$ | 2.5 ± 0.08$^{bc}$ | 2.3 ± 0.01$^{cd}$ | 0.366 | 0.027 | 0.057 | 0.210 | <0.001 | <0.001 | <0.001 |
| **C18:1** | 9.1 ± 0.28 | 8.5 ± 0.05 | 9.1 ± 0.05 | 8.9 ± 0.17 | 7.6 ± 1.13 | 8.9 ± 0.35 | 8.9 ± 0.43 | 8.2 ± 0.06 | 0.067 | 0.427 | 0.822 | 0.764 | 0.158 | 0.143 | 0.035 |
| **C18:2** | 7.6 ± 0.13 | 7.4 ± 0.12 | 7.6 ± 0.11 | 7.5 ± 0.14 | 6.4 ± 1.05 | 6.3 ± 0.54 | 7.7 ± 0.41 | 7.2 ± 0.04 | 0.018 | 0.029 | 0.372 | 0.046 | 0.758 | 0.693 | 0.701 |
| **C18:3n-6** | 0.2 ± 0.04 | 0.1 ± 0.01 | 0.2 ± 0.02 | 0.1 ± 0.01 | 0.1 ± 0.04 | 0.2 ± 0.01 | 0.2 ± 0.05 | 0.1 ± 0.03 | 0.455 | 0.864 | 0.132 | 0.453 | 0.269 | 0.685 | 0.623 |
| **C18:3n-3** | 3.4 ± 0.05 | 3.4 ± 0.08 | 3.4 ± 0.10 | 3.2 ± 0.05 | 2.9 ± 0.48 | 3.0 ± 0.21 | 3.4 ± 0.14 | 3.3 ± 0.01 | 0.096 | 0.141 | 0.485 | 0.068 | 0.754 | 0.355 | 0.750 |
| **C20:1** | 0.2 ± 0.02$^{abc}$ | 0.2 ± 0.01$^{bc}$ | 0.2 ± 0.03$^{ab}$ | 0.1 ± 0.03$^{c}$ | 0.2 ± 0.03$^{abc}$ | 0.3 ± 0.00$^{a}$ | 0.2 ± 0.04$^{abc}$ | 0.2 ± 0.02$^{bc}$ | 0.050 | 0.154 | 0.085 | 0.362 | 0.011 | 0.003 | 0.145 |
| **SFA** | 76.4 ± 0.42 | 77.4 ± 0.31 | 76.4 ± 0.37 | 77.0 ± 0.36 | 80.1 ± 3.00 | 78.4 ± 1.15 | 76.6 ± 1.16 | 78.0 ± 0.12 | 0.047 | 0.115 | 0.629 | 0.168 | 0.478 | 0.302 | 0.203 |
| **MUFA** | 12.4 ± 0.28 | 11.7 ± 0.09 | 12.3 ± 0.15 | 12.1 ± 0.19 | 10.4 ± 1.42 | 12.1 ± 0.40 | 12.2 ± 0.57 | 11.4 ± 0.13 | 0.067 | 0.278 | 0.998 | 0.545 | 0.133 | 0.130 | 0.035 |
| **PUFA** | 11.1 ± 0.14 | 10.9 ± 0.22 | 11.2 ± 0.23 | 10.9 ± 0.18 | 9.4 ± 1.58 | 9.4 ± 0.76 | 11.3 ± 0.59 | 10.6 ± 0.01 | 0.036 | 0.053 | 0.379 | 0.054 | 0.950 | 0.573 | 0.707 |
| **n6/n3** | 2.29 ± 0.01$^{ab}$ | 2.26 ± 0.01$^{b}$ | 2.29 ± 0.03$^{ab}$ | 2.36 ± 0.01$^{a}$ | 2.23 ± 0.01$^{b}$ | 2.12 ± 0.03$^{c}$ | 2.29 ± 0.04$^{ab}$ | 2.26 ± 0.01$^{b}$ | <0.001 | <0.001 | 0.046 | 0.046 | 0.002 | 0.003 | 0.719 |
| **PUFA/SFA** | 0.15 ± 0.00 | 0.14 ± 0.00 | 0.15 ± 0.00 | 0.14 ± 0.00 | 0.12 ± 0.02 | 0.12 ± 0.01 | 0.15 ± 0.01 | 0.14 ± 0.00 | 0.035 | 0.058 | 0.373 | 0.064 | 0.949 | 0.508 | 0.572 |
| **AI** | 4.39 ± 0.05 | 4.59 ± 0.08 | 4.40 ± 0.10 | 4.52 ± 0.11 | 5.60 ± 1.06 | 4.94 ± 0.35 | 4.45 ± 0.24 | 4.71 ± 0.04 | 0.059 | 0.116 | 0.924 | 0.147 | 0.398 | 0.333 | 0.255 |
| **TI** | 2.07 ± 0.05 | 2.09 ± 0.02 | 2.10 ± 0.05 | 2.12 ± 0.02 | 2.29 ± 0.40 | 2.41 ± 0.17 | 2.11 ± 0.06 | 2.11 ± 0.03 | 0.122 | 0.238 | 0.647 | 0.121 | 0.809 | 0.680 | 0.714 |

*Different letters between the treatments within a row mean significant differences.

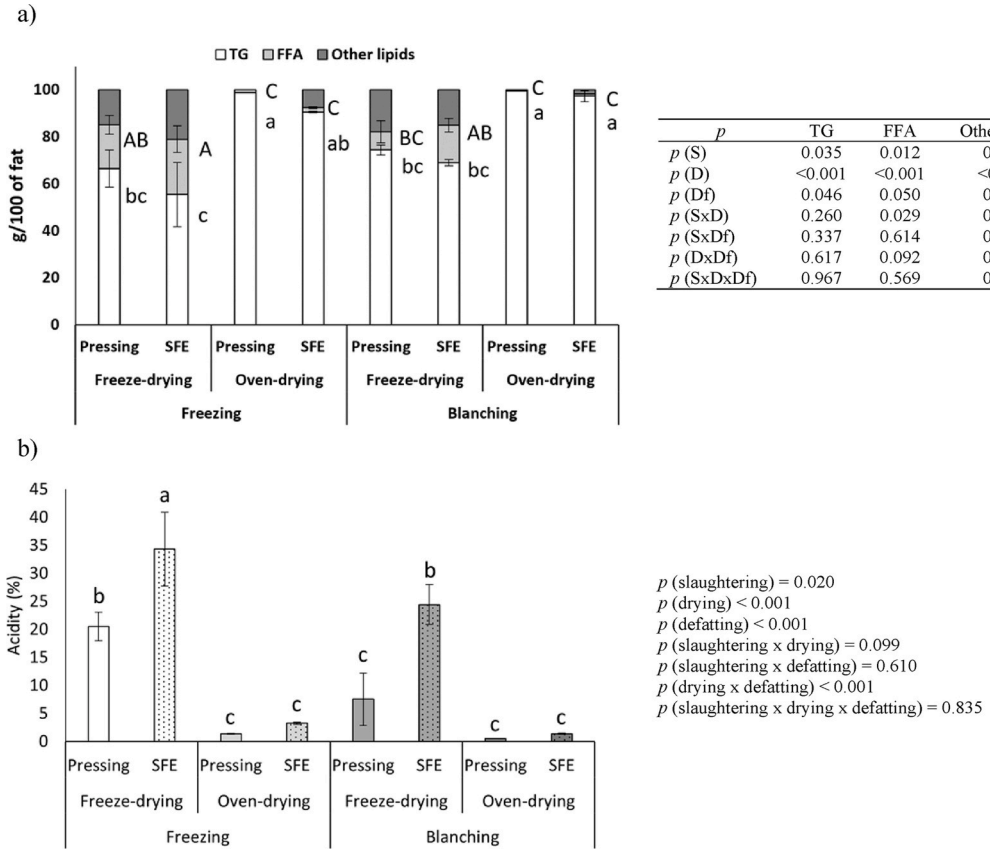
respective binary interactions, were observed for the n6/n3 ratio. Although a lack of triple interaction effect of the three factors was obtained. As remarkable result, a significant reduction in the n6/n3 value was observed for blanching, followed by freeze-drying and SFE. Nevertheless, despite these differences, they should be considered negligible, as all n6/n3 values were within the same range, close to 2, which is considered, in general, a high value for all samples.

Therefore, the different modes employed for slaughtering, drying and defatting of BSFL did not practically impact on the fatty acid profile and the nutritional quality of the fat obtained as a co-product during the production of BSFL meals.

### 3.3. Effect of the larvae processing on the lipid species profile

The different modes of slaughtering and drying of BSFL affected the major lipid species of the obtained fats, as shown in Fig. 3 a. Those major species as TG and FFA are presented, while the rest of lipids are shown as "other lipids", which correspond to minor components like partial glycerides or phospholipids. As shown in Fig. 3 a, the most relevant result was the presence of a remarkable amount of FFA and a subsequent lower TG content after some specific treatments and their combinations. Thus, in general, slaughtering by freezing and drying by freeze-drying caused the greatest modification of the lipid species profile throughout an increase in FFA. Additionally, a significant interaction effect was observed between the slaughtering and drying factors concerning the FFA content. Therefore, the combination of slaughtering by freezing and drying by freeze-drying led to the most remarkable modification of the lipid profile (mean value of 21% of FFAs and 61% of TGs). On the contrary, regardless of the method of slaughtering, larvae that were dried by oven-drying showed a lipid species profile more consistent with what is expected for insects, that is, a minor content of FFA (<1%) and a major content of TG (≥90%) (Horne et al., 2009). Therefore, considering this lipid composition of the oven-dried samples as reference, those samples that presented higher FFA and lower TG content would suggest that a TG lipolysis phenomena took place for such samples. This effect could be explained by the existence of active lipolytic enzymes in these samples. In fact, there is previous evidence in the scientific literature that has shown the relevance of lipase activities in edible insects, being especially intense in the case BSFL (Caligiani et al., 2019; Leong et al., 2022; Ravi et al., 2020). During the slow slaughter of BSFL by freezing, Leni et al. (2019) identified different metabolites that indicated that energetic metabolisms are stimulated, such as the hydrolysis of glucose, production of lactic acid, consumption of citric acid, and lipolysis of TGs. In contrast, these authors detected lower related metabolites due to the brief dying and deactivation of enzymes by blanching. Thus, it is expected that any processing treatment of BSFL that involves the application of high temperatures, such as slaughtering by blanching, and drying by oven drying, would limit such lipolytic activity, as shown in Fig. 3 a. This same reasoning would explain why the degree of lipolysis observed in the case of slaughtering by blanching followed by freeze-drying was slightly lower, compared to the same drying derived from slaughtering by freezing (Fig. 3). In this case, the short heat treatment of blanching (40 s at 90 °C) may have partially deactivated the lipase activity, causing slightly less lipolysis compared to slaughtering by freezing.

Different examples can be found about the strong lipolysis process in BSFL when non-thermal methods of slaughtering are employed. Thus, Leong et al. (2022) and Ravi et al. (2020) already suggested the necessary thermal slaughtering of BSFL to avoid an evident lipolysis, since even more restrictive freezing temperatures of −80 °C were insufficient




| p | TG | FFA | Other lipids |
|---|---|---|---|
| p (S) | 0.035 | 0.012 | 0.312 |
| p (D) | <0.001 | <0.001 | <0.001 |
| p (Df) | 0.046 | 0.050 | 0.166 |
| p (SxD) | 0.260 | 0.029 | 0.734 |
| p (SxDf) | 0.337 | 0.614 | 0.105 |
| p (DxDf) | 0.617 | 0.092 | 0.480 |
| p (SxDxDf) | 0.967 | 0.569 | 0.703 |

**Fig. 3.** Lipid species (a) and acidity (b) of the fat from BSFL processed by different methods of slaughtering (S), drying (D), and defatting (Df). Lowercase letters in (a) indicate significant differences in TG between treatments and uppercase letters indicate significant differences in FFA between treatments. Different letters in (b) between the treatments mean significant differences.

to inactivate the lipolytic enzymes. Furthermore, Caligiani et al. (2019) demonstrated that the BSFL lipase remains active, and lipolysis continues, during the freezing time of storage, although the greatest activity seems to be found immediately after slaughtering. Alternatively, Montevecchi et al. (2020) proposed direct grinding as a better slaughtering method for preserving fat integrity, in terms of lower amount of FFA compared to freezing or blanching.

Therefore, most of the studies have demonstrated a clear lipolysis process of BSFL fat due to the mode of slaughtering, in agreement with our findings. Additionally, the noteworthy novel contribution of the present study is that the drying method, specifically freeze-drying, can also affect such lipolysis reaction, both individually, but also in relation to the slaughtering mode. This advancement is of considerable importance as the two stages of slaughter and drying are typically performed sequentially during the production of BSFL meals and cannot be viewed as independent of each other.

Finally, concerning the last step of defatting in BSFL meal production, the present study likewise evidenced that this step also slightly contributed to the final lipolysis of the obtained fat, since the amount of TG was significantly lower due to SFE, together with a higher content on FFA, regardless of the mode of slaughtering and drying (Fig. 3a). Nevertheless, this effect of defatting was not as pronounced as that evidenced for slaughtering and drying. This influence of the SFE process may be explained by a higher lipolysis caused by the residual moisture content in the starting material together with a long residence time at 40 °C within the extraction cell. Nevertheless, reducing the time of SFE defatting may be possible by increasing the CO2 flow/larvae ratio used, as we already demonstrated (Cantero-Bahillo et al., 2022), achieving around 85% fat recovery in just 30 min under the same used pressure and temperature conditions (450 bar and 60 °C) but with a CO2 flow/larvae ratio in the range of 0.6–1.0 $min^{-1}$. Another potential factor reinforcing the obtained result might be the increased solubility of FFA in supercritical $CO_2$ compared to TG (Vázquez et al., 2009).

As complementary information, the acidity of the samples was also determined (Fig. 3b), which agreed with the different lipolysis evidenced by the FFA content of the samples as affected by the treatments.

Concerning the impact of the FFA content and acidity of the fats on the other quality parameters, it should be noted that, in general, higher FFA contents used to be related to poor oxidative stability, since these species are more prone to oxidation than esterified fatty acids. In this sense, we recently performed a detailed study about the oxidative quality of the same fat samples obtained in the present study as affected by slaughtering, drying and defatting methods (Hurtado-Ribeira et al., 2023). We effectively demonstrated a significant negative correlation between the oxidative stability measured by the accelerated Rancimat method and the acidity of the samples. However, such relation was non-linear, suggesting that the acidity of the samples would only partially explain the differences in oxidative stability between samples. In fact, when the oxidative stability of the fat samples was measured under normal conditions of storage, the acidity of the samples did not determine the stability of the samples (Hurtado-Ribeira et al., 2023). Indeed, the samples obtained through freeze-drying, which had the highest acidity (Fig. 3), were the most stable during the storage experiment, but only when combined with mechanical pressing and not when combined with SFE (Hurtado-Ribeira et al., 2023). Therefore, in such previous study, we concluded that the acidity of the fat samples from BSFL may have a role on lipid oxidation under accelerated oxidation conditions, whereas the initial acidity would not have a major impact on the oxidation of BSFL fat under storage conditions. In this last case, the presence of antioxidant compounds in the fat samples depending on the processing methods was evidenced as one of the most plausible hypotheses.

As summary, the optimal method of processing of BSFL for preserving the integrity of the fat is the combination of slaughtering by blanching, drying by oven-drying, and mechanical pressing being preferred for defatting.

### 3.4. Effect of the larvae processing on other minor lipid compounds

Two minor compounds, as phytosterols and squalene, were identified in BSFL fat (Table 2). Concerning squalene, we have previously described the presence of this minor compound in lipids from BSFL (Cantero-Bahillo et al., 2022; Fornari et al., 2023). Nevertheless, the estimated content of squalene of the BSFL fats, in the range of 0.02%, was not remarkable compared to other edible oils that are typical sources of this compound, such as vegetable oils (Beltrán et al., 2016). On the contrary, the content of phytosterols of BSFL is noteworthy, with values in the range of 0.2–0.4%. This fraction mainly consisted of sitosterol (mean value of 0.2%), followed by campesterol (mean value of 0.07%) and trace amounts of stigmasterol (mean value of 0.03%). Phytosterols are not sterols commonly found in animal fats, including insects. However, BSFL have the ability to accumulate phytosterols (Boukid et al., 2021; Fornari et al., 2023; Matthäus et al., 2019), typically showing a profile as the detected in the current study, namely sitosterol > campesterol > stigmasterol. As shown in Tables 2 and in agreement with other studies (Boukid et al., 2021; Fornari et al., 2023; Matthäus et al., 2019), the phytosterol content of BSFL fat can reach values comparable to or even superior than other vegetable oils, such as palm oil (0.08%), olive oil (0.2%), soybean oil (0.3%) or sunflower oil (0.3–0.4%) (Verleyen et al., 2002).

Concerning the impact of the treatments of BSFL, it is remarkable that none of the methods of slaughtering, drying, or defatting resulted in significant differences in the content of these minor compounds. Therefore, it can be concluded that, in general, the minor compounds of BSFL fat, such as phytosterols and squalene, are preserved at their typical levels, regardless of the different modes of slaughtering, drying, and defatting of BSFL.

### 3.5. Effect of the larvae processing on the moisture content of fat

As already explained in Section 3.1, the initial larvae contained different moisture content depending on the mode they were processed, especially due to drying. Therefore, we considered of interest to explore whether the resulting BSFL fats might have a variable moisture content depending on the processing of larvae. Although low levels of moisture were expected in the fats, this analysis is relevant as it is a quality indicator of the fats, with lower moisture content in the fat samples being the preferred.

As represented in Fig. 4, the different processing of drying and defatting, as well as their interaction, caused variable moisture content in the obtained fats, whereas the slaughtering mode did not determine this parameter. Thus, concerning drying, the freeze-drying method resulted in fats with higher moisture content than oven-drying (mean value of 0.8% and 0.2%, respectively). Therefore, this would confirm that larvae which had higher initial moisture content due to the drying method lead to derived fats with higher moisture content.

Concerning the mode of defatting, the SFE method yielded fats with significantly higher levels of moisture content compared to mechanical pressing (mean value of 0.7% and 0.3%, respectively). This result may be explained by the fact that during the SFE process, supercritical $CO_2$ may drag certain levels of water from the sample, co-extracting it with the fat. We recently evidenced this phenomenon in a specific study about the impact of the initial moisture content of BSFL on the subsequent moisture content of the derived fat when extracted by SFE (Fornari et al., 2023).

Therefore, considering the interaction effect of the drying and defatting methods, the recommended processing for BSFL to minimizing moisture content in the derived fats would be drying by oven-drying, and defatting by mechanical pressing, regardless of the slaughtering method. However, in case of using other processing methods, further studies would be needed to reduce the initial moisture content in the larvae, and consequently, in the fats. In our previous study, we concluded that to obtain BSFL fat with proper moisture quality using

**Table 2**
Minor lipid compounds of the fat (%) from BSFL processed by different methods of slaughtering, drying, and defatting.

| Compounds | Freezing | | | | Blanching | | | |
|---|---|---|---|---|---|---|---|---|
| | Freeze drying | | Oven drying | | Freeze drying | | Oven drying | |
| | Pressing | SFE | Pressing | SFE | Pressing | SFE | Pressing | SFE |
| Squalene | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.02 ± 0.00 | 0.01 ± 0.00 |
| Phytosterols | 0.27 ± 0.01 | 0.31 ± 0.04 | 0.32 ± 0.03 | 0.22 ± 0.10 | 0.23 ± 0.04 | 0.39 ± 0.13 | 0.25 ± 0.01 | 0.21 ± 0.02 |
| *Campesterol* | 0.07 ± 0.00 | 0.09 ± 0.02 | 0.09 ± 0.01 | 0.06 ± 0.02 | 0.06 ± 0.01 | 0.10 ± 0.03 | 0.07 ± 0.00 | 0.06 ± 0.01 |
| *Stigmasterol* | 0.03 ± 0.01 | – | 0.03 ± 0.01 | 0.03 ± 0.03 | 0.02 ± 0.01 | 0.05 ± 0.03 | 0.02 ± 0.00 | 0.02 ± 0.01 |
| *Sitosterol* | 0.17 ± 0.02 | 0.20 ± 0.02 | 0.21 ± 0.01 | 0.14 ± 0.04 | 0.14 ± 0.03 | 0.23 ± 0.06 | 0.15 ± 0.01 | 0.13 ± 0.01 |

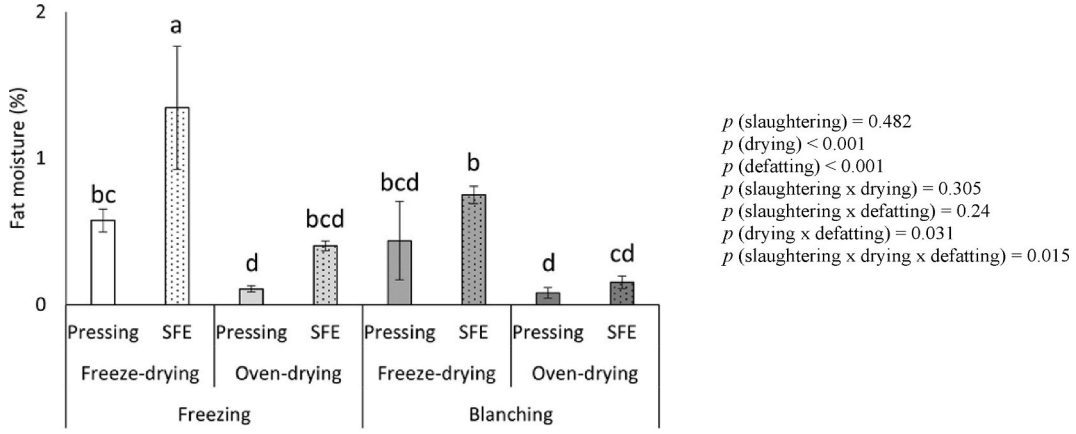



**Fig. 4.** Moisture content of the fat (g/100 g of fat) from BSFL processed by different methods of slaughtering, drying, and defatting. Different letters between the treatments mean significant differences.

SFE, an efficient drying of the initial larvae to reach values ≤ 5% seemed necessary (Fornari et al., 2023).

Currently, there is a lack of specific recommendations or standards for moisture content of oils from edible insects. Nevertheless, the Codex Alimentarius standard for "edible fats and oils not covered by individual standards" recommends a matter volatile at 105 °C of 0.2% (Codex Alimentarius, 2021). Taking this value as reference, most samples from oven-drying showed proper quality in terms of moisture content, such as those from freezing plus oven-drying and mechanical pressing (0.11%), blanching plus oven-drying and mechanical pressing (0.08%), and blanching plus oven-drying and SFE (0.15%) (Fig. 4).

### 3.6. Effect of the larvae processing on the thermal properties of fat

The modification of the lipid species profile produced in BSFL fat due to the processing, as well as the moisture content, may directly impact the thermal properties of the fat, such as the melting and crystallization temperatures. These properties are technologically relevant as they determine the handling of the fat in the different possible applications at different temperatures. Additionally, the thermal behavior of fats affects other aspects such as palatability or digestibility. Therefore, the melting and crystallization profiles of the different fat samples were determined. Fig. 5 shows the thermograms showing the zones of endothermic changes, which correspond to phase changes due to melting, as well as the zones of exothermic changes, which correspond to phase changes to crystallization.

Most of the samples started to crystalize at around 8 °C, with the major exothermic peak varied in the range of 3.0–7.0 °C, depending on the sample. No significant effect due to the slaughtering, drying and defatting methods, as independent variability factors, was found on such exothermic peak. However, an interaction effect of the three factors was obtained ($p = 0.010$). Thus, the sample with the highest crystallization temperature peak resulted from the combination of blanching, freeze-drying and mechanical pressing (6.96 °C). In contrast, the sample with the lowest crystallization temperature was that derived from the combination of blanching, freeze-drying and SFE (2.99 °C). In order to find a relationship between these results and the composition of the samples, a Pearson's correlation test was performed between crystallization temperatures and factors such as fatty acid profile, lipid species as well as moisture content. A positive significant correlation was found between the exothermic peak and the total lauric acid content ($r = 0.522$, $p = 0.038$). Thus, this would suggest that the crystallization behavior of the BSFL fat samples would be partially conditioned by the lauric acid content, which was significantly higher for those samples processed by blanching, freeze-drying and mechanical pressing (Table 1). In contrast, the content of lipid species and moisture in the fats did not appear to be related to the crystallization temperatures of the samples.

The thermal behavior of BSFL fat has received limited attention and only a few examples can be found. Matthäus et al. (2019) described a major crystallization peak at 8.98 °C, which was mainly attributed to the major TG of this fat as trilaurin. These authors used a particular way of simultaneous killing and drying by vacuum dryer, followed by fluidized bed dryer, and mechanically pressed for defatting. Additionally, these authors also found a second major crystallization peak at 3.57 °C, which was related to the minor content in other forms of esterified TG different to lauric acid, mainly unsaturated fatty acids. In the current study, we did not find two clear crystallization peaks, but we effectively observed some samples with a major crystallization peak displaced to the range of 6–7 °C, while other samples had this peak in the range of 3–4 °C (Fig. 5). This may be determined by the specific types of TG present, in agreement with the explanation proposed by Matthäus et al. (2019).

Concerning the melting profile, most of the samples started to melt at around 5 °C, but relevant significant differences ($p < 0.05$) were observed in the thermograms depending on slaughtering, drying, and defatting methods, as well as their interactions. Respect to slaughtering, in general, those samples from freezing showed a lower melting temperature; while in case of drying, those from freeze-drying, in general, melted at a lower temperature. The significant interaction of both

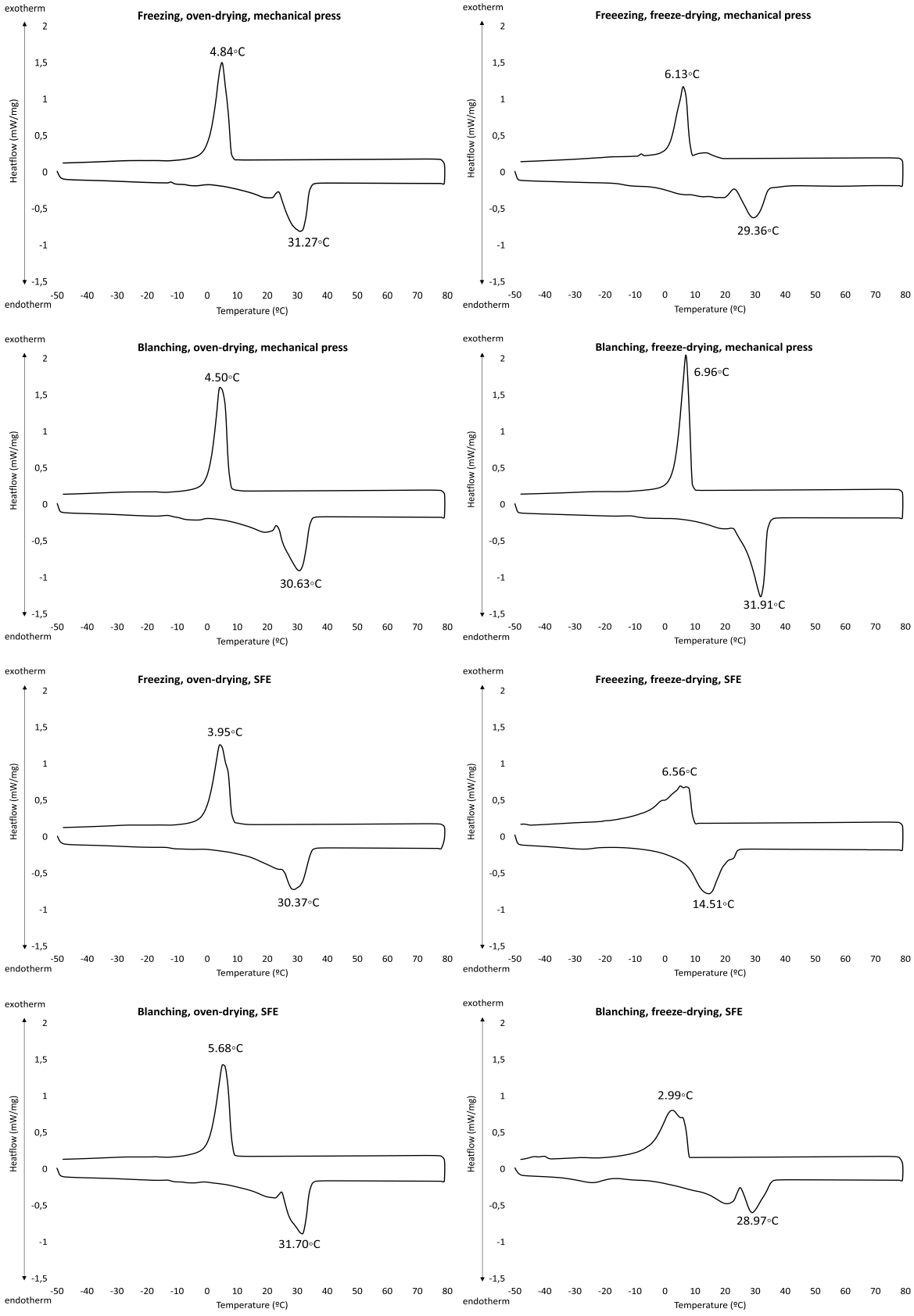



**Fig. 5.** Thermograms of crystallization and melting of the fat from BSFL processed by different methods of slaughtering, drying, and defatting.

factors caused that those samples slaughtered by freezing and dried by freeze-drying melted at a lower temperature than those from blanching plus oven-drying. Concerning the effect of defatting method, those samples from mechanical pressing melted at higher temperature compared to those from SFE, in general. Due to all these results, the triple interaction between all the three factors caused a remarkable effect on the melting behavior ($p < 0.05$). Consequently, the samples from slaughtering by freezing, drying by freeze-drying, and defatting by SFE clearly showed a different thermal melting thermogram, with a melting peak at 14.51 °C, while the rest of the samples melted in the range of 30 °C. In case of melting, Pearson's test did not show a correlation with the fatty acid profile of the samples, unlike what was observed for crystallization peaks. In contrast, moisture content was found to be strongly related to the melting peaks ($r = -0.825$, $p < 0.001$). Thus, the higher the moisture content, the lower the melting temperature. Specifically, the sample with the lowest melting peak had a moisture content higher than 1% (Figs. 4 and 5). Additionally, a significant correlation was also found with the lipid species content. Thus, the higher the FFA content and the lower the TG content, the lower the melting temperature ($r = -0.706$, $p = 0.002$ and $r = 0.619$, $p = 0.011$, respectively). This result agreed with previous findings that have reported slightly lower melting ranges with an increase in FFA content, as observed for palm oil (Jacobsberg and Ho., 1976). However, the similar high melting point that the pure lipids lauric acid and trilaurin have (40.57 °C and 46.29 °C, respectively, according to Knothe and Dunn (2009)), would not explain the extreme low melting point obtained for the sample at 14.51 °C. Therefore, whether this result was due to a combined effect of the moisture content, the lipid profile, or other components of this sample, would need further studies to understand the so different thermal behavior after processing by freezing, plus freeze-drying and SFE. In any case, despite the differences observed, most of the samples showed a major melting peak similar to those recently described for BSFL fat by Matthäus et al. (2019) at 27.23 °C, or by Lawal et al. (2022) at 31.33 °C (Lawal et al., 2022; Matthäus et al., 2019).

To complete the study, the solid fat content (SFC) as a function of temperature was calculated from the partial integration of the thermograms. As shown in Fig. 6a, differences were observed between the samples, especially in the range of 10–30 °C, reflecting the different thermal behavior of the sample from freezing, plus freeze-drying and SFE already shown in Fig. 5. Thus, the most remarkable result was the near-fluid state of this sample at room temperature conditions

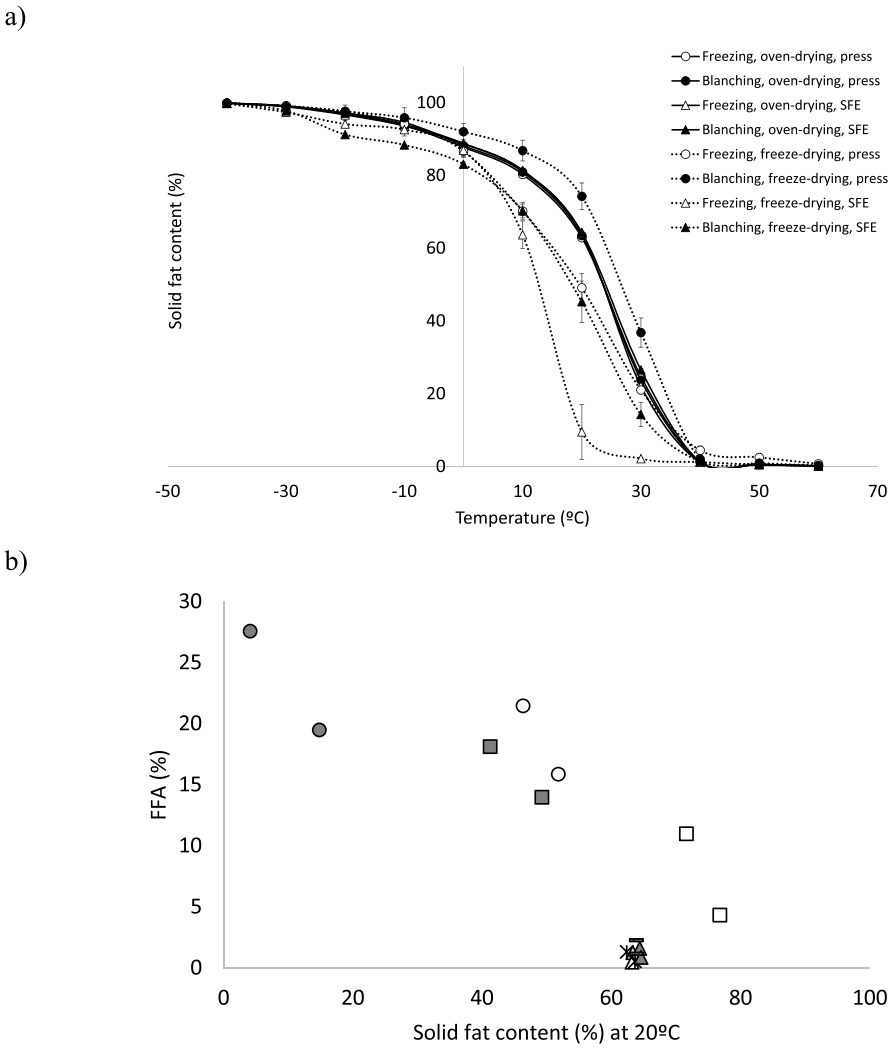



**Fig. 6.** Solid fat content (a) and its relationship with the FFA content (b) of the fat from BSFL processed by different methods of slaughtering, drying, and defatting. In case of figure (b): empty circles (freezing, freeze-drying, mechanical pressing), full circles (freezing, freeze-drying, SFE), empty triangles (freezing, oven-drying, mechanical pressing), empty squares (blanching, freeze-drying, mechanical pressing), full squares (blanching, freeze-drying, SFE), full triangles (freezing, oven-drying, SFE), asterisks (blanching, oven-drying, mechanical pressing), line (blanching, oven-drying, SFE).

(SFC<10% at 20 °C) compared to the predominantly solid state of the other samples (SFC>40% at 20 °C). Considering the value of SFC at 20 °C for all the samples, and similar to the observed for the melting points, strong significant correlations were found with the FFA ($r = -0.826, p < 0.001$) and moisture ($r = -0.810, p < 0.001$) contents of the samples. In fact, the interesting effect of the FFA content on the SFC at 20 °C of BSFL is illustrated in Fig. 6b. It can be clearly observed that the different lipolysis degree of the freeze-dried samples to yield variable FFA content, determined such linear relationship with the melting behavior, while those samples from oven-drying, and negligible FFA content, were almost irrelevant on such correlation. The technological implication of this result is indisputable, since the physical state of the fats obtained, depending on the mode of BSFL processing, would condition their technological handling in the different industrial processes, either for edible or non-edible applications. Additionally, organoleptic and digestibility might be also conditioned by these different thermal behaviors. Therefore, further studies are needed to thoroughly understand the specific components that explain the different behavior of the fats, in general those from freeze-drying, and specifically, those from freezing, freeze-drying and SFE.

## 4. Conclusions

In general, the different modes of slaughtering, drying, and defatting, as well as their interactions, affects the process yields, lipid species, moisture content and thermal properties of the derived fat of BSFL, whereas they do not affect the overall fatty acid profile in SFA, MUFA and PUFA and the content of minor lipid compounds.

Specifically, the combination of blanching, freeze-drying and mechanical pressing is the least favorable option from the process yields point of view. In contrast, the rest of processes are better and similar, although SFE is preferable for defatting. For preserving the integrity of the fat, the combination of thermal methods as blanching and oven-drying, is preferred, since non-thermal methods of slaughtering and drying causes intense lipolysis. The thermal drying by oven-drying, especially combined with mechanical pressing, is also desired for lower moisture content in the fat. Finally, the melting temperature is much lower for samples with higher lipolysis and moisture, as that derived from freezing, freeze-drying and SFE.

Therefore, this study provides valuable insights into selecting the most interesting combinations of slaughtering, drying, and defatting methods of BSFL to selectively produce fat as co-product, taking into consideration factors such as process efficiency, quality, or technological properties of the fat. Thus, depending on the priorities or purposes among those parameters, this study shows that different options of processing of BSFL can be adopted.

## CRediT authorship contribution statement

**Raúl Hurtado-Ribeira:** Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **David Villanueva-Bermejo:** Methodology, Investigation. **Mónica R. García-Risco:** Methodology, Investigation. **M. Dolores Hernández:** Conceptualization, Funding acquisition adquisition. **María José Sánchez-Muros:** Conceptualization. **Tiziana Fornari:** Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing. **Luis Vázquez:** Methodology, Investigation, Formal analysis, Writing – review & editing, Visualization, Supervision. **Diana Martin:** Conceptualization, Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

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