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ORIGINAL PAPER

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EFFECT OF DRYING REGIMES ON THE QUALITY AND SAFETY OF ALTERNATIVE PROTEIN SOURCES – YELLOW MEALWORM LARVAE (*TENEBRIO MOLITOR* L.)

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ABSTRACT

Background. The growing population and the increase in protein hunger have highlighted a need to search for an alternative protein source. The European Food Safety Agency (EFSA) stated that yellow mealworm larvae (*Tenebrio molitor* L.) are a potential source of protein, fat, and fibre. Their processing involves different thermal treatments to ensure the quality and safety of the insect products. Therefore, the aim of this study was to explore the effect of four processing regimes.

Materials and methods. The applied regimes were: F.M. (fast frozen and microwave dried), S.M. (slow frozen and microwave dried), F.C. (fast frozen and conventionally dried), and S.C. (slow frozen and conventionally dried). The quality and safety of the flours from *Tenebrio molitor* L. were evaluated by the changes in proximate composition, technological properties, oxidative stability, fatty acids' composition, and microbial status.

Results. The applied regimes had a slight effect on the proximate composition. Moisture and water activity (a_w) were significantly affected by the applied regimes. The Saturated to Unsaturated fatty acids ratio was influenced by the type of drying. The microwave drying initiated hydrolytic and oxidative changes, but the limits for primary and secondary oxidation products were not exceeded. Higher survival rate of microorganisms was evaluated after fast freezing but low a_w should guarantee microbial safety.

Conclusion. Both of the freezing and drying regimes tested slowly influence the proximate composition of mealworm flours. Summarizing the changes in lipid fraction of microwave dried flours it can be concluded that the induction period has passed and ongoing oxidation has been evaluated. Despite the positives of microwave drying as a faster and more energy efficient processing method, the decreased oxidative stability of the obtained flour could be a drawback. The results confirm the theory about the higher survival rate of microorganisms after fast freezing. At the same time, microbial safety could be ensured by a low moisture content and a_w.

Keyword: edible insect, microwave drying, oxidation, insect flours

INTRODUCTION

Protein deficiency has increased manifold in recent decades (Janssen et al., 2017). As the population grows and surpasses 8 billion, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) warn of looming future shortages of food resources (Hong et al., 2020). According to the UN, the global livestock industry generates over 14.5% of global greenhouse gas emissions. By comparison,

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rearing insects is more efficient as a source of protein than livestock. In addition, organic by-products can be utilized for their rearing (Hong et al., 2020).

Mealworms are the first insects approved for human consumption by the European Food Safety Agency (EFSA) (Turck et al., 2021). The name "mealworm" refers to the larva of *Tenebrio molitor (Tenebrio molitor* L.), belonging to the Family *Tenebrionidae* (Darkling beetle). According to EFSA, the larvae of the yellow mealworm (*Tenebrio molitor* L.) are extremely rich in protein (47–60%), fat (23–35%), and fibre (5–10%), and that they can be used raw, dried, and ground in the form of flour (Hong et al., 2020; Lenaerts et al., 2018; Turck et al., 2021). The larvae are an excellent source of minerals, containing high amounts of potassium, calcium, iron, and magnesium. They are also a valuable source of riboflavin (B_2), pantothenic acid (B_5), and biotin (B_7), as well as vitamin B_{12} (Lenaerts et al., 2018). From behavioural studies, it is clear that a large proportion of consumers are reluctant to consume whole insects (Niva and Vainio, 2021). A solution was proposed by Melgar-Lalanne et al. (2019), consisting in the incorporation of insects in the form of "flour" in various everyday food products.

From growing the mealworm (*Tenebrio molitor* L.) to obtaining flour from it, the production goes through certain stages all associated with huge costs. This is the reason for developing a new optimized process, either by shortening the processing time or by minimizing the required electricity (Lenaerts et al., 2018; Vandeweyer et al., 2017). The nutritional value of the obtained flours depends on the larvae's development stage (Hernández-Álvarez et al., 2021). Freezing could be used as a procedure to unify the production process and assure constant quality. For this reason, larvae that have reached the optimal development stage (around 60–70 days old) could be frozen (Vandeweyer et al., 2017; Selaledi and Mabelebele, 2021). Intensified freezing gives significantly good results for a variety of food products, as in addition to the formed ice crystals being smaller in size and the microstructure being significantly preserved, it is several times faster than conventional freezing, which in turn reduces costs (James et al., 2015). Later on, frozen larvae are blanched to minimize their microbial load (Selaledi and Mabelebele, 2021). Before being ground into flour, the mealworms are dried. Conventional drying is at around 60°C and takes a considerable amount of time, which is associated with the consumption of large amounts of electricity. On the other hand, microwave drying is quicker and requires much less electricity, which characterizes it as a "green" technology (Lenaerts et al., 2018). But we have to take into consideration that the irradiation of fat-rich foods has a rather negative image, due to the initiation of lipid peroxidation and reduction of the shelf life (Kröncke et al., 2018). Therefore, the aim of the present work is to determine the influence of freezing regimes, namely, intensified or conventional, and also drying regimes, namely, microwave or conventional and their interaction on the quality of flour from *Tenebrio molitor* L. larvae as an alternative protein source.

MATERIALS AND METHODS

The mealworms were reared in plastic containers (60 × 40 × 12 cm, meeting the requirements of Commission Regulation (EU) No 10/2011 of 14 January 2011) at the insect farm, Petrich, Bulgaria ("Vi Bi Ef PRO" LTD). The temperature was maintained at 25 ±2°C with 50–70% of relative humidity. The mealworms were fed up to day 70 with wheat bran. Food with water content (fruits and vegetables) was given 2–3 times a week, and 3 days prior to the experiment mealworms were not fed. For the purposes of the experiment, the mealworms were separated as presented in Table 1. F.M. and F.C. were fast frozen (super chilled at –20°C and flow 3 m/s at pilot station IS51H-OPRO,

Table 1. Processing regimes

	F.M. (fast frozen + microwave dried)	S.M. (slow frozen + microwave dried)	F.C. (fast frozen + conventionally dried)	S.C. (slow frozen + conventionally dried)
Freezing	2 h until –18°C	7 h until –18°C	2 h until –18°C	7 h until –18°C
Drying	7 min at 825 W	7 min at 825 W	6 h at 60°C	6 h at 60°C

UFT-Plovdiv, Bulgaria), and groups S.M. and S.C. were conventionally (slow) frozen (chilled at –18°C without blowing, QUIGG, MD 37072) (1 kg each group).

All four groups were frozen and stored for 72 h until blanching. The larvae were blanched in boiling water for 45 s, drained out, and dried in a microwave (825W, 7 min, Whirlpool, MWD307/WH) or a conventional drying rack (60°C, 6 h, LUMEL) (Table 1). The dried mealworms were ground into homogeneous flour using a Nutribullet blender (model NB-WL046A-02) and stored in sealed plastic bags until the analysis.

Proximate composition

Total lipid extraction was performed using Soxhlet apparatus according to ISO 1444:1996. The nitrogen quantity was determined by the AOAC (1996), and the protein content was calculated by nitrogen-to-protein conversion factor 4.76, purposed by Janssen et al. (2017) to compensate for the presence of non-protein nitrogen – chitin derivate nitrogen. Total ash content was evaluated by incineration (Lenaerst et al., 2018). Carbohydrate content was calculated using the following equation:

$$\text{Cabohydrates} = 100 - \text{Protein} - \text{Fat} - \text{Ash} - \text{Mouistute}$$

Determination of pH value

The pH value was measured using a pH-meter Microsyst MS 2004 (Microsyst, Bulgaria), equipped with pH electrode Sensorex Combination Recorder S450CD (Sensorex, USA) (Young et al., 2012). Two-point calibration with 4.04 and 6.86 pH buffer solutions (Hanna instruments, USA) was carried out.

Water activity (a_w) and moisture content

Water activity was determined using a Novasina AG CH-8853 (Novasina, Switzerland) at 20°C. The moisture content of the flours was calculated after drying at 104–105°C using a KERN MLS 65-3A – moisture analyzer (Kern & Sohn Gmbh, Germany) until it had a constant weight (Vandeweyer et al., 2017).

Water binding capacity (WBC)

The procedure was performed according to Luo et al. (2019). A 0.5 g of mealworm flour is briefly mixed with 10 ml of distilled water in centrifuge tube. Tubes are left for 30 min at room temperature, followed by centrifuging at 3500 rpm for 25 min. The supernatant is removed and the tubes are weighed. Water binding capacity of mealworm flour is calculated using the following equation:

$$\text{WBC} = \frac{\text{hydrated flour after centrifugion, g}}{\text{initial weight of flour, g}} \cdot 100, \%$$

Yield

This is calculated using the following equation:

$$\text{Yield} = \frac{\text{dryed sample weight}}{\text{raw sample weight}} \cdot 100, \%$$

Instrumental colour determination

The Konica Minolta CR-410 colourimeter (Konica Minolta Inc., USA) equipped with standard observer 2° and light source D65 at aperture of 8 mm was used to determine the colour parameters L^*, a^*, and b^* (Young et al., 2012). L^* – lightness of the colour varies from 0 (black) to 100 (white); a^* – represents the balance between red (positive value) and green (negative value) and b^* between yellow (possitive value) and blue (negative value). A calibration step before measurement was completed using white reference standard no.18833116 ($Y = 94.3$, $x = 0.3134$ and $y = 0.3197$).

Fatty acid composition

The fat was extracted following the method of Bligh and Dyer (1959) with modifications. 50 g of mealworm flour is mixed with 50 ml of chloroform and 50 ml methanol and shaken vigorously for 2 min. Another 50 ml of chloroform is added, followed by 30 s of shaking, and finally, distilled water is added. The volume of water is calculated taking into account the moisture of the flour, to final corrected moisture content of 80%. The mixture is shaken for 30 s and filtrated through a Filtrax filter (grade 388) in a Büchner funnel. A separatory funnel is used, and the bottom (chloroform-methanol) layer is drained. Evaporation is completed at 40–50°C and 0.05 atm. The obtained fat is stored at –18 °C until analysis. The fatty acid composition was determined after transmethylation of fat with 2% H_2SO_4 in CH_3OH at 50°C. Thermo

Scientific GC gas chromatographic system Trace GC (Thremo Fisher Scientific Inc., USA) ultra equipped with a fid detector was used (ISO 12966-4:2015). The column conditions are: 140°C (held 5 min), at 4°C/min to 240°C (held 3 min); the temperature of the injector and detector is 250°C.

Lipolytic and oxidative changes in lipid fraction

The degree of lipolysis was evaluated by the acid value (AV) according to EN ISO 660:2020. The quantity of primary products of lipid peroxidation was expressed using the peroxide value (POV) (Shantha and Decker, 1994). The thiobarbituric value (TBA) determination followed the method of Botsoglou et al. (1994) and modified by Kolev et al. (2022). Both POV and TBA values were measured using a dual beam UV-VIS spectrophotometer Camspec M550 (Spectronic CamSpec Ltd, United Kingdom)

Determination of microbial status

All of the preparation and decimal dilutions of samples suspension were done according to ISO 6887-4:2017. The microbiological status was presented by the Total Plate Count (TPC), Coliforms count, and *E. coli* count, following the procedure of ISO 4833-1:2013/Amd 1:2022.

Data analysis

Statistical analysis was conducted with Microsoft Excel 2016 software applying two-way ANOVA with replications. The whole experimental design was in duplicate, and the following results are presented as means ±SEM. The influence of the variables, namely, type of freezing and type of drying and their interaction were determined at significance level of $P < 0.05$ ($n = 5$).

RESULTS AND DISCUSSION

The protein content varies insignificantly ($P > 0.05$) from 61.65% to 63.89% (Fig. 1), which is higher than what was previously reported at around 41–58% (Hernández-Álvarez et al., 2021; Lenaerst et al., 2018). The applied regimes didn't affect the fat content of the obtained flours ($P > 0.05$). At the same time, all the samples were characterized by a lower fat content than reported by others (Kröncke et al., 2018; Lawal et al., 2021). One possible reason for this deviation in protein content is that the protein to fat ratio is dynamic and directly linked with age of larvae (Turck et al., 2021).

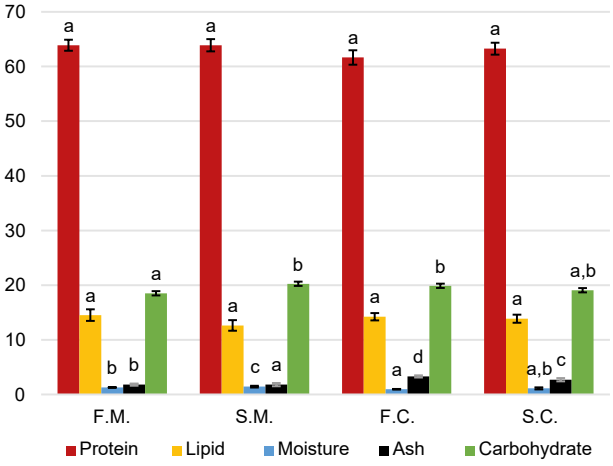

Fig. 1. Proximate composition, %s; a, b, c, d – indicate significant ($P < 0.05$) differences between means for each parameter separately.

The results for ash content and moisture correspond to each other (Fig. 1). The ash content was 1.5 times higher ($P < 0.05$) after conventional drying (F.C and S.C), compared to microwave dried samples (F.M and S.M). The ash content reported in literature often varies from 2% to 4%, which is confirmed by our results (Hernández-Álvarez et al., 2021; Selaledi and Mabelebele, 2021). The calculated carbohydrates were higher than those in the literature, but at the same time, moisture was lower compared to other studies (Pan et al., 2022; Selaledi and Mabelebele, 2021). This suggests that the applied drying regimes are efficient and suitable for production of mealworm flour with low moisture and potentially longer shelf-life.

The results show no significant effect of the type of freezing before blanching on pH value ($P > 0.05$), but the influence of drying was significant ($P < 0.05$) (Table 2). Potentially, the longer time of heat exposure initiates changes in the matrix, leading to changes in pH value. The thermal denaturation of proteins is associated with changes in their solubility, which could explain our results (Lee et al., 2019). On the other hand, both the type of freezing and the type of drying have a significant ($P < 0.05$) effect on the water activity (a_w).

Table 2. Technological parameters and properties of the mealworm flours

Sample / Parameter	F.M.	S.M.	F.C.	S.C.
pH value, (–)	6.90 ±0.01[a]	6.90 ±0.01[a]	6.98 ±0.02[b]	6.96 ±0.04[b]
$a_{W,}$ (–)	0.41 ±0.02[c]	0.56 ±0.02[d]	0.10 ±0.03[a]	0.19 ±0.03[b]
WBC, %	69.96 ±0.51[c]	72.00 ±1.66[c]	62.26 ±1.69[b]	55.90 ±0.49[a]
Yield, %	22.63 ±0.34[b]	24.13 ±0.29[c]	21.94 ±0.44[a]	22.73 ±0.49[b]
Colour components				
*L**, (–)	50.46 ±0.28[a]	49.74 ±0.40[a]	51.23 ±0.17[b]	49.78 ±0.25[a]
*a**, (–)	7.20 ±0.10[d]	6.88 ±0.08[c]	6.17 ±0.12[b]	5.24 ±0.14[a]
*b**, (–)	12.50 ±0.33[c]	13.92 ±0.38[d]	11.13 ±0.28[b]	8.44 ±0.36[a]

a, b, c, d – indicate significant ($P < 0.05$) differences between means for each parameter separately.

The evaluated a_w is below 0.60–0.65, which is considered crucial for microbial growth (Melgar-Lalanne et al., 2019; Vandeweyer et al., 2017) effectively preventing microbiological spoilage. The lower water activity of both conventionally dried samples is in agreement with the evaluated moisture content (Fig. 1). Compared to microwave drying, water activity was 2–3 times lower ($P < 0.05$) after conventional drying (Table 2). The results were similar after drying in a hot air rack, obtained by Kröncke et al. (2018). The water binding capacity/ WBC (Table 2) of the flours differ in a broad range, showing that the applied freezing and drying regimes had a significant effect ($P < 0.05$), this is assuming that the applied freezing and drying regimes affect the functional properties of the proteins like solubility and aggregation (Hernández-Álvarez et al., 2021). The temperature of 60°C used for the conventional drying (Table 1) was potentially enough for denaturation of the protein, which could have led to decreased WBC (Hernández-Álvarez et al., 2021). In contrast, Lee et al. (2019) report that neither different temperature (55, 75 or 95°C) nor time of treatment (20, 40 or 60 min) had a significant effect on WBC of mealworm protein extracts. The established yields (Table 2) are in close relation with the moisture content (Fig. 1). This can be summarized as follows: low moisture equals low yield and *vice versa*.

Significant difference in the lightness of the colour (*L**) was only found in F.C. (Table 2), which is slightly higher ($P < 0.05$) than the rest of the samples. Kröncke et al. (2018) and Lenaerst et al. (2018) suggested that enzymatic browning may lead to a decrease in the lightness of colour, which in our case was not evaluated. Both types of freezing and drying affect ($P < 0.05$) the red component of the colour (*a**) visible from the results in Table 2. The combination of higher values of red (*a**) and yellow (*b**) component of the colour in samples F.M. and S.M. lead to a pleasant golden-brown colour. At the same time, fast frozen and conventionally dried flours (F.C.) have a more light and yellow colour than slow frozen and conventionally dried (S.C.). These results confirm the data described by Lenaerst et al. (2018) except for the lightness of the colour. Lower *L** values, possibly a result of higher enzymatic browning, were found after conventional (slow) freezing. The fast freezing before blanching followed by drying—conventional or microwave—potentially inhibits or may even destroy part of the enzymes responsible for browning (Azzollini et al., 2016), and in samples F.M and F.C, *L** values were 3% higher ($P < 0.05$) compared to S.M and S.C (Table 2).

The FAC of all investigated samples was estimated to be represented by fatty acids with 8 carbon atoms up to 23 (Table 3). The majority of the fatty acids' composition was presented by the Palmitic ($C_{16:10}$), Oleic ($C_{18:1}$), and Linoleic ($C_{18:2}$) acids. Our results confirm those reported by Selaledi and Mabelebele (2021). The ratio of Saturated to Unsaturated FA was significantly affected by the drying regime. In both microwave dried samples (F.M. and S.M.), the portion of

Table 3. Fatty acids' composition FAC, g/100 g fatty acids

Fatty acid	F.M.	S.M.	F.C.	S.C.
$C_{8:0}$	0.10 ±0.03	N.I.	0.10 ±0.02	0.10 ±0.03
$C_{10:0}$	0.10 ±0.01	N.F.	N.F.	N.F.
$C_{12:0}$	0.20 ±0.02	0.20 ±0.02	0.20 ±0.03	0.30 ±0.03
$C_{13:0}$	0.10 ±0.03	0.10 ±0.02	0.10 ±0.02	0.10 ±0.03
$C_{14:0}$	3.40 ±0.02	3.60 ±0.03	4.30 ±0.04	4.00 ±0.04
$C_{14:1}$	0.10 ±0.01	0.10 ±0.01	0.10 ±0.02	0.10 ±0.02
$C_{15:0}$	0.20 ±0.03	0.20 ±0.03	0.20 ±0.01	0.30 ±0.03
$C_{15:1}$	N.F.	0.10 ±0.01	0.10 ±0.02	0.10 ±0.01
$C_{16:0}$	17.00 ±0.05	17.80 ±0.04	20.50 ±0.05	18.60 ±0.05
$C_{16:1}$	0.60 ±0.02	0.70 ±0.03	0.70 ±0.02	0.80 ±0.04
$C_{17:0}$	0.60 ±0.04	0.40 ±0.03	0.6 ±0.04	0.60 ±0.05
$C_{17:1}$	0.40 ±0.03	0.30 ±0.02	0.40 ±0.04	0.40 ±0.03
$C_{18:0}$	7.90 ±0.05	6.90 ±0.06	8.40 ±0.05	8.10 ±0.05
Trans-$C_{18:1}$	0.10 ±0.02	N.F.	N.F.	N.F.
$C_{18:1}$	37.30 ±0.11	41.20 ±0.09	42.70 ±0.10	35.90 ±0.13
Trans-$C_{18:2}$	0.30 ±0.02	0.30 ±0.03	0.30 ±0.02	0.30 ±0.04
$C_{18:2}$	29.50 ±0.12	26.60 ±0.10	19.80 ±0.15	28.40 ±0.11
$C_{18:3}$	0.60 ±0.05	0.50 ±0.06	0.30 ±0.04	0.50 ±0.05
$C_{20:0}$	0.70 ±0.06	0.30 ±0.04	0.20 ±0.04	0.20 ±0.05
$C_{20:1}$	0.10 ±0.02	0.20 ±0.02	0.10 ±0.03	0.20 ±0.01
$C_{20:2}$	0.10 ±0.01	0.10 ±0.03	0.10 ±0.02	0.10 ±0.02
$C_{22:0}$	0.40 ±0.02	0.20 ±0.02	0.20 ±0.04	0.20 ±0.03
$C_{22:1}$	N.F.	0.10 ±0.02	0.10 ±0.03	0.20 ±0.02
$C_{22:6}$	0.10 ±0.02	0.10 ±0.03	0.40 ±0.02	0.40 ±0.04
$C_{23:0}$	0.10 ±0.02	N.F.	0.10 ±0.04	0.10 ±0.03
Saturated	30.80 ±0.16^{b}	29.70 ±0.20^{a}	34.90 ±0.18^{d}	32.60 ±0.14^{c}
Unsaturated	69.20 ±0.21^{c}	70.30 ±0.19^{d}	65.10 ±0.23^{a}	67.40 ±0.16^{b}
Monounsaturated	38.60 ±0.13^{b}	42.70 ±0.11^{c}	44.20 ±0.17^{d}	37.70 ±0.09^{a}
Polyunsaturated	30.60 ±0.27^{d}	27.60 ±0.30^{b}	20.90 ±0.33^{a}	29.70 ±0.25^{c}

a, b, c, d – indicate significant ($P < 0.05$) differences between means for each parameter separately; N.F. – not found (below limit of detection); N.I. – not identified.

unsaturated FA is higher by around 7% ($P < 0.05$), compared to the conventionally dried (F.C. and S.C). The results confirm the Saturated to Unsaturated FA ratio reported by Lenaerts et al. (2018). Lawal et al. (2021) also reported the lipid fraction is mainly composed of monounsaturated fatty acids (MUFA), followed by saturated (SFA) and polyunsaturated (PUFA). As previously testified, the shorter the heat exposure, the lower nutritional losses (Pan et al., 2022). This could be a potential explanation for the higher content of Unsaturated FA in microwave dried samples.

The results presented in Figure 2 show that the type of freezing does not affect ($P > 0.05$) the degree of lipolysis (AV), but the type of drying had a significant ($P < 0.05$) effect. In conventionally dried samples (F.C. and S.C), there were around 13% more free fatty acids ($P < 0.05$), confirming that longer heat exposure initiates lipolytic processes (Lenaerts et al., 2018). This could confirm the evaluated decrease of Linoleic ($C_{18:2}$) and increase of Oleic ($C_{18:1}$) and Palmitic ($C_{16:0}$) acids (Table 3) due to transformation of long chain fatty acids (Moschopoulou et al., 2019). In the meantime, AV was lower in microwave dried flours F.M. and S.M., but the peroxide value (POV) of F.M. was the highest ($P < 0.05$) (Fig. 3), but it still did not exceed the limit of 2.0 meqO_2/kg (Lenaerts et al., 2018). The highest TBA value was also evaluated in sample F.M. (two times higher than S.C., $P < 0.05$).

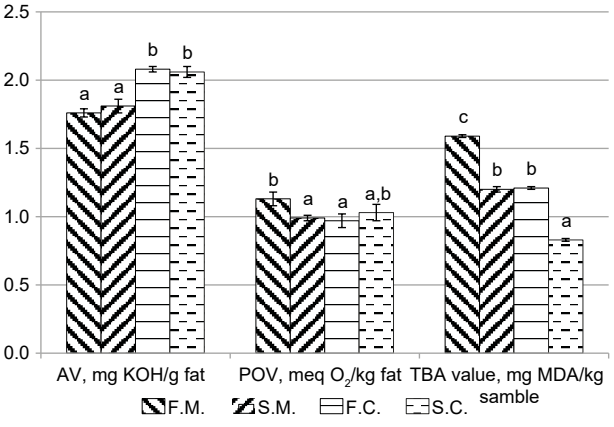

Fig. 2. Oxidative stability of the lipid fraction; a, b, c, d – indicate significant ($P < 0.05$) differences between means for each parameter separately

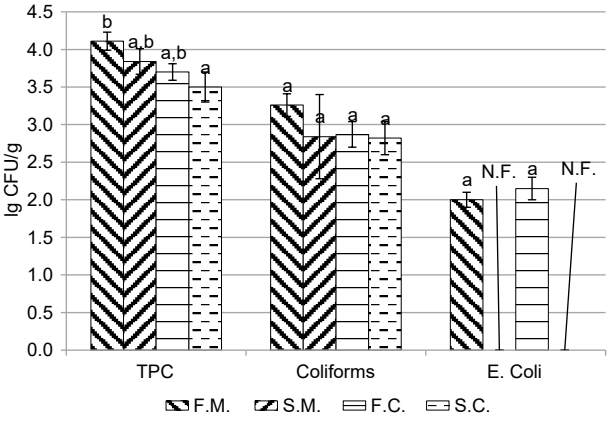

Fig. 3. Microbial status. a, b, c, d – superscripts show significant ($P < 0.05$) differences between means for each parameter separately; N.F. – not found

These results suggest that microwave drying initiates faster oxidation changes and accumulation of greater amounts of secondary products, in particular, MDA.

Fast freezing (F.C. and F.M.) led to higher TPC ($P < 0.05$) (Fig. 3). On the other hand, the shorter drying process (microwave drying, F.M., and S.M.) led to a greater survival rate of microorganisms. The Coliforms count of all the samples do not differ significantly ($P > 0.05$), but in both samples F.M. and F.C., up to 2 CFU/g *E. Coli* were found. This confirmed that despite all the positive aspects of fast freezing, like shorter processing time, lower costs etc., it comes with disadvantages. In theory, the formation of ice crystals is faster and they has a higher count but with smaller size; therefore, they aren't able to destroy the structure of the cells (James et al., 2015). Despite the potential opportunity for a higher survival rate of the microorganisms, after fast freezing followed by microwave drying, the low obtained a_W can guarantee the microbial safety of F.M. mealworm flour.

CONCLUSION

Overall, the applied regimes showed positive and negative influences on the evaluated parameters. Both freezing and drying regimes tested slowly influenced the proximate composition of mealworm flours. The microwave drying preserve both MUFA and PUFA to a higher extent. Summarizing all changes in lipid

fraction of microwave dried flours, it could be concluded that the low AV may be a consequence of the already passed lipid oxidation, explaining the higher POV and TBA values. Despite the positives of microwave drying as a faster and energy efficient processing method, the decreased oxidative stability of the obtained flour could be a drawback. Further evaluation of oxidative stability during storage would be necessary to guarantee the quality and safety of mealworm flours. The results for the microbiological status only confirm the theory about the higher surviving rate of microorganisms after fast freezing but the low a_W should guarantee microbial safety.

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