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Liquid and encapsulated duckweed (*Lemna minor* L.) extracts differentially shape metabolomic fingerprints of packaged beef burgers during shelf-life

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ABSTRACT

Oxidative deterioration and color loss are critical factors limiting the shelf-life and consumer acceptance of fresh beef burgers under modified atmosphere packaging (MAP). This study evaluated *Lemna minor* (duckweed) extracts, applied in liquid form (LLE) and encapsulated with gum Arabic (AGL) or maltodextrin (ML), as natural antioxidants in beef burgers stored at 4 °C for 14 days. Extracts were tested at 0.1 %, 0.5 %, and 1 % (*w*/w), and their effects were assessed through physicochemical, microbiological, and untargeted metabolomics analyses. Encapsulation yields were high (~80 %) for both carriers, with gum Arabic retaining higher antioxidant capacity. The liquid extract at 1 % and the encapsulated extracts at 0.1 % were the most effective treatments, preserving redness, reducing lipid oxidation below the sensory off-flavour threshold, and limiting oxygen consumption by day 7. Conversely, higher doses of encapsulated extracts (0.5–1 %) were less effective and in some cases induced pro-oxidant effects. Untargeted metabolomics revealed modulation of oxidative biomarkers, including glutathione, hemin, and tryptamine, supporting the antioxidant role of duckweed extracts in stabilising lipid and protein oxidation pathways. No antimicrobial effect was observed. These findings indicate that duckweed extracts can serve as sustainable, plant-based antioxidants for fresh beef burgers, with recommended application levels of 0.1 % (*w*/w) for encapsulated forms and 1 % (w/w) for liquid extracts. This represents the first demonstration of duckweed-based antioxidants in meat systems, offering a promising alternative to synthetic preservatives and supporting the transition toward clean-label strategies in the meat industry.

1. Introduction

In recent years, the search for natural antioxidants from plant sources has intensified as the food industry pursues sustainable alternatives to synthetic preservatives (Munekata et al., 2020). Among these plant-based bioactive compounds, *Lemna minor*, commonly known as duckweed, has drawn attention for its remarkable content of polyphenols, flavonoids, and phenolic acids, such as caffeic acid, followed by carotenoids and glucosinolates (Zhang et al., 2023). These compounds have potent antioxidant properties and play a key role in mitigating oxidative stress through scavenging reactive oxygen species (ROS) and chelating metal ions that are pro-oxidants (Muscolo, Oliva, Torello, & Russo, 2024).

Recently, the European Commission approved duckweed for human consumption through Commission Implementing Regulation (EU) 2025/153, adopted on 29 January 2025, adding *L. minor* and *L. gibba* to the Union list of novel foods. This decision followed EFSA's safety evaluation, supported by evidence from the Wageningen Plant Research Institute, which in 2024 showed that lowering manganese in the cultivation medium reduced plant levels to about 6 mg/kg fresh weight, comparable to spinach and resolving the main safety concern. With appropriate controls, several studies now confirm that duckweed products can be safely incorporated into the human diet (Muller, Cournoyer, & Bazinet, 2025). Among its main fields of application, duckweed has

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long been used as animal and insect feed, showing no adverse effects (Ujong et al., 2025). Additionally, it has been investigated as a promising phytoremediation agent for removing heavy metals from air, soil, and water (Ekperusi, Sikoki, & Nwachukwu, 2019), as a valuable tool for producing bioenergy (such as bio-oil, bioethanol, and biogas) (Chen et al., 2022), and as a biostimulant in agriculture (Regni et al., 2021). A recent review by Ujong et al. (2025) highlighted duckweed as a promising and sustainable aquatic plant, offering an eco-friendly alternative for synthesizing high-value bio-products with applications across the food industry, pharmaceuticals, and bioenergy production. Similarly, Sosa et al. (2024) emphasized its significant potential in both the food and feed industries, while Ofoedu, Bozkurt, and Mortimer (2025) recently outlined how duckweed can be used in food formulation for human consumption, serving as a sustainable plant-based protein source.

Although powdered duckweed and its derivatives show great potential in meat science (Ofoedu et al., 2025), direct incorporation of *L. minor* into meat remains challenging due to the susceptibility of bioactives to oxidation, light, and thermal stress (Rezagholizade-shirvan et al., 2024; Rocchetti et al., 2023). Encapsulation has therefore emerged as an effective way to improve stability, bioavailability, and controlled release of plant antioxidants (Smaoui et al., 2021). Among the most reliable carriers are maltodextrins and gum Arabic; the former offers high solubility and neutral taste, while the latter provides strong emulsifying properties and stable protective films (Akdeniz, Sumnu, & Sahin, 2017; Smaoui et al., 2021). Building on this background, the present study investigated the encapsulation of *L. minor* extracts with maltodextrin and gum Arabic using spray drying, a well-established food industry technique for producing stable powders with controlled particle size (Assadpour & Jafari, 2019). This method was chosen based on our previous work, which indicated potential pro-oxidant effects of liquid duckweed extracts in beef burgers at different dosages (Rocchetti et al., 2023). The main objective was to assess whether encapsulation could preserve antioxidant capacity more effectively than non-encapsulated extracts in a beef burger system. To this end, untargeted metabolomics was combined with classical biochemical and microbiological assays to elucidate the biochemical changes and antioxidant mechanisms associated with both forms of extract.

2. Materials and methods

2.1. Plant material and duckweed liquid extract (LLE) preparation

The details related to duckweed collection, growth, and optimal conditions to obtain the liquid extracts (LLE) were defined according to the RSM study previously published by Zhang et al. (2023). The preparation of LLE was done at Università Cattolica del Sacro Cuore (Cremona, Italy). Briefly, 1 g of dried duckweed tissues was extracted in 100 mL of solvent using ultrasound-assisted extraction (UAE; DU-32 ARGOLab, Milan, Italy) for 15 min, setting 50 °C (temperature), 120 W (power), and 50 % ethanol (solvent). After sonication, the samples were homogenized using a PT1200E (Polytron) blender and centrifuged at 6000 ×*g* for 10 min at 4 °C. The supernatants were then filtered through 0.22 μm syringe filters and collected. Following extraction, ethanol was removed using a vacuum rotary evaporator (55 °C, 30 min), and the solution was reconstituted to its original volume with water. Overall, the extraction procedure provided an average yield of ~220 mg dry extract per g of dried plant material (~22 % *w*/w).

2.2. Encapsulation of duckweed extracts with gum Arabic and maltodextrin

The encapsulation process was carried out at Clever Bioscience Srl (Pavia, Italy). Briefly, 20 g of duckweed dried biomass was extracted in 2 L of Ethanol 50 % (*v*/v) using an ultrasonic probe (15 min, 300 W, 65 % of the maximum power, 10 s active, 50 s Off). The mixture was centrifuged twice for 15 min at 5 °C to maximize the recovery of phytochemicals and then filtered to collect the supernatants. Ethanol was then removed by rotary-evaporator under vacuum at 55 °C, and then 50 g of both gum Arabic (OENO® Kordofan) and maltodextrin (GLUCIDEX® 19; dextrose equivalent = 19) were dissolved respectively to 500 mL of concentrated extracts (10 % *w/v*) under magnetic agitation for 30 min to ensure complete dissolution. The carrier ratio was chosen based on preliminary trials and previous literature, to ensure proper encapsulation efficiency and powder stability (Sarabandi, Jafari, Mahoonak, & Mohammadi, 2019). Spray drying was performed using a Pilotech YC-018 laboratory spray dryer (Shanghai Pilotech Instruments & Equipment Co., Ltd.; rated capacity 3500 mL·h^{-1}; rated airflow 2 m^3·min^{-1}). The unit was equipped with a two-fluid nozzle (standard jet 1.5 mm) and operated using the instrument's compressed-air supply (32 L·min^{-1} at 0.5–2 bar). The inlet air temperature was set at 130 °C, the outlet temperature was 67 °C, pump speed was fixed at 20 rpm, and the automatic de-blocking system was activated every 10 s. The volumetric feed rate (mL·h^{-1}) depends on feed properties and was controlled by pump speed; the pump speed was maintained constant across runs to ensure comparable processing conditions.

2.3. Evaluation of the encapsulation yields of bioactive compounds

The total and surface bioactive compounds were determined following the method reported by Saénz, Tapia, Chávez, and Robert (2009), with minor modifications. Briefly, to quantify bioactive compounds, 100 mg of encapsulated powder were dispersed in 1 mL of ethanol, acetic acid, and water (50:8:42), vortexed for 1 min, and sonicated for 40 min at 120 W and room temperature. After centrifugation at maximum speed for 10 min, the supernatant was filtered through 0.22 μm syringe filters, and bioactive compounds were measured using the Folin-Ciocalteu (FC) assay, through an estimation of the total reducing equivalents (expressed as gallic acid equivalents; GAE). FC is known to measure total reducing capacity and is not fully specific for phenolics, considering that it can react with other reducing substances, such as ascorbate or certain amino acids. Therefore, Folin values are intended as an index of extract reducing/antioxidant pool rather than an absolute sum of phenolic molecules. Additionally, for surface bioactive compounds, 100 mg of encapsulated powder were mixed with 1 mL of ethanol and methanol (1:1), vortexed for 1 min at room temperature, and filtered through 0.22 μm syringe filters. The FC assay was used again for quantitative purposes. The surface bioactive compounds percentage (SB) and bioactive compounds microencapsulated yield (BMY) were calculated according to eqs. (1) and (2):

$$SB\ (\%) = (Surface\ bioactive\ compounds / Total\ bioactive\ compounds)^{*} 100 \quad (1)$$

$$BMY\ (\%) = 100 - SB\ (\%) \quad (2)$$

2.4. In vitro antioxidant potential of LLE and encapsulated extracts (AGL and ML)

The in vitro antioxidant activity of the prepared duckweed extracts (LLE, AGL, and ML) was investigated using complementary assays, namely DPPH, ABTS, CUPRAC, FRAP, and Metal Chelating activity. Three independent batches of each extract type (LLE, AGL, ML) were prepared and analyzed by different antioxidant assays. Trolox equivalents (TE) were measured for DPPH, ABTS radical scavenging, CUPRAC, and FRAP. The metal chelating activity was measured in EDTAE (ethylenediaminetetraacetic acid equivalents). Full experimental details are available in a previous research article (Grochowski et al., 2017).

2.5. Preparation of beef burgers for the shelf-life study under MAP conditions

Beef burgers were produced by Indal S.r.l. (Montichiari, Brescia, Italy), while the storage process was conducted at the meat pilot plant of Università Cattolica del Sacro Cuore (Cremona, Italy). Three independent meat batters, each weighing 13.2 kg, were prepared on separate days. The formulation consisted of 99 % beef meat (silver side and top side cuts, containing approximately 5 % fat) and 1 % added salt (NaCl). The meat was minced using an industrial meat grinder (Risco, Thiene VI, Italy) equipped with a 4 mm perforated plate, followed by salt incorporation using an industrial mixer (Risco, Thiene VI, Italy). The prepared meat mixture, with 1 % NaCl evenly distributed, was divided into 11 experimental batches based on the study design: CTR- (negative control, containing only 1 % NaCl without additives), CTR+ (positive control, including 1 g/kg ascorbic acid in addition to 1 % NaCl), LLE 0.1 %, LLE 0.5 %, LLE 1 % (*L. minor* extract added at 1, 5, and 10 g/kg, respectively), AGL 0.1 %, AGL 0.5 %, AGL 1 % (*L. minor* extract encapsulated in gum Arabic, added to at 1, 5, and 10 g/kg, respectively), and ML 0.1 %, ML 0.5 %, ML 1 % (*L. minor* extract encapsulated in maltodextrin, added at 1, 5, and 10 g/kg, respectively). All extract concentrations (0.1 %, 0.5 %, and 1 % *w*/w) were calculated on a meat basis, i.e., relative to the total mass of minced beef used in the batter before burger formation. Each batch was individually mixed to ensure homogeneity using a planetary mixer (Kenwood Major Pro) for 5 min at medium speed to ensure uniform distribution of liquid and powdered extracts. Then, burgers weighing 100 g were manually shaped using a burger press. Subsequently, each burger was packaged in polypropylene thermosealable trays under modified atmosphere packaging (MAP) conditions, consisting of 66 % oxygen (O_2), 25 % carbon dioxide (CO_2), and 9 % nitrogen (N_2). The packaged samples were stored at 4 °C under light exposure, mimicking supermarket conditions. The chosen high-oxygen MAP (66 % O_2 / 25 % CO_2 / 9 % N_2) reflects retail display conditions typically used to maintain beef redness (Djenane & Roncalés, 2018), while also providing an oxidising environment suitable for evaluating antioxidant efficacy. Sampling was conducted after 1 (T_1), 7 (T_7), and 14 (T_{14}) days of storage. In total, 396 burger samples were produced, corresponding to four burgers per treatment × eleven treatments × three storage times × three independent replications (performed on different days with varying raw meat batches but identical ingredient compositions).

2.6. Microbiological, pH, water activity (a_w), and gas composition analyses

Microbial assessment followed ISO methodologies for each microbial group. Decimal dilutions were plated on selective growth media (Oxoid, Milan, Italy) and incubated according to ISO standards: a) Total Microbial Counts (TMC) for psychrotrophic and mesophilic bacteria were determined on Plate Count Agar, incubated at 7 °C for 10 days and at 30 °C for 72 h, respectively; b) Lactic acid bacteria (LAB) were enumerated on MRS Agar under anaerobic conditions at 37 °C for 48 h (ISO 15214:1998); c) Staphylococci were counted on Baird Parker Agar with egg yolk tellurite emulsion, aerobically at 37 °C for 48 h (ISO 6888-1:2018); d) *Enterobacteriaceae* were assessed on VRBGA, incubated at 37 °C for 24 h (ISO 21528-2:2017); e) Yeasts were determined on Rosa Bengal Agar with chloramphenicol (100 mg/L), incubated at 30 °C for 4 days. Microbial counts were expressed as Log CFU/g.

For pH measurements, the electrode tip (pH 127-m; 692 pH/Ion Meter, Metrohm, Laramie, Wyoming, USA) was inserted into the −1 dilution of each sample. Calibration was performed using buffers at pH 4.01 and 7.00 at 22 °C. Water activity (a_w) was measured at 25 °C with an AQUALAB 4TE a_w-meter (Decagon Devices, Inc., Pullman, WA, USA) following ISO procedures (ISO 18787:2017). pH and a_w measurements were conducted in triplicate for each sampling point ($n = 3$).

Finally, changes in MAP composition were evaluated by measuring CO_2 and O_2 percentage values at each sampling point (T_1, T_7, and T_{14}) by using the headspace gas analyzer for quality control of MAP Dansenor CheckMate 3 (Ametek Mocon, Ringsted, Denmark).

2.7. Color analysis and thiobarbituric acid reactive substances (TBARS) assay

Color measurements were performed in triplicate for each batch using a HunterLab D25 NC colorimeter (HunterLab, Reston, Virginia) after opening the packaging and following a 30-min blooming time. Results were recorded in the CIELab* color space, utilizing a D65 illuminant, an aperture size of 25.4 mm, and a 10° viewing angle. The three-dimensional color parameters included L^* (lightness), where $L^* = 0$ represents black and $L^* = 100$ represents white; a^*, which measures chromaticity along the red-green axis ($-a^*$ for greenness, $+a^*$ for redness); and b^*, which represents the blue-yellow axis ($-b^*$ for blueness, $+b^*$ for yellowness) (Biró, Fodor, Szedljak, Pásztor-Huszár, & Gere, 2019). Additionally, total color differences (ΔE*) were determined following the equation outlined by Biró et al. (2019).

Lipid oxidation was assessed through the thiobarbituric acid reactive substances (TBARS) assay, following the method of Vyncke (1975) with minor modifications. The results were expressed as milligrams of malonaldehyde (MDA) per kg sample, based on three independent replicates ($n = 3$).

2.8. Untargeted metabolomics profiling of meat metabolites

Meat metabolites were extracted as reported by Rocchetti et al. (2023). Burger samples (1 g) were diluted ten-fold with 80 % methanol (*v*/v) acidified with 0.1 % formic acid and extracted using a PT1200E (Polytron) homogenizer. Extracts were centrifuged (6000 ×*g*, 15 min, 4 °C) and filtered through 0.22 μm cellulose syringe filters into UHPLC vials. UHPLC-HRMS analysis was performed on a Q-Exactive™ Focus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific, USA) coupled to a Vanquish UHPLC system using a HESI-II probe. The mobile phases were water and acetonitrile (LC-MS grade, Sigma-Aldrich) with 0.1 % formic acid, following a gradient elution from 6 % to 94 % acetonitrile in 35 min. Separation was achieved on an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) at 35 °C. Full-scan MS (*m/z* 80–1200) was done in positive ionization mode (70,000 resolution at *m/z* 200) with a 200 μL/min flow rate and 6 μL injection volume. Pooled quality control (QC) samples were randomly injected and analyzed in data-dependent MS/MS mode (Top $N = 3$), evaluating reproducibility, with a resolution of 17,500 at m/z 200 and 23.3 eV as normalized collision energy. Data were processed using MS-DIAL (version: 4.90) (Tsugawa, Cajka, Kind, et al., 2015), for peak finding, LOWESS normalization, and metabolite annotation via spectral matching against FooDB. Full details on software parameters and identification workflow are reported in Rocchetti et al. (2021). Annotation was based on mass accuracy, isotopic profile, and spectral matching, achieving level 2 confidence according to COSMOS metabolomics standards (García-Pérez, Becchi, Zhang, Rocchetti, & Lucini, 2024).

2.9. In silico docking study: Focus on caffeic acid

An in silico docking study based on "AutoDock Vina" and "Attracting cavities" was then performed on SwissDock (Bugnon et al., 2024) to study the main interactions existing between caffeic acid and the myoglobin protein (UniProt ID: P02192). Particularly, the binding free energy (deltaG) and affinity of the ligand-protein interaction, together with other factors (e.g., ligand solubility, desolvation energy, and overall physicochemical compatibility with the binding environment) were evaluated by inspecting AC Score and SwissParam Score values, respectively.

2.10. Statistical analyses

The in vitro antioxidant activity values of the different duckweed extracts and encapsulating agents (LLE, AGL, ML, AG powder, and M powder) were analyzed using R (version 4.4.1). Estimated marginal means (EMMs) were calculated using a linear mixed-effects model (lmer), with extract type as a fixed factor and batch as a random effect. Pooled standard errors of the mean (SEM) values were derived from the residual variance of each model, and significant differences were determined through pairwise comparisons with compact letter display grouping ($P < 0.05$). Regarding the other parameters determined on beef burgers (i.e., pH, aw, microbial counts, MAP gases, L^*, a^*, b^*,and MDA values), a two-way ANOVA was carried out using IBM SPSS Statistics (version 26.0) considering storage time and treatment as fixed factors, while manufacturing repetition was treated as a random effect. When ANOVA indicated significance ($P < 0.05$), Duncan's post hoc test was applied to determine differences between means. The interaction between the fixed factors (storage time × treatment) was also analyzed.

For untargeted metabolomics, data processing was carried out using multiple software tools, including Mass Profiler Professional B.12.06 (Agilent Technologies), SIMCA 13 (Umetrics), and MetaboAnalyst 6.0. Raw UHPLC-HRMS data were median-centered, $\log_{10}$-transformed, and Pareto-scaled. Hierarchical cluster analysis (HCA) based on Euclidean distance was used for unsupervised clustering, whereas supervised classification was performed using orthogonal projections to latent structures discriminant analysis (OPLS-DA), with storage time as the discriminating factor. Details regarding validation and permutation testing can be found in Rocchetti et al. (2023). Key discriminant metabolites were identified using the variable importance in projection (VIP) algorithm (threshold >1), while their variations were analyzed through Fold-Change (FC) analysis (cut-off >1.2) using Mass Profiler Professional software. Additionally, the "rAMOPLS" package (version 0.2) in R-Studio was used to perform multifactorial ANOVA coupled with OPLS prediction modeling (AMOPLS) to better evaluate the potential interactions existing between ripening time and treatments. The model built was then inspected in terms of statistical and predictive assessments through different parameters, such as the residual sum of squares (RSS), residual structure ratio *P*-value based (RSR *P*-value) and goodness of fit (R^2Y).

3. Results and discussion

3.1. Phytochemical content and in vitro antioxidant potential of liquid and encapsulated duckweed extracts

The UHPLC-HRMS analysis confirmed that the liquid *L. minor* extract (LLE) was abundant in polyphenols, with a total content of 3340 μg equivalents (Eq.)/g (Supplementary Table 1). Specifically, the extract contained 1826 μg Eq./g of phenolic acids, 1065 μg Eq./g of flavonoids, 401 μg Eq./g of other polyphenols (including lignans, tyrosols, and coumarins), and 47.1 μg Eq./g of stilbenes. Among these, caffeic acid and hydroxycaffeic acid were predominant, in line with previous reports on *Lemna* species (Baek, Saeed, & Choi, 2021; Del Buono, Bartucca, Ballerini, et al., 2022; Petrova-Tacheva, Ivanov, & Atanasov, 2020; Zhang et al., 2023).

The linear mixed-effects models confirmed significant differences in the in vitro antioxidant potential among extracts for all assays (Table 1), while properly accounting for batch-to-batch variability. For DPPH and ABTS radical scavenging, the LLE extract showed the highest activity (47.71 and 68.44 mg TE/g, respectively), significantly outperforming the encapsulated (AGL, ML) and encapsulating agent-only controls (AG and M powders). Similar trends were observed for CUPRAC and FRAP assays, where LLE and AGL achieved the highest reducing power, indicating that the liquid extract and gum Arabic encapsulation retained higher antioxidant capacity. The metal chelating activity followed the same pattern, with LLE (23.43 mg EDTAE/g) showing the strongest

Table 1
Estimated marginal means (EMMs) for each in vitro antioxidant assays. EMMs were obtained from a linear mixed-effects model including extract type as a fixed factor and batch as a random effect. A pooled standard error of the mean (SEM) is reported for each assay, based on the residual variance and the number of independent batches ($n = 3$).

Sample	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)	Metal Chelating (mg EDTAE/g)
LLE	47.71^{e}	$68.4\ 4^{e}$	111.29^{d}	68.13^{c}	23.43^{e}
AG powder	10.96^{b}	11.07^{b}	12.30^{a}	8.32^{a}	4.82^{a}
M powder	6.26^{a}	8.95^{a}	11.70^{a}	7.55^{a}	3.53^{a}
AGL	44.73^{d}	60.93^{d}	95.81^{c}	66.58^{c}	15.75^{d}
ML	25.05^{c}	43.47^{c}	83.65^{b}	55.58^{b}	11.56^{c}
SEM	0.296	0.466	0.863	0.800	0.494

Different superscript letters within each column indicate significant differences among extract types according to pairwise comparisons with the compact letter display (CLD) method ($P < 0.05$). *Abbreviations:* LLE (*L. minor* liquid extract); AG (gum Arabic); M (maltodextrin); AGL (*L. minor* liquid extract encapsulated with gum Arabic); ML (*L. minor* liquid extract encapsulated with maltodextrin); TE = Trolox Equivalents; EDTAE = Ethylenediaminetetraacetic acid Equivalents.

effect, highlighting its broad-spectrum antioxidant potential. These findings demonstrate that the in vitro antioxidant capacity of the duckweed extracts is markedly affected by the extraction form and encapsulation type, and that batch effects were successfully controlled through the mixed model approach. Interestingly, encapsulation with gum Arabic preserved antioxidant capacity more efficiently than maltodextrin under the tested conditions, and this was true mainly for FRAP values (Table 1). These results are in agreement with previous studies demonstrating that gum Arabic, due to its complex branched structure and good emulsifying properties, forms a more stable matrix that better protects polyphenols from oxidation and degradation (Rezende, Nogueira, & Narain, 2018; Smaoui et al., 2021). Raw polysaccharide powders alone showed minimal antioxidant interference (Table 1), confirming that the observed activities were due to the duckweed extract itself.

Total bioactive content (TBC) was highest for LLE (19.46 mg GAE/g), while AGL and ML retained significant levels (17.41 and 14.23 mg GAE/g, respectively) (Supplementary Table 2). Although the FC assay provides a non-specific measure of reducing capacity, our UHPLC-HRMS profiling confirmed that phenolic acids (notably caffeic acid) dominate the extract composition, supporting the use of Folin values as a practical comparative index for TBC. The encapsulation yield (BMY) was similar for gum Arabic (81 %) and maltodextrin (79 %), indicating both carriers effectively entrapped the bioactive compounds. However, their release behaviour in the meat matrix may differ, as gum Arabic's higher molecular weight and complex branching can lead to a slower, more controlled release compared to maltodextrin, which is more readily soluble and may release polyphenols faster (Rezende et al., 2018; Tolun, Altintas, & Artik, 2016). This controlled release is particularly relevant for meat applications, where prolonged antioxidant action during storage is desired. Various factors can influence the performance of encapsulated systems, including the ratio of core to coating material, the dextrose equivalent (DE) of maltodextrin, the solubility of the carrier in the meat matrix, and process conditions such as inlet temperature during spray drying (Tolun et al., 2016). For example, Tolun et al. (2016) found that combining maltodextrin and gum Arabic improved polyphenol retention and antioxidant stability in grape pomace extract compared to maltodextrin alone. While combined carriers can offer synergistic effects, in this study a single coating was chosen to simplify formulation and focus on comparing the release profiles of the two carriers under identical conditions. Our findings highlight that encapsulation with gum Arabic may provide better protection and controlled release of polyphenols in the meat matrix, potentially enhancing antioxidant effectiveness throughout the burger's shelf-life. This supports

the rationale for testing both carriers under MAP storage, as the carrier type can directly influence how bioactives interact with muscle pigments and lipids, thereby impacting color stability and oxidative status.

3.2. Changes in a_w, pH, and microbiological counts during storage of beef burgers

The results on pH, a_w, and microbial counts are shown in Table 2. A significant interaction effect between treatment and storage time was found ($P < 0.001$). Therefore, mean comparisons were performed across all treatment × time combinations. pH is a key factor for meat color stability, influencing OxyMb preservation during storage. At T_1, pH ranged from 5.54 (CTR+) to 5.66 (LLE 0.5 %), regardless of whether liquid or encapsulated extracts were added. This indicates that the intrinsic pH of the ingredients (measured as 3.8 for AGL and 5.7 for ML and LLE) did not strongly influence the initial pH of the burgers. By T_7, pH values varied more widely, from 5.36 (CTR-, LLE 0.5 %) to 5.93 (ML 0.5 %). Treatments with encapsulated extracts occasionally showed higher pH values (up to 5.93), which coincided with increased lipid oxidation (MDA) and poorer redness retention. Conversely, slightly lower pH values, as seen in LLE 1 %, ML 0.1 %, and AGL 0.1 %, were associated with better color stability and oxidative control. These findings contrast with Zareian et al. (2019), who reported that lower pH accelerated MetMb formation. The discrepancy may reflect differences in matrix composition and the way bioactive compounds interact with the meat system, potentially influencing local redox conditions (Oliveira et al., 2025). It has been reported that carrier substances such as gum Arabic and maltodextrin can alter the microenvironment around proteins (Rezende et al., 2018), and their indirect influence together with microbial metabolism may have contributed to the observed pH dynamics, as also noted in studies on other encapsulated plant extracts in meat (Conte-Junior, Monteiro, Patrícia, Mársico, et al., 2020). During storage, pH decreased, particularly in samples with initially higher values (e.g., AGL 1 % dropped from 5.85 at T_7 to 5.26 at T_{14}), mainly due to microbial activity and carbonic acid formation from dissolved CO_2. Overall, these results suggest that while encapsulation may have some impact on early pH behaviour, spoilage-related processes and MAP conditions remain the dominant drivers of pH decline over time.

Water activity (a_w) showed minor but significant variations over storage (Table 2). Overall, a_w ranged from 0.9880 to 0.9995, with slight increases by T_{14}, likely due to reduced water-holding capacity as pH approached the isoelectric point of myofibrillar proteins (Kowalczyk, Domaradzki, Ziomek, et al., 2024). This aligns with reports that oxidation and proteolysis can lead to structural changes in muscle proteins, affecting water migration (Kowalczyk et al., 2024). Measured water activity values confirmed that the maximum extract addition (i.e., 1 % *w*/w) did not materially alter sample hydration; therefore, observed differences among treatments can be attributable to the extracts and their carriers rather than to differences in bulk moisture.

Microbiological analyses showed no antimicrobial effects from the duckweed treatments (Table 2). Total mesophilic counts (TMC) increased over time, ranging from 6 to 7 log CFU/g, typical of MAP-stored fresh meat. Psychrotrophic bacteria dominated at 7 °C, while Lactic acid bacteria (LAB) progressively increased from ~4 log CFU/g at T_1 to ~5 log CFU/g at T_{14}, in line with the decreasing pH. No treatment inhibited LAB growth, indicating that the phenolic content and antioxidant activity were not sufficient to exert bacteriostatic effects under these conditions. This agrees with previous studies showing that polyphenol-rich extracts encapsulated in polysaccharides tend to have more impact on oxidative rather than microbiological stability (Pilatti-Riccio et al., 2019). Staphylococci remained below 3 log CFU/g, indicating minimal contamination (supplementary Table 3), consistent with Kowalczyk et al. (2024), who evaluated the microbiological quality of beef packaged in vacuum and MAP. Enterobacteriaceae counts decreased during storage, likely due to MAP and pH decline, while yeasts remained low (< 2.5 log CFU/g), confirming that MAP's elevated

Table 2
pH and water activity (a_w) values, together with microbial counts (log CFU/g) for Total Microbial Counts (TMC) at 30 °C and 7 °C, and Lactic Acid Bacteria (LAB) of burger samples during storage period (SP).

Parameter	SP (days)	CTR-	CTR+	LLE 0.1 %	LLE 0.5 %	LLE 1 %	AGL 0.1 %	AGL 0.5 %	AGL 1 %	ML 0.1 %	ML 0.5 %	ML 1 %	SEM	T	SP	TxSP
pH	1	5.60^{pq}	5.54^{m}	5.60^{qr}	5.66^{t}	5.62^{s}	5.59^{p}	5.57^{o}	5.56^{n}	5.58^{o}	5.59^{p}	5.61^{r}	0.005	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	5.36^{fg}	5.43^{k}	5.41^{hi}	5.35^{f}	5.43^{k}	5.41^{hi}	5.90^{v}	5.85^{u}	5.42^{j}	5.93^{w}	5.85^{u}	0.041			
	14	5.44^{l}	5.36^{g}	5.43^{k}	5.41^{ij}	5.33^{e}	5.40^{h}	5.31^{d}	5.26^{c}	5.33^{e}	5.18^{b}	5.10^{a}	0.018			
	SEM	0.035	0.026	0.031	0.047	0.043	0.031	0.085	0.085	0.036	0.108	0.110				
a_w	1	0.9910^{cdef}	0.9914^{cdef}	0.9908^{bcde}	0.9919^{defg}	0.9918^{def}	0.9912^{cdef}	0.9928^{efg}	0.9926^{defg}	0.9880^{a}	0.9922^{defg}	0.9961^{hi}	0.0004	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	0.9913^{cdef}	0.9915^{cdef}	0.9928^{efg}	0.9925^{defg}	0.9908^{bcde}	0.9913^{cdef}	0.9904^{bcde}	0.9925^{defg}	0.9933^{fg}	0.9933^{fg}	0.9924^{defg}	0.0003			
	14	0.9887^{ab}	0.9981^{ij}	0.9985^{j}	0.9995^{j}	0.9927^{efg}	0.9977^{ij}	0.9892^{abc}	0.9963^{hi}	0.9902^{bcd}	0.9913^{cdef}	0.9942^{gh}	0.0007			
	SEM	0.0006	0.0012	0.0013	0.0013	0.0004	0.0012	0.0005	0.0009	0.0010	0.0005	0.0006				
TMC 30 °C	1	6.20^{bcdefg}	5.95^{a}	6.03^{abc}	6.15^{abcdef}	6.15^{abcdef}	6.10^{abcd}	6.14^{abcde}	6.03^{abc}	6.23^{cdefg}	6.04^{abc}	6.15^{abcd}	0.018	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	6.06^{abc}	6.04^{abc}	6.32^{defg}	6.61^{hi}	6.26^{cdefg}	6.43^{gh}	6.72^{i}	7.11^{j}	7.15^{j}	6.38^{fg}	6.36^{efg}	0.067			
	14	6.81^{i}	5.98^{ab}	7.33^{jk}	7.24^{jk}	6.37^{efg}	6.83^{i}	7.14^{j}	7.31^{jk}	7.41^{k}	6.74^{i}	7.17^{j}	0.077			
	SEM	0.117	0.051	0.202	0.160	0.031	0.125	0.149	0.200	0.179	0.106	0.163				
TMC 7 °C	1	7.36^{fg}	7.15^{bcd}	7.53^{hi}	7.22^{def}	7.06^{bc}	7.02^{ab}	7.22^{def}	7.14^{bcd}	6.91^{a}	7.00^{ab}	7.10^{bcd}	0.031	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	7.15^{bcd}	7.35^{fg}	7.68^{ijk}	7.57^{hij}	7.33^{efg}	7.07^{bcd}	7.47^{gh}	7.75^{kl}	7.45^{gh}	7.19^{cde}	7.58^{hij}	0.039			
	14	7.58^{hij}	7.64^{ijk}	8.02^{n}	7.87^{lm}	7.44^{gh}	7.69^{jk}	8.05^{n}	8.31^{o}	7.59^{hij}	7.67^{ijk}	7.95^{mn}	0.046			
	SEM	0.065	0.083	0.074	0.099	0.060	0.108	0.124	0.171	0.105	0.104	0.123				
LAB	1	4.24^{fghi}	4.05^{cd}	4.23^{fghi}	4.20^{efgh}	4.15^{defg}	3.87^{a}	4.17^{efgh}	4.09^{cde}	4.24^{fghi}	4.00^{bc}	4.13^{def}	0.022	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	4.54^{lm}	4.62^{mnop}	4.37^{jk}	4.49^{l}	4.23^{fghi}	4.35^{ij}	4.57^{lmn}	4.59^{lmno}	4.29^{hij}	4.26^{ghij}	4.48^{kl}	0.026			
	14	4.73^{p}	4.13^{ab}	4.63^{mnop}	4.68^{nop}	4.70^{op}	4.74^{p}	4.85^{q}	4.93^{q}	4.71^{op}	4.37^{jk}	4.88^{q}	0.048			
	SEM	0.073	0.109	0.061	0.073	0.089	0.129	0.100	0.122	0.076	0.056	0.110				

Different superscript letters within each analytical parameter indicate significant differences within treatment and storage period combinations. SEM, T, SP and TxSP stand out for the standard error of the mean, the fixed factors treatment and storage period, and the interaction between them, respectively. CTR- (negative control, containing only 1 % NaCl without additives), CTR+ (positive control, including 1 g/kg ascorbic acid in addition to 1 % NaCl), LLE 0.1 %, LLE 0.5 %, LLE 1 % (*L. minor* extract added at 1, 5, and 10 g/kg, respectively), AGL 0.1 %, AGL 0.5 %, AGL 1 % (*L. minor* extract encapsulated in gum Arabic, added to at 1, 5, and 10 g/kg, respectively), and ML 0.1 %, ML 0.5 %, ML 1 % (*L. minor extract* encapsulated in maltodextrin, added at 1, 5, and 10 g/kg, respectively).

CO_2 controlled their growth (supplementary Table 3). These microbial dynamics confirm that the observed improvements in oxidative stability and color must be primarily attributed to the antioxidant action of the encapsulated extracts rather than antimicrobial effects, as also observed by Sirini et al. (2025) working on encapsulated kiwifruit peel extracts. Our findings differ from those reported by Najjaa et al. (2020), evaluating the impact of garlic essential oil, encapsulated with gum Arabic and maltodextrin, on quality of beef meat, and revealing a strong antimicrobial effect, likely due to the abundance of allicin. This suggests a pivotal role exerted by the key bioactive compounds of different plant matrices. Accordingly, our results imply that while the encapsulation strategy effectively modulates oxidation and pH trends, it does not extend shelf-life by controlling spoilage microorganisms and thus should be combined with strict hygienic practices and adequate MAP conditions to ensure microbial safety during storage.

3.3. Changes in color, MDA and MAP gas compositions during storage of beef burgers

The analysis of color parameters, TBARS, and MAP gas composition highlighted how LLE and its encapsulated forms (AGL and ML) affected the oxidative stability and appearance of beef burgers during storage (Fig. 1). Particularly, a significant interaction effect between treatment and storage time was found ($P < 0.001$). Therefore, mean comparisons were performed across all treatment × time combinations. Significant differences were identified according to Duncan's test ($P < 0.05$), as indicated by different superscript letters (Table 3). Overall, the MDA content of fresh meat (prior to burger preparation and packaging) was not detectable, thus confirming the expected absence of oxidative processes in freshly minced beef. After one day (T_1), all samples exhibited a bright red color, confirming the initial protective effect of MAP. However, clear differences emerged: CTR+ displayed the highest redness ($a^* = 18.87$) and the lowest lipid oxidation (MDA = 0.14 mg/kg), while LLE 1 % improved redness ($a^* = 17.93$) and moderately reduced MDA levels. Among the encapsulated forms, AGL 0.1 % showed a balanced effect on redness ($a^* = 17.40$) and oxidative control (MDA = 0.78 mg/kg), whereas ML 1 % performed poorly, exhibiting low redness and elevated MDA, suggesting suboptimal antioxidant release at higher doses. By day 7, oxidative deterioration became more pronounced (Table 3), with CTR- exhibiting the most severe discoloration ($a^* = 3.89$). LLE 1 % maintained the highest redness ($a^* = 11.39$) and the lowest MDA fold increase, confirming its antioxidant effectiveness. Low-dose encapsulated treatments (AGL 0.1 %, ML 0.1 %) also preserved color and inhibited oxidation better than higher doses, likely due to a more controlled release of antioxidants. Conversely, high concentrations (1 %) led to increased MDA and greater color loss, possibly due to the pro-oxidant activity of excessive polyphenols (Procházková, Boušová, & Wilhelmová, 2011; Rocchetti et al., 2023). The faster release from maltodextrin compared to gum Arabic may have contributed to these effects, with gum Arabic's emulsifying properties enabling a more gradual antioxidant release. At day 14 (T_{14}), none of the treatments effectively preserved redness, and off-flavors were detected once removing the packaging. All samples showed advanced myoglobin oxidation ($a^* < 3.3$) but lower MDA values. The decrease in TBARS values at T_{14} compared to T_7 across all treatments, although appearing counterintuitive, it is consistent with the known dynamics of lipid oxidation. After an initial accumulation of primary and secondary oxidation products, including free MDA, subsequent reactions can lead to their transformation into less reactive compounds (Papuc, Goran, Predescu, & Nicorescu, 2017). In particular, MDA may form Schiff bases and protein adducts, undergo polymerisation, or be converted into volatile aldehydes that are not quantified by the TBARS assay. Consequently, the assay may underestimate the actual oxidative status of the meat at later storage times. This interpretation is further supported by the following metabolomics data, which highlighted a progression toward more complex lipid oxidation products at T_{14}. Therefore, the decrease in TBARS values should not be interpreted as an improvement of oxidative stability, but rather as a methodological limitation inherent to the measurement of free, TBA-reactive MDA. T_7 thus represents the critical time point for evaluating treatment efficacy, as oxidative processes became predominant thereafter. Looking at similar works available in scientific literature, elderberry encapsulated extracts (EE), using maltodextrin and spray-drying, have been evaluated as meat extenders against lipid and protein oxidation during the 13 days-shelf-life of beef burgers (5 % fat content) under MAP conditions (80 % O_2 and 20 % CO_2) (Rocchetti et al., 2022). The authors demonstrated that substitution levels of EE at 2.5 and 5 g/kg were able to significantly reduce MDA levels when compared with control samples, reaching at T_{13} levels of 2.570 and 2.029 mg MDA/kg, respectively, thus overcoming the accepted deterioration level.

The gas composition analysis showed that initial O_2 levels (66 %) gradually decreased due to oxidative reactions and microbial respiration (Table 3). By T_7, LLE 1 % and ML 0.1 % retained the highest O_2 levels, indicating slower oxidative degradation. The progressive decline in O_2 and rise in CO_2 across all samples reflected microbial metabolism, particularly by aerobic and facultative anaerobic bacteria such as LAB and *Brochothrix thermosphacta* (Conte-Junior et al., 2020).

In summary, LLE 1 % proved to be the most effective liquid treatment for preserving color and oxidative stability for up to 7 days. Low-dose encapsulated extracts (AGL 0.1 %, ML 0.1 %) also revealed good performances, achieving a balance between controlled antioxidant release and oxidative inhibition. High-dose encapsulated treatments (1 %) were less effective, likely due to not optimal release or pro-oxidant effects. These findings agree with previous reports describing the biphasic behaviour of plant polyphenols in meat systems. Pro-oxidant effects of phenolics have been widely reported both in vitro and in meat systems (Estévez, 2021), especially when pure compounds are used at high doses (Castaneda-Arriaga, Pérez-González, Reina, Alvarez-Idaboy, & Galano, 2018). For instance, Nowak et al. (2022) showed that 5 out of 18 tested phenolics (gallic acid, phloroglucinol, pelargonidin, ellagic acid,

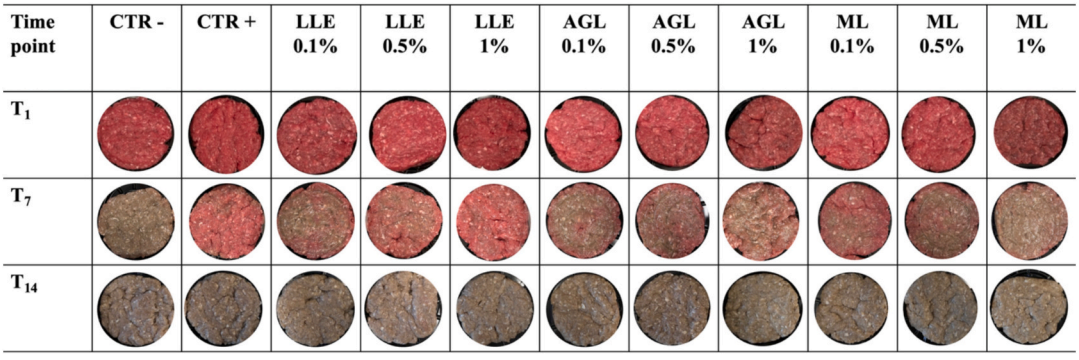

Fig. 1. Visual overview of the formulated beef burgers at different storage time points (T_1, T_7, and T_{14}).

Table 3
Color parameters (L^*, a^*, b^*), malondialdehyde (MDA) content (mg/kg), and changes in MAP composition (O_2% and CO_2%) of beef burgers formulated with different treatments (T) during storage period (SP).

Parameters	SP (days)	CTR-	CTR+	LLE 0.1 %	LLE 0.5 %	LLE 1 %	AGL 0.1 %	AGL 0.5 %	AGL 1 %	ML 0.1 %	ML 0.5 %	ML 1 %	SEM	T	SP	TxSP
L*	1	40.81^{efgh}	39.39^{cde}	38.76^{bcd}	39.46^{cde}	39.20^{bcde}	38.83^{bcd}	38.40^{abc}	38.60^{bcd}	37.41^{ab}	38.92^{bcde}	36.65^{a}	0.224	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	41.92^{ghij}	38.99^{bcde}	40.40^{defg}	39.32^{bcde}	39.96^{cdef}	42.34^{hijk}	41.43^{fghi}	41.67^{fghij}	41.68^{fghij}	40.81^{efgh}	42.48^{hijkl}	0.272			
	14	44.08^{klmno}	42.93^{ijklm}	43.36^{jklmn}	44.30^{lmno}	45.76^{opq}	44.6^{mnop}	46.32^{pq}	48.31^{rs}	45.21^{nop}	47.05^{qr}	49.52^{s}	0.367			
	SEM	0.580	0.714	0.719	0.830	1.074	0.920	1.166	1.473	1.187	1.266	1.878				
a*	1	17.57^{q}	18.87^{r}	15.31^{n}	17.48^{pq}	17.93^{q}	17.40^{q}	16.19^{o}	14.45^{m}	16.86^{op}	16.39^{o}	12.43^{l}	0.323	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	3.89^{d}	10.55^{j}	6.36^{gh}	8.40^{i}	11.39^{k}	5.39^{f}	6.06^{fgh}	5.68^{fg}	6.58^{h}	5.88^{fg}	4.63^{e}	0.399			
	14	2.93^{bc}	3.24^{c}	2.45^{ab}	2.34^{ab}	2.74^{abc}	2.48^{ab}	2.02^{a}	2.49^{ab}	2.16^{a}	2.27^{ab}	2.01^{a}	0.074			
	SEM	2.365	2.260	1.910	2.200	2.206	2.375	2.108	1.792	2.181	2.120	1.566				
b*	1	14.68^{ijklm}	15.58^{m}	13.87^{efghij}	15.35^{lm}	15.56^{m}	15.25^{m}	14.75^{jklm}	14.25^{ghijk}	14.75^{jklm}	14.18^{fghijk}	13.21^{cdef}	0.161	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	12.58^{bcd}	13.36^{defg}	12.39^{abc}	12.47^{abcd}	13.08^{cde}	11.94^{ab}	12.42^{abcd}	13.35^{defg}	11.58^{a}	12.46^{abcd}	12.97^{cde}	0.110			
	14	13.74^{efghi}	13.08^{cde}	13.72^{efghi}	14.17^{fghijk}	14.18^{fghijk}	13.66^{efgh}	14.08^{fghijk}	14.98^{klm}	13.81^{efghij}	14.11^{fghijk}	14.58^{hijkl}	0.103			
	SEM	0.319	0.426	0.267	0.424	0.403	0.574	0.363	0.277	0.495	0.309	0.266				
MDA	1	1.05^{cde}	0.14^{a}	1.03^{cd}	1.03^{cd}	0.97^{bcd}	0.78^{b}	1.07^{cdef}	0.84^{bc}	1.02^{cd}	0.84^{bc}	0.98^{bcd}	0.046	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	1.85^{j}	1.28^{fghi}	1.67^{j}	1.36^{hi}	1.33^{ghi}	1.44^{i}	1.46^{i}	1.71^{j}	1.44^{i}	1.47^{i}	1.69^{j}	0.038			
	14	1.09^{def}	0.78^{b}	1.26^{efghi}	1.04^{cde}	1.35^{hi}	1.18^{defgh}	1.26^{efghi}	1.27^{efghi}	1.12^{defg}	1.11^{defg}	1.02^{cd}	0.032			
	SEM	0.132	0.166	0.100	0.064	0.066	0.100	0.065	0.132	0.081	0.098	0.121				
O_2 (%)	1	62.43^{ijkl}	69.23^{o}	62.80^{jkl}	67.70^{no}	67.90^{no}	62.07^{ijkl}	68.00^{no}	67.53^{no}	66.87^{mno}	68.27^{no}	67.53^{no}	0.442	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	57.23^{gh}	59.73^{hij}	54.10^{fg}	54.93^{fg}	65.33^{lmn}	59.57^{hij}	60.80^{hijk}	58.87^{hi}	63.83^{klm}	59.13^{hij}	51.47^{f}	0.823			
	14	32.23^{bc}	35.77^{cd}	27.67^{a}	33.20^{bc}	45.23^{e}	37.00^{d}	34.90^{cd}	33.33^{bc}	44.07^{e}	37.90^{d}	30.80^{ab}	0.920			
	SEM	4.676	5.107	5.277	5.059	3.619	4.079	5.032	5.174	3.631	4.541	5.367				
CO_2 (%)	1	11.00^{a}	12.37^{ab}	10.77^{a}	11.67^{ab}	11.73^{ab}	10.17^{a}	11.87^{ab}	11.90^{ab}	11.40^{ab}	12.03^{ab}	12.10^{ab}	0.111	$P < 0.001$	$P < 0.001$	$P < 0.001$
	7	22.53^{fg}	17.27^{cde}	23.80^{g}	19.30^{def}	14.93^{bc}	17.80^{cde}	19.07^{de}	19.60^{def}	16.13^{cd}	17.53^{cde}	19.80^{ef}	0.481			
	14	31.97^{ijk}	27.47^{h}	33.27^{jk}	34.47^{k}	28.97^{hi}	28.73^{hi}	33.97^{k}	35.20^{k}	30.07^{hij}	34.27^{k}	39.70^{l}	0.738			
	SEM	3.042	2.403	3.354	3.420	2.647	2.756	3.266	3.450	2.794	3.362	4.146				

Different superscript letters within each analytical parameter indicate significant differences within treatment and storage period combinations. SEM, T, SP and TxSP stand out for the standard error of the mean, the fixed factors treatment and storage period, and the interaction between them, respectively. CTR- (negative control, containing only 1 % NaCl without additives), CTR+ (positive control, including 1 g/kg ascorbic acid in addition to 1 % NaCl), LLE 0.1 %, LLE 0.5 %, LLE 1 % (*L. minor* extract added at 1, 5, and 10 g/kg, respectively), AGL 0.1 %, AGL 0.5 %, AGL 1 % (*L. minor* extract encapsulated in gum Arabic, added to at 1, 5, and 10 g/kg, respectively), and ML 0.1 %, ML 0.5 %, ML 1 % (*L. minor extract* encapsulated in maltodextrin, added at 1, 5, and 10 g/kg, respectively).

pelargonidin-3-*O*-rutinoside) exerted pro-oxidant activity at 5–50 μmol/L, while others such as ferulic acid, chlorogenic acid and cyanidin-3-*O*-glucoside could switch between antioxidant and pro-oxidant roles within the same range. Such behaviour depends on several factors, including dose, solubility, pH, and the presence of transition metals or other redox-active compounds. Phenolic acids, the dominant class in duckweed extracts, generally act as reducing agents but can become pro-oxidants in the presence of oxygen and metal ions (Mu, Yao, Wang, & Kitts, 2023). Their structure is critical, with hydroxyl groups in ortho position enhancing redox reactivity (Rajashekar, 2023), while smaller phenolics tend to be stronger pro-oxidants. Under our conditions, both encapsulated extract at higher concentrations and elevated pH values were linked to a shift toward pro-oxidant activity, consistent with this evidence. The significant interactions for 'storage time × treatment' ($P < 0.001$; Table 3) also confirmed that both liquid and encapsulated extracts influenced color and oxidation dynamics during storage. From a practical standpoint, the antioxidant strategies tested successfully extended the oxidative shelf-life of beef burgers under MAP conditions for up to 7 days, which aligns with consumer expectations for refrigerated fresh meat where color and oxidative stability are critical quality attributes (Domínguez et al., 2019). After this period, oxidation progressed in all samples, irrespective of treatment, highlighting the need for further stabilisation measures and sensory validation.

3.4. Differentiating beef burgers over storage using multivariate statistical analyses

UHPLC-HRMS metabolomics putatively annotated 3126 compounds, with 246 confirmed by MS/MS (Supplementary Table 4). Hierarchical cluster analysis (HCA) showed that storage time was the main factor driving changes in metabolite profiles (supplementary Fig. 1). At T_1, samples clustered separately from T_7 and T_{14}, indicating that metabolic changes intensified over time. Treatments also influenced metabolomic patterns within each storage group. Particularly, at T_1, CTR+ samples showed lower metabolite abundance (mainly blue shades on the heatmap), reflecting the antioxidant effect of ascorbic acid. Duckweed-treated samples had higher metabolite levels, consistent with their polyphenol content. By T_7 and T_{14}, metabolite abundance increased further, reflecting ongoing oxidative and biochemical processes. Principal Component Analysis (PCA) confirmed these trends (supplementary Fig. 1), showing three distinct clusters corresponding to storage times. The separation between T_7 and T_{14} was less clear, suggesting some stabilisation or nonlinear progression of metabolic pathways during extended storage.

Additionally, the AMOPLS analysis (supplementary Table 5) clearly demonstrated that all the experimental factors considered in the study, namely the treatment (T), storage period (SP), and their interaction (T × SP), significantly contributed to the overall metabolic variability observed in the beef burger samples ($P < 0.01$ for all effects). Among them, SP showed the highest relative explained variability (RSS = 26.1 %), confirming the strong influence of shelf-life on the meat metabolome. Interestingly, the T factor alone explained 12.0 % of the total variability, while the interaction between T and SP accounted for 17.6 % of the variance. This indicates that the effect of duckweed extracts (in both liquid and encapsulated forms) on the beef meat metabolome was strongly modulated by the storage time, highlighting the importance of jointly evaluating both factors. The significant interaction also aligns with our two-way ANOVA results for physicochemical parameters, supporting the need to interpret T effects in the context of SP. Overall, the residual variance was moderate (44.3 %), reflecting acceptable experimental variability given the biological nature of the samples and the untargeted UHPLC-HRMS approach. The score plot components (supplementary Table 5) further showed that the main predictive terms for each factor contributed strongly to the model discrimination (R^2Y values >97 %), while the orthogonal components captured residual structured variation, indicating the robustness of the AMOPLS decomposition for complex metabolomics datasets with multi-factorial designs. These results highlight that the antioxidant effect of the duckweed extracts cannot be discussed without considering the storage time, which is fully consistent with the experimental design based on multiple treatment levels and time points. This outcome aligns with our previous findings on liquid duckweed extracts added to beef meat under the same MAP conditions (Rocchetti et al., 2023).

3.4.1. Focus on the metabolomic profile and marker compounds at T_7

Based on the significant interaction highlighted by the AMOPLS results, we subsequently focused our metabolomic interpretation on the 7-day storage time, where the antioxidant treatments still exhibited an ability to retain meat redness, likely stabilising the myoglobin redox state. This time point was therefore considered the most representative for elucidating the specific metabolic pathways modulated by the duckweed extracts. The heatmap in Fig. 2A (T_7) revealed two distinct clusters among the treatments. Cluster 1 grouped CTR− together with high-dose encapsulated extracts (ML 0.5 %, ML 1 %, AGL 0.5 %, AGL 1 %), indicating limited oxidative protection. Cluster 2 included low-dose encapsulated treatments (ML 0.1 %, AGL 0.1 %) and LLE 1 %, highlighting their higher antioxidant efficacy. CTR+ formed a separate cluster, reflecting its short-lived protective effect, as also indicated by its high MDA fold increase (9.28) from the TBARS analysis. Notably, lower concentrations of encapsulated extracts (0.1 %) performed comparably to the liquid extract (LLE 1 %), while higher doses clustered closer to CTR−, confirming a clear dose-dependent pattern.

The OPLS-DA score plot (Fig. 2B) supported these trends, showing clear separation of liquid and low-dose encapsulated treatments from high-dose encapsulated forms. CTR− remained central, serving as the oxidative degradation baseline. VIP analysis (VIP > 1) identified 462 discriminant metabolites, including amino acids (116), fatty acids (114), glycerolipids (63), phospholipids (82), and other minor classes (Supplementary Table 4). This metabolic fingerprint provided mechanistic insights into treatment effects. Lipid oxidation intermediates, such as 9-hydroperoxy-10,12-octadecadienoate and 13-oxo-9-octadecenoic acid, accumulated in antioxidant-treated samples relative to CTR−, suggesting that antioxidant addition slowed oxidation, stabilising intermediate compounds (Amaral, da Silva, & da Silva Lannes, 2018; Domínguez et al., 2019; Hadidi et al., 2022). In contrast, advanced oxidative degradation in CTR− likely resulted in their further breakdown. Effective treatments (LLE 1 %, ML 0.1 %, AGL 0.1 %) also showed elevated glutathione and cystine levels, indicating activation of endogenous antioxidant pathways (Al-Temimi et al., 2023; Paulsen & Carroll, 2013). These compounds mitigate oxidative stress by scavenging reactive oxygen species and regenerating antioxidant capacity. A similar increase in glutathione was previously observed by Rocchetti et al. (2022), evaluating the untargeted metabolomic profile of beef meat packaged for 13 days under MAP conditions and added with encapsulated elderberry extracts.

Interestingly, hemin concentrations (Table 4, Supplementary Table 4) were significantly reduced in AGL 0.5 % and AGL 1 % ($\log_2$ FC: −1.50 and − 1.72), suggesting gum Arabic's potential to chelate hemin and limit its pro-oxidant activity (Carlsen, Møller, & Skibsted, 2005; Zhu et al., 2022). This effect likely relates to gum Arabic's emulsifying behaviour, which stabilises polyphenols and promotes their interaction with heme iron (Lisboa et al., 2025; Zhang, Fan, Li, Chen, & Liang, 2019). However, excessive polyphenol release at higher doses may have overwhelmed antioxidant pathways, shifting toward pro-oxidant effects (Procházková et al., 2011), thus revealing a complex dynamics.

Other important indicators, such as the polyamine spermine and its oxidative catabolite spermine dialdehyde (Ha et al., 1998), were more abundant in CTR− and in high-dose encapsulated treatments (ML 1 %, AGL 1 %; supplementary Table 4). Conversely, their lower levels in LLE 1 % and low-dose encapsulated samples suggest reduced proteolytic activity and microbial spoilage, as well as limited polyamine oxidation. Similarly, tryptamine, a marker of proteolytic activity and microbial

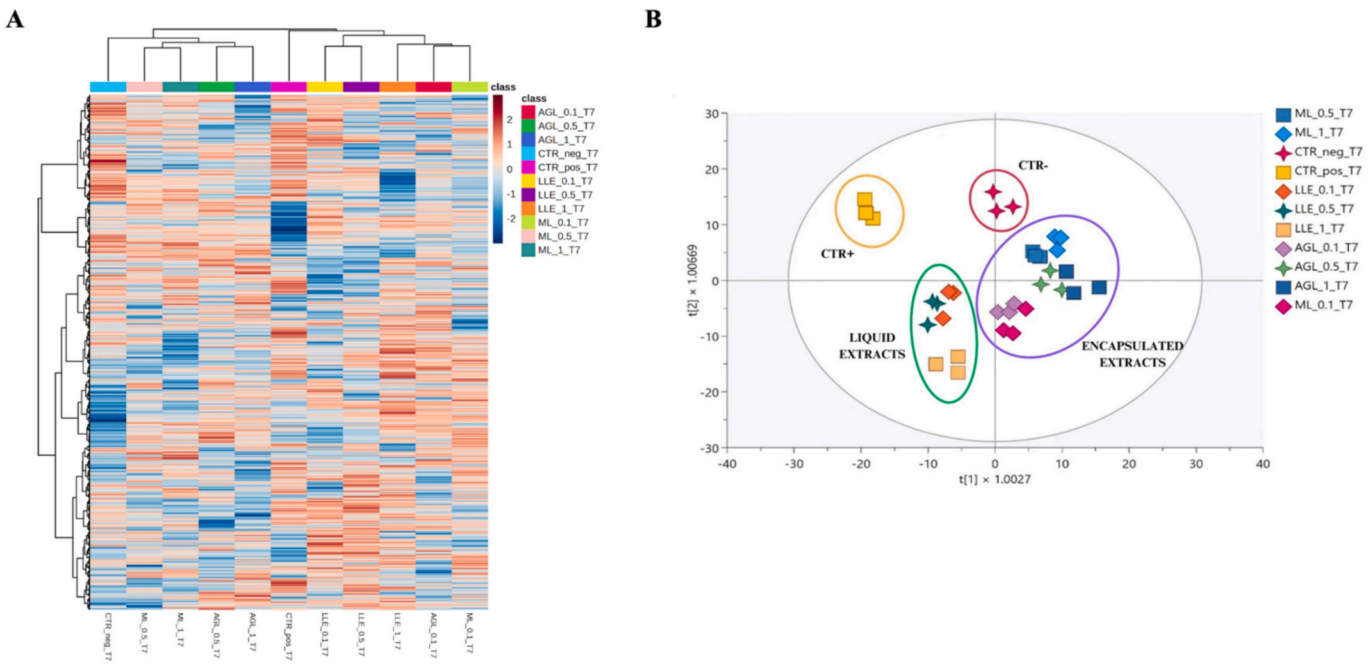

Fig. 2. Heat map (A) from unsupervised hierarchical clustering and score plot (B) from the OPLS-DA supervised modeling, considering 7 days of storage period.

Table 4
Log_2 Fold-Change variations of meat metabolites better associated with redox impairment at T_7 of storage period when considering the best performing treatments (i.e., CTR+, LLE 0.1 %, LLE 0.5 %, LLE 1 %, AGL 0.1 %, and ML 0.1 %) vs the CTR-. ns = not significant.

Meat metabolites	CTR+ vs CTR-	LLE 0.1 % vs CTR-	LLE 0.5 % vs CTR-	LLE 1 % vs CTR-	AGL 0.1 % vs CTR-	ML 0.1 % vs CTR-
4-hydroxy-2-nonenal-[L-Cys] conjugate	0.09	0.41	−0.13	0.18	0.27	−0.27
4-Hydroxy-2-oxoglutaric acid	−1.23	0.02	−1.27	−0.48	−0.11	0.11
Acetylcarnosine	0.58	0.73	−0.03	−0.18	−0.25	0.08
(9S,10E,12Z)-9-hydroperoxy-10,12-octadecadienoate	2.65	2.09	2.76	3.62	2.59	4.94
(Z)-13-Oxo-9-octadecenoic acid	0.28	0.66	−0.73	0.02	0.17	0.10
9-Oxooctadecanoic acid	1.87	−0.66	0.18	1.33	2.47	−2.47
alpha-Linolenic acid	−3.25	−0.15	0.17	−0.32	−0.13	−0.42
Anserine	ns	0.51	0.30	−0.83	0.48	0.82
Glutathione	0.89	0.82	1.34	1.33	1.17	1.24
Glutathione disulfide	−1.11	0.40	−1.94	−0.33	1.32	−2.02
Hemin	−0.56	0.38	0.37	0.44	0.26	−0.62
Asparagine	−0.23	−0.80	−0.38	−0.27	−0.04	0.29
Cystine	0.79	1.64	0.58	1.93	1.64	1.44
Lysine	0.10	0.94	0.95	1.07	0.71	0.92
Serine	0.14	−1.57	−1.38	−1.63	−1.48	−1.74
Tryptophan	−1.42	−2.33	−2.37	−0.57	−0.30	−2.20
Tyrosine	1.23	0.96	1.25	1.82	0.80	1.08
Leucine	−0.87	−0.13	−0.08	0.18	0.82	−0.86
Ornithine	−0.04	0.07	−0.23	−2.06	−0.24	0.17
Putrescine	−0.42	0.54	0.45	0.52	0.70	−0.06
Spermic acid 2	−0.07	−0.13	0.17	0.75	0.44	0.45
Spermine	0.67	1.21	1.35	1.52	1.34	1.11
Spermine dialdehyde	−0.31	−0.36	0.71	1.29	1.03	1.08
Sperminidine	−0.52	−0.13	−0.37	−1.02	−1.30	−0.77
Tryptamine	−1.68	−1.78	−4.49	−3.17	−1.73	−2.76
2-nonenoic acid	−1.38	−0.37	0.22	0.18	0.33	−0.43
Nonadecenoic acid	−2.59	−1.30	−0.09	−1.39	0.06	1.46
Octacosanal	−0.87	0.69	−0.38	0.26	−0.84	−2.14

decarboxylation, also decreased in all antioxidant-treated samples.

Overall, the metabolomic snapshot at T_7 demonstrates that CTR− experienced the most advanced lipid and protein oxidation, while LLE 1 % and low-dose encapsulated extracts effectively mitigated oxidative stress. These findings emphasize that not only the presence of antioxidants, but also their dosage and release kinetics, are critical to maintaining oxidative stability and avoiding pro-oxidant outcomes during refrigerated storage.

3.4.2. *In silico binding affinity and interactions of caffeic acid with myoglobin*

Given the high abundance of caffeic acid (CA) in the duckweed extracts (Supplementary Table 1), we carried out docking against myoglobin to explore a molecular basis for the observed antioxidant effects. CA's antioxidant activity is primarily due to its ability to chelate Fe^{2+} ions, thus preventing hydroxyl radical formation via the Fenton reaction (Genaro-Mattos, Maurício, Rettori, Alonso, & Hermes-Lima, 2015). This chelation is pH-dependent, being most effective at neutral pH (~80 % at pH 7.2) and still significant (~36 %) under slightly acidic conditions like those in meat (pH 5.5). By disrupting the Fenton reaction, CA helps preserve oxymyoglobin and reduce meat discoloration.

An in silico docking study (SwissDock, Fig. 3A) was therefore useful to evaluate the potential interaction between CA and myoglobin. The binding free energy (ΔG) was −5.6 kcal/mol, with a highly favorable AC score of −27.41 kcal/mol, indicating strong ligand-protein affinity. The SwissParam score (−6.19) reflected good interaction despite some solubility-related penalties. CA established four hydrophobic contacts (Val-69, Leu-90, Ile-108, Leu-105) and one ionic interaction with His-94 (Fig. 3B). These interactions are compatible with localisation near the heme environment and may reduce hemin dissociation, aligning with observation of reduced hemin levels in gum Arabic-encapsulated samples (AGL 0.5 % and AGL 1 %) and with the improved redness and lower TBARS observed in the best performing treatments, thereby linking molecular binding, redox metabolite changes and macroscopic oxidative stability. CA's interaction with myoglobin and haemoglobin is known to inhibit oxidative reactions in meat (Kassa, Whalin, Richards, & Alayash, 2021; Park, Undeland, Sannaveerappa, & Richards, 2013). Its antioxidant effect is mainly attributed to stabilising the heme group, preventing hemin dissociation from metHb, a process that otherwise generates free

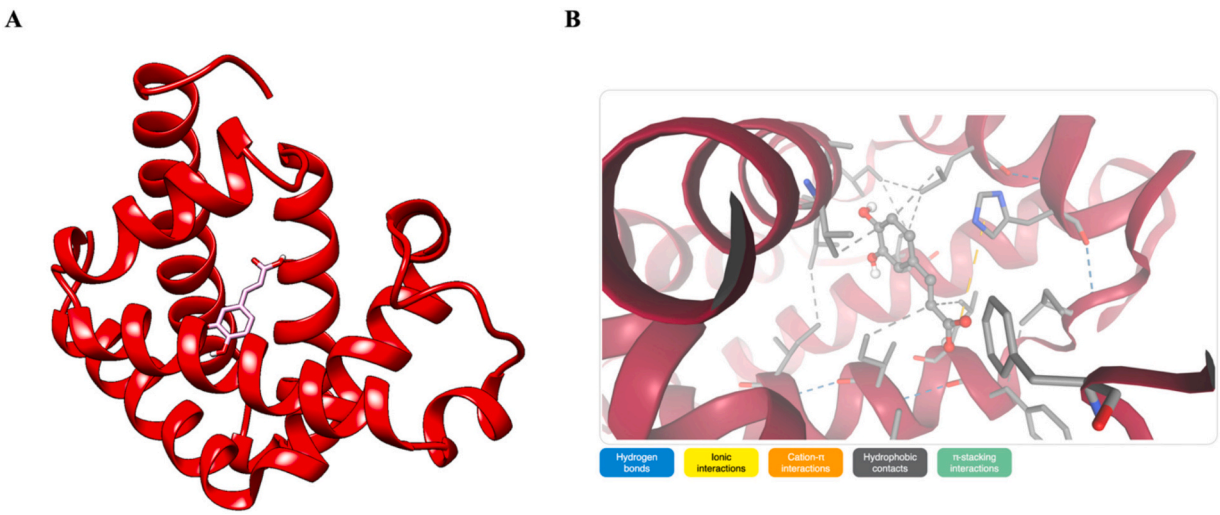

Fig. 3. In silico docking visualization (A) showing also the interactions between caffeic acid and the myoglobin protein (B).

radicals and accelerates lipid oxidation. By binding to hemin or metHb, CA can limit oxidative damage and contributes to maintaining meat color during storage (Park et al., 2013). In this work, we demonstrated that this technological role is strictly dependent from the percentage of encapsulated extract added to beef meat acting 14 days under MAP conditions.

4. Conclusions

This study demonstrated that *L. minor* extracts, applied in both liquid (LLE) and encapsulated forms (AGL, ML), significantly influenced the oxidative stability and color preservation of beef burgers stored under modified atmosphere packaging (MAP). The liquid extract at 1 % (*w*/w) and the encapsulated extracts at 0.1 % (w/w) were identified as the most effective dosages, maintaining redness and reducing lipid oxidation up to 7 days of storage. In contrast, higher concentrations of encapsulated extracts (0.5–1 %) were less effective and in some cases promoted pro-oxidant effects, suggesting that excessive polyphenol release may impair oxidative balance. Encapsulation yields were high (~80 %) for both gum Arabic and maltodextrin, but gum Arabic provided superior retention of antioxidant activity and a more controlled release of bioactives. Untargeted metabolomics confirmed these results, highlighting modulation of oxidative pathways such as glutathione metabolism, hemin chelation, and reduced accumulation of proteolysis-derived compounds (e.g., tryptamine, spermine dialdehyde). These metabolic insights further explain the observed improvements in color stability and oxidative control. From a practical perspective, the results suggest that *L. minor* extracts, particularly when encapsulated at low concentrations, can serve as sustainable, plant-based antioxidants to partially replace synthetic preservatives in fresh meat products. The optimal application appears to be 0.1 % (w/w) for encapsulated forms and 1 % (w/w) for liquid extracts, as these dosages maximised oxidative protection without inducing pro-oxidant effects. While biochemical assays and metabolomics demonstrate that duckweed extracts modulate redox homeostasis and mitigate oxidation, the docking results offered a plausible molecular mechanism; targeted follow-up experiments (e.g., spectroscopic binding studies or hemin-release assays) would be valuable to validate the predicted interaction in vitro. Nevertheless, some limitations must be acknowledged; no antimicrobial effect was observed, and sensory or technological parameters were not evaluated in this study. Now that *L. minor* has been authorised as a novel food in the EU, future research should focus on consumer acceptability, sensory quality, and technological traits (e.g., texture, cooking behaviour), as well as synergistic combinations with other natural preservatives. Overall, this work provides the first evidence that duckweed extracts, supported by encapsulation strategies, represent a novel and sustainable approach to extend the oxidative shelf-life of beef burgers while aligning with the clean-label and circular bioeconomy trends in the meat industry.

Consent form

This work involves no experimentation with human subjects.

CRediT authorship contribution statement

Gabriele Rocchetti: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Annalisa Rebecchi:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Michele Dallolio:** Writing – original draft, Methodology. **Daniele Del Buono:** Writing – review & editing, Conceptualization. **Giorgio Freschi:** Writing – review & editing. **Gokhan Zengin:** Writing – original draft, Methodology, Data curation. **Luigi Lucini:** Writing – review & editing, Supervision, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meatsci.2025.109975.

Data availability

Data will be made available on request.

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