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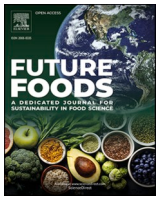

Plant-based burgers: effects of protein source, type of extrusion and cooking technology on oxidation status and in vitro digestibility

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ABSTRACT

The effects of cooking method (microwaving/pan frying) and in vitro digestion of plant-based burgers formulated with different protein types (soy/pea) and extrusion types (low/high moisture) were assessed. A conventional beef burger was used as control. Every formulation was adjusted to 18.1–19.5 % of protein. Plant-based burgers showed lower cooking losses (4–13 %) than beef burgers (26–30 %), with microwaving resulting in the highest losses across all samples. Plant-based burgers formulated with high moisture extrusion showed the lowest protein digestibility (66.8–73.2 %). No effect of the cooking method was observed in lipid and protein digestibility in any sample. Soluble peptides increased during in vitro digestion in all samples, with beef burgers showing the lowest levels. The amount of malondialdehyde increased whereas carbonyls decreased in the bioaccessible fraction in all samples during in vitro digestion. Burgers formulated with low moisture extrusion showed the highest lipid oxidation, both after cooking and after in vitro digestion. DPPH values decreased (17–59 %) and ABTS increased (277–810 %) during in vitro digestion. These results suggest that the protein digestibility and lipid oxidation of plant-based burgers (before and after digestion), are more strongly influenced by the type of extrusion than by the type of protein or cooking method applied.

Introduction

The use of protein alternatives to reduce the consumption of foods from animal origin is increasing. The need to decrease the environmental impact of livestock production as well as the growing concern about health and animal welfare, have led to the development of new ingredients and products based on non-animal raw materials. One of the most interesting alternatives, from the economic perspective, consumer acceptability and support for the rural world, is the use of plant proteins as substitutes for animal proteins (Astiasarán et al., 2022). According to consumer perception, plant-based meat alternatives already seem to be fairly well established (Lanz et al., 2024).

Plant-based meat analogues (PBMAs) can be defined as textured plant protein products that aim at mimicking meat in appearance, nutrient content, and sensory properties, such as color, texture, mouthfeel, and nutrient bioavailability (Ishaq et al., 2022; Vatansever et al., 2020). Moreover, they can be a sustainable and healthy part of our future protein landscape (Bryant, 2022). However, it is a challenge to develop meat product analogues due to the differences in the nature and technological properties between animal and plant proteins.

Extrusion technology is applied to transform globular proteins from plant-based concentrates or isolates into more fibrous structures. This process aims to mimic the textural properties of products made with animal proteins. The low moisture extrusion process (10–35 % moisture) results in a textured dried protein with a not well-defined fiber structure, requiring hydration before consumption. In contrast, high moisture extrusion (40–80 % moisture) produces a textured protein with a wet, fibrous structure, that closely resembles conventional meat (Samard and Ryu, 2019). The source of protein and the conditions of the technological process -such as extrusion- used to optimize the technological and sensory properties of the proteins used, can positively or negatively influence the bioaccessibility of nutrients (Peñaranda et al., 2025). Indeed, to date, no studies have investigated the effect of the different extrusion technologies on the oxidation status of the final plant-based meat analogs, particularly after in vitro digestion.

Regarding ingredients, protein is the most important component in meat analogues due to its structuring, texturizing, emulsifying and water-holding capacity properties (McClements and Grossmann, 2021).

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Soy and pea proteins have good nutritional quality and are the most used proteins for meat analogues (Singh et al., 2021), giving a good fibrous texture to the final products (Ishaq et al., 2022).

Besides the textured and non-textured protein, a typical plant-based meat analogue includes vegetable oils, binder agents, and several additives, flavors, or seasonings (Xie et al., 2024). The type of oil used determines the fat quality of the final product, as it is the primary lipid source. Binder agents, such as polysaccharides like methylcellulose, gums, or starches are required to maintain cohesion and prevent the loss of water and oil. Additives extend shelf life, provide antioxidants, and improve sensorial attributes such as color, smell and flavor.

The cooking process is essential for ensuring food safety, inhibiting anti-nutrient components, and achieving desirable organoleptic characteristics, such as flavor and texture (Bhat et al., 2021). However, it can also cause some changes in the composition and oxidation of lipids and proteins, which could affect nutrient digestibility. Factors such as the cooking technique, temperature, and heating exposure time, are key determinants of the quality of the final products. As for the cooking method is concerned, microwaves, previously used by our group in patties (Ansorena and Astiasaran, 2024), has been shown to improve the safety and quality of foods, involving rapid heating and short cooking time, and facilitating nutrient absorption (Meenu et al., 2024; Nguyen and Songsermpong, 2022). Pan frying is also a common cooking method for this type of samples (Cutroneoet al., 2024; Zhou et al., 2022), but the consequences of its application on oxidative processes remains to be explored. Notably, oxidation occurs not only during storage or cooking, but also during the gastrointestinal track, where the pro-oxidant environment promotes the formation of oxidation products (Dong et al., 2020; Nieva-Echevarría et al., 2020). This phenomenon is well known in meat products, but much less studied in plant-based meat analogues, making it an interesting subject for further research.

The aim of this work was to study the influence of various factors, including the type of protein, type of extrusion, and cooking technique, on the protein and lipid fractions of self-made plant-based burgers before and after being subjected to an in vitro digestion process. A comparison with conventional beef burgers was also carried out.

Materials and methods

Raw materials

Four types of extruded products (pea or soy, both obtained by low or high extrusion processes) were obtained from Sanygran S.L (Tudela, Spain). According to the product specification sheets on wet matter basis, the soy-based high moisture extruded product consisted of 65 % of water, 24 % protein, 7.9 % dietary fiber, 1.7 % fat, 0.6 % carbohydrates, and 0.8 % salt. The soy-based low moisture extruded product consisted of 50 % protein, 22 % carbohydrates, 16 % dietary fiber, 10.5 % of water, and 1.5 % fat. The pea-based high moisture product consisted of 64.7 % of water, 29 % protein, 3.9 % fat, 1.43 % salt, and 1 % carbohydrates. The pea-based low moisture extruded product consisted of 60 % protein, 10 % dietary fiber, 17.5 % carbohydrates, 5.85 % of water, 5.7 % fat, and 1 % salt. The isolated pea protein (pea protein F85M) was obtained from the Roquette company, and the isolated soy protein from T. Aliment, Espècies Teixidor S.L. (Barcelona, Spain). The cacao (RV 4 V) was from Indcresa S.A. (Barcelona, Spain), and the beetroot powder from Nanosalud (Saludviva S.L., Elche, Spain).

Beef minced meat was purchased in a local butcher. All the ingredients used for burger formulations were purchased from commercial companies. The chemical reagents, enzymes, and bile used in the in vitro digestion were purchased from Sigma Chemical (Co. St. Louis, Mo, USA).

Beef and plant-based burgers formulation

The four plant-based burgers - low moisture extruded soy protein burger (LS), low moisture extruded pea protein burger (LP), high moisture extruded soy protein burger (HS), and high moisture extruded pea protein burger (HP) – were formulated to supply the same protein amount as the beef burger (BB). All ingredients and their respective quantities are listed in Table 1. Minor adjustments in water, gelled emulsion, protein isolates, binding agents, and spices were needed to ensure comparable protein content and appropriate technological and sensory characteristics in all plant-based burgers. Low moisture extruded products required hydration with boiling water before mixing with the rest of ingredients. High moisture extruded products were grinded before use (Bosch chopper MMR08R2). The use of cocoa and beetroot extract in the plant-based formulations aimed to mimic the color of meat burgers; therefore, they were not added to the beef burger. The spices and extract included in each formulation were selected to achieve the desired sensory properties, and the required amounts for each formulation were determined through preliminary laboratory tests. The gelled emulsion used contained 40 % virgin olive oil, and it was prepared according to Alejandre et al. (2016). Figure S1 of Supplementary material describes the elaboration of the different types of burgers.

For every type of burger formulation, 20 units (110 g/ unit) were prepared. Two units were separated for analysis in the raw state, and the rest (18 units) for being cooked (9 units for pan frying and 9 units for microwaving). All samples were cooked (pan fried or microwaved) in three different days, giving rise to three different batches.

For microwave cooking, each burger was individually cooked for 1 min per side at 900 W. For pan frying, the pan was first pre-heated with a negligible amount of olive oil, and burgers were cooked, turning them every two minutes (internal temperature 72 °C). All samples were minced, mixed, and kept in vacuum conditions at −20 °C until analysis or until being subjected to in vitro digestion.

Proximate composition

Moisture, ash and protein content of raw and cooked burgers were analyzed following official methods 950.46, 920.153, and 928.08 (AOAC, 2002b, 2002a, 2002c). The nitrogen to protein conversion factor used was 6.25. For fat content determination, Soxhlet extractor B-811 Büchi Extraction System (BÜCHI Labortechnik AG, Flawil, Switzerland) was used (Ariz et al., 2024). Carbohydrate percentage was calculated by difference: 100 % - (protein % + ash % + moisture % + fat %) and energy value with conversion factors (4 kcal/ g of protein or carbohydrate and 9 kcal/ g of fat). Each parameter was measured per triplicate in each batch.

Table 1
Formulations of the five types of burgers.

g per 100 g of product	LS	LP	HS	HP	BB
Minced beef meat	–	–	–	–	98.3
Soy-based low moisture extruded product	22.7	–	–	–	–
Pea-based low moisture extruded product	–	23.6	–	–	–
Soy-based high moisture extruded product	–	–	56.2	–	–
Pea-based high moisture extruded product	–	–	–	51.2	–
Water	43.1	42.5	9.7	18	–
Gel emulsion (60:40 water: virgin olive oil)	22.9	20.5	23.3	20	–
Methylcellulose	1	2	1.9	1.9	–
Guar gum	1	2	1.9	1.9	–
Isolated soy protein	6.9	–	3.9	–	
Isolated pea protein	–	7.3	–	4.4	–
Salt	0.9	1	1.2	1.1	0.8
Black pepper	0.2	0.2	0.2	0.2	0.2
Red pepper	0.1	0.1	0.1	0.1	0.1
Dried garlic	0.4	0.4	0.5	0.5	0.2
Dried onion	0.4	0.4	0.4	0.5	0.4
Cocoa	0.1	0.1	0.4	0.1	–
Beetroot extract	0.4	0.4	0.4	0.3	–

LS: Low Moisture Extruded Soy Protein Burger; LP: Low Moisture Extruded Pea Protein Burger; HS: High Moisture Extruded Soy Protein Burger; HP: High Moisture Extruded Pea Protein Burger; and BB: beef Burger;.

Cooking loss

Each burger was weighed both before and after being cooked. After cooking, the burgers were weighed once they cooled to room temperature. The cooking loss (%) was calculated according to the Eq. (1):

$$\text{Cooking loss } (\%) = \frac{\textit{Weight before cooking } (g) - \textit{Weight after cooking } (g)}{\textit{Weight before cooking } (g)} x\,100 \quad (1)$$

Each analysis was performed in triplicate.

In vitro digestion

Cooked samples were subjected to an in vitro digestion process, that was done according to the INFOGEST method (Brodkorb et al., 2019). This included oral, gastric and intestinal digestions steps. For the oral phase, 5 g of sample (burger) was mixed with 4 mL simulated salivary fluid, 0.475 mL of water, 25 μL of 0.3 M $CaCl_2(H_2O)_2$, and 0.5 mL of α-amylase (equivalent to 75 U/mL final digestion volume) and incubated at 37 °C for 2 min in a water bath. Secondly, gastric digestion phase was carried on at 37 °C during 2 h adding 8 mL of simulated gastric fluid, 5 μL of 0.3 M $CaCl_2(H_2O)_2$, 0.5 mL of pepsin (equivalent 2000 U/mL final digestion volume) and 0.5 mL of lipase (equivalent 60 U/ mL final digestion volume). The pH was adjusted to 3 with HCl and water was added to rise 20 mL. For the intestinal phase, 3.5 mL of simulated intestinal fluid, 2.5 mL bile salt (10 nM), 40 μL of 0.3 M $CaCl_2(H_2O)_2$, 5 mL of pancreatin from porcine pancreas (equivalent 100 U/mL of trypsin), and 5 mL of lipase (equivalent 2000 U/mL final digestion volume) were added. The pH was adjusted to 7 with NaOH and water was added to rise final volume to 40 mL. Resulting digested samples were centrifuged at 2000 rcf, at 4 °C for 50 min (A-4–62 Rotor, Eppendorf centrifuge 5810R Eppendorf, Barcelona, Spain) to separate the bioaccessible phase (micellar phase) from the residual fraction (pellet). The fractions were stored at −20 °C until analysis. Three in vitro digestions were done per each type of cooking sample. A blank digestion was also carried out, replacing the sample by water.

In vitro dry matter digestibility (IVDMD)

To calculate the in vitro dry matter digestibility the pellet of each digested sample (including the blank) was dried in an oven at 80 °C/6 h and then at 45 °C until constant weight.

The Eq. (2) was used to calculate IVDMD (%), according to Khemiri et al. (2021) and Fradinho et al. (2020):

$$\text{IVDMD } (\%) = \frac{DWS\ (g) - DWP(g)}{DWS\ (g)} x100 \quad (2)$$

Where DWS refers to the dry weight of sample submitted to digestion and DWP refers to the dry weight of the pellet. The pellet weight was blank-corrected.

In vitro protein digestibility (IVPD)

The protein content of dried pellets (including the blank) was analyzed using the Kjeldahl method and then IVPD was calculated using the Eq. (3) (Khemiri et al., 2021):

$$\text{IVPD } (\%) = \frac{PDS\ (g) - PDP\ (g)}{PDS\ (g)} x100 \quad (3)$$

Where PDS refers to the protein content of the dry sample submitted to digestion and PDP refers to the protein content of the dry pellet. The protein content of pellet was blank-corrected.

In vitro lipid digestibility (IVLD)

The fat content of the micellar phase was extracted using chloroform: methanol (2:1 v/v) and then a titration with NaOH 0.01 M was done. Ethanol: diethyl ether (1:1 v/v) was used to dissolve the fat content and phenolphthalein was used as indicator. IVLD was calculated using the Eq. (4) (Zhou et al., 2021):

$$\text{IVLD (g FFA / 100 g fat)} = \frac{\textit{Free fatty acids amount in digested micellar phase } (g)}{\textit{Fat amount in sample submitted to digestion } (g)} x100 \quad (4)$$

The free fatty acids (FFA) amount of micellar phase was blank-corrected.

Fatty acids profile

The total fatty acid profile was determined in raw, cooked and digested (micellar phase) samples according to a previous paper (Ariz et al., 2024). Briefly, the fat was extracted (Folch method), saponified and derivatized into fatty acids methyl esters (FAME), which were analyzed by a gas chromatography with a flame ionization detector (FID) on a Perkin Elmer Clarus 500 (Perkin Elmer, Shelton, CT, USA). The results were expressed as g of fatty acids/ 100 g of total fatty acids. The analysis was performed in triplicate.

Lipid oxidation by MDA determination

Malondialdehyde quantification was used as a lipid oxidation indicator. The analysis was performed in cooked samples and in the digested micellar phase, following the method described by Sobral et al. (2020). Results were expressed as mg MDA/ kg sample. The analysis was performed in triplicate.

Protein oxidation by total carbonyls determination

Carbonyls measurement was analyzed in cooked samples and in the digested micellar phase using the method described by Ganhão et al. (2010). Carbonyl concentration, expressed as nmol carbonyl per mg of protein, was calculated using the following equation:

$$\text{Carbonyls (nmol carbonyl / mg Protein)} = \frac{A370}{\varepsilon hydrazone,\ 370\ x\ (A280 - A370\ x\ 0.43)} x10^6 \quad (5)$$

Where, $\varepsilon_{hydrazone}$, 370 is 22,000 M^{-1} cm^{-1}, and $\varepsilon_{hydrazone}$, 280/ $\varepsilon_{hydrazone}$, 370 is 0.43.

The analysis was performed in triplicate.

TCA soluble peptides

The TCA soluble peptides concentration in cooked samples and in digested micellar phase was obtained using the protocol described by Ketnawa & Ogawa (2019) and the results were expressed as mg of tyrosine/ g of sample. The analysis was performed in triplicate.

Antioxidant capacity

To analyze the antioxidant capacity of cooked and digested samples, an extract of 2.5 g of lyophilized sample (cooked burgers or micellar phase) in 50 mL of 70 % ethanol was prepared. Then, the DPPH and ABTS assays were carried out as described previously (Ariz et al., 2024). Results were expressed as μg of Trolox/ g of sample and the analysis was performed in triplicate.

Statistical analysis

Tables and figures depict mean and standard deviations of all data obtained for each sample. The normality of the variables was checked by the Shapiro–Wilk test. The batch effect (samples processed on different days) was analyzed (ANOVA) and no significant differences were found ($p > 0.05$). A Student t-test was applied to evaluate statistically significance differences ($p < 0.05$) between samples before and after digestion. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test, was applied to evaluate statistically significant differences ($p < 0.05$) among sample types for each parameter. The Pearson correlation coefficient was used to assess the linear relationship between the amount of spices and the antioxidant capacity (DPPH and ABTS). The software used for all the analysis was the STATA 15 program (Stata Corp LLC, TX, USA).

Results and discussion

Proximate composition

The proximate composition of raw and cooked burgers is presented in Table 2. Regarding the composition of raw samples, protein content showed similar results in all samples (18.1–19.5 %). Since the goal of PBMA is to replace meat as the primary protein source, the formulations developed in this study aimed to match the protein content of a conventional beef burger. Therefore, considering the different protein concentrations of the extruded products used, different formulations were used, resulting in some compositional differences. Although a limitation of this work was the need to modify the formulation of the developed plant-based burgers in order to achieve comparable protein content and acceptable sensory characteristics, this issue has been addressed and justified in the discussion.

The fat content ranges from 8 to 10 %, being much lower than in beef burgers (16.8 %). Fatty acid profiles were also very different when comparing plant-based and beef burgers as a consequence of the different raw materials used (see supplementary material Table S1). Plant-based burgers showed very high monounsaturated fatty acid (MUFA) values (60–66 %), due to the use of olive oil as an ingredient in the formulations. The amount of polyunsaturated fatty acids (PUFAs) ranged from 14 % to 20.5 % in plant-based products, and it was only 3.5 % in beef burgers. Regarding saturated fatty acids (SFAs), their amount was very similar in all plant-based burgers (19–19.5 %) and much lower than in beef burgers (47 %).

Unlike meat products, plant-based meat analogues need binder agents such as starch, guar gum, pectin, or methylcellulose, among others, to stabilize the food matrix. In addition, extruded plant products have a significant carbohydrate amount, in contrast to the negligible amount present in meats. As a consequence, noticeable differences were observed in the amount of carbohydrates: 9.1–14.7 % in plant-based burgers and 1.3 % in beef ones. Although the dietary fiber was not determined, it is assumed that part of the carbohydrates correspond to the fiber from the binders and the extruded products. The amount of binders (methylcellulose and guar gum) included in the products was 2–4 % which implied a significant source of non-digestible carbohydrates. The ash content in raw plant-based burgers was also higher than in beef burgers (2.5 % and 1.7 %, respectively).

As a consequence of the differences found in the macronutrient contents, the energy values were significantly lower in plant-based burgers (206 kcal/ 100 g) than in beef burger (254 kcal/ 100 g).

The five types of burgers were submitted to two different heating technologies, microwaving (Mw) and pan frying (PF), and their acceptability was internally tasted by the staff of the laboratory. Cooking losses were significantly lower in plant-based products (4–13 %) than in beef products (26–30 %) (Table 2). These results agree with those from Zhou et al. (2022), who found that cooking loss, when applying pan frying technology, was much higher in beef burgers than in plant-based

Table 2
General composition of raw and cooked burgers and cooking loss.

		Protein (%)	Fat (%)	Ash (%)	Water (%)	Carbohydrates (%)	Cooking loss (%)
LS	Raw	18.3 ± 0.2^{a}	10.1 ± 0.2^{cd}	2.4 ± 0.3^{bc}	56.9 ± 0.2^{gh}	12.3 ± 0.4^{de}	NA
	PF	20.1 ± 0.8^{b}	10.4 ± 0.7^{cd}	3 ± 0.1^{gh}	53.6 ± 0.6^{de}	12.9 ± 0.6^{de}	4.2 ± 0.5^{a}
	Mw	22.5 ± 1.1^{d}	11.5 ± 0.4^{e}	3.5 ± 0.1^{i}	48.4 ± 0.8^{b}	14.1 ± 0.8^{ef}	13.0 ± 1.4^{c}
LP	Raw	18.1 ± 0.5^{a}	9.8 ± 0.3^{bc}	2.6 ± 0^{cdef}	54.8 ± 0.2^{ef}	14.7 ± 0.5^{f}	NA
	PF	20.6 ± 0^{bc}	9.9 ± 0.5^{c}	2.9 ± 0.1^{efgh}	50.9 ± 0.9^{c}	15.6 ± 0.8^{f}	4.5 ± 0.6^{a}
	Mw	22.1 ± 1.1^{d}	10 ± 0.8^{c}	3.1 ± 0^{h}	46.8 ± 0.2^{a}	17.9 ± 1.5 g	11.8 ± 1.4^{c}
HS	Raw	18.2 ± 0.5^{a}	10.2 ± 0^{cd}	2.6 ± 0^{cdef}	59.1 ± 0.1^{i}	9.9 ± 0.3^{bc}	NA
	PF	18.7 ± 0.3^{a}	10.7 ± 0.5^{cde}	2.6 ± 0.2^{cd}	56.1 ± 0.6^{fg}	11.9 ± 0.2^{d}	4.2 ± 0.5^{a}
	Mw	21.7 ± 0.7^{cd}	11.1 ± 0.4^{de}	2.9 ± 0.1^{fgh}	52.7 ± 0.8^{d}	11.6 ± 0.7^{cd}	11.8 ± 1.3^{c}
HP	Raw	19.5 ± 0.9^{ab}	8.1 ± 0.3^{a}	2.6 ± 0^{cde}	60.7 ± 0.1^{ij}	9.1 ± 0.8^{b}	NA
	PF	22.3 ± 0.9^{d}	8.8 ± 0.5^{ab}	2.8 ± 0^{defg}	57.3 ± 0.8^{h}	8.7 ± 0.1^{b}	6.9 ± 1.0^{b}
	Mw	22.5 ± 0.5^{d}	8.3 ± 0.6^{a}	3.1 ± 0.3^{h}	54 ± 1^{e}	12.1 ± 0.6^{de}	12.1 ± 1.4^{c}
BB	RAW	18.2 ± 0.1^{a}	16.8 ± 0.4^{f}	1.7 ± 0^{a}	62 ± 0.1^{j}	1.3 ± 0.3^{a}	NA
	PF	26.2 ± 0.7^{e}	15.8 ± 0.8^{f}	2.1 ± 0.2^{b}	56 ± 0.8^{fg}	0 ± 0.6^{a}	26.7 ± 1.8^{d}
—	MW	27.4 ± 0.6^{e}	16 ± 0.7^{f}	1.8 ± 0^{a}	54.2 ± 1^{e}	0.7 ± 0.3^{a}	29.6 ± 1.5^{e}

LS: burger with low moisture extruded soy protein; LP: burger with low moisture extruded pea protein; HS: burger with high moisture extruded soy protein; HP: burger with high moisture extruded pea protein; BB: beef burger; PF: pan frying; Mw: microwave; NA: Not applicable. Carbohydrates were obtained by difference. ANOVA and Tukey post hoc test were applied to analyze, for each parameter, differences among the samples (raw, pan fried and microwaved samples of 5 burger types). Different letters within the same column indicate significant differences ($p < 0.05$).

meat analogues (40 and 10 %, respectively). Those authors explained these results by the protein denaturation of muscle fibers in beef burgers, which causes shrinkage and fluid leakage, whereas in plant-based samples, protein was already denatured before cooking. In addition, the use of hydrocolloids in the formulation of plant-based burgers was probably the cause of their higher water-holding capacity. Also, in the present work, it was shown that microwaving gave rise to higher cooking losses than pan frying in all types of burgers, similarly to what was found in other works (Domínguez et al., 2014; Oz et al., 2017). These cooking losses were mainly driven by dehydration, but also by certain fat losses observed in the leaking fluid. As compared to pan frying, that forms a firm crust that contributes to retain water inside the burgers, the high electromagnetic field applied in microwaving cause protein denaturation, facilitating water and fat release (Domínguez et al., 2014). Water losses between 5–7 % were observed for pan fried products and 11–15 % for microwaved ones, in the case of plant-based burgers. In beef burgers, water losses were 10 % (for pan frying) and 12 % (for microwave cooking).

As a consequence of these cooking losses, cooked samples showed some modifications in their composition when compared to raw samples. These changes especially affected the amount of protein in beef burgers, which increased significantly (up to a final content of 27 %), whereas in plant-based samples, the increase was more subtle (to 18.7–22.5 %). These increases were, in general, more relevant in samples cooked in the microwave, except for beef burgers and burgers with high moisture extruded pea protein, that did not show differences when compared to pan fried samples. The fat content did not change with cooking, and neither fatty acid fractions underwent any relevant

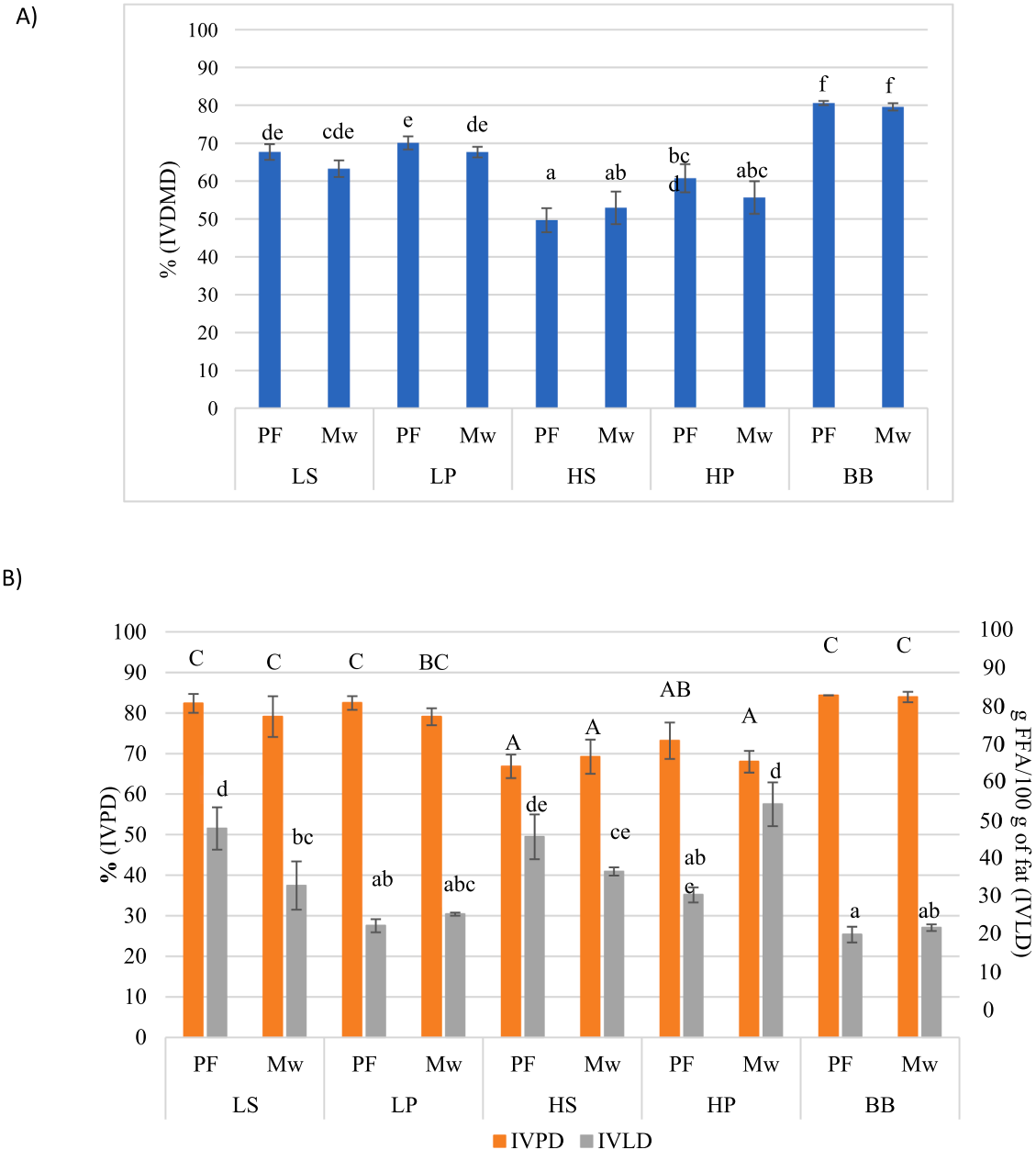

Fig. 1. A) In vitro dry matter digestibility (IVDMD) and **B)** I*n vitro* protein digestibility (IVPD) and in vitro lipid digestibility (IVLD) of burgers. LS: burger with low moisture extruded soy protein; LP: burger with low moisture extruded pea protein; HS: burger with high moisture extruded soy protein; HP: burger with high moisture extruded pea protein; BB: beef burger; PF: pan frying; Mw: microwave; FFA: free fatty acids. ANOVA and Tukey post hoc test were applied to analyze differences among samples (capital letters for IVPD and small letters for IVLD) (different letters indicate significant differences, $p < 0.05$).

changes (see Supplementary Material Table S1). Regarding carbohydrates, some differences were observed with cooking in plant-based products, with slight increases when microwave was used in the case of pea protein products. However, no differences were noticed for the beef burgers.

In vitro dry matter, protein, and lipid digestibility

All samples were submitted to an in vitro digestion process to evaluate the potential availability of nutrients and bioactive compounds. The in vitro dry matter digestibility (Fig. 1A) was clearly lower for plant-based burgers (< 70 %) compared to beef burgers (around 80 %). This parameter is expressed on a dry matter basis, and therefore was not influenced by the differences observed in the moisture content among the cooked samples. The lower digestibility of plant-based foods is attributed to the presence of fibers and antinutritional factors, which could inhibit the enzymatic activity and reduce further nutrients absorption. Ariz-Hernandez et al. (2025) found lower values in beef-based dishes than those obtained in this work for beef burgers. In the case of foods formulated with vegetables, a wide range of total digestibility values has been found (50–90 %) (Ariz-Hernandez et al., 2025; Giura et al., 2024; Khemiri et al., 2021). The different formulations used in these types of foods could explain these differences.

The digestibility of the two main macronutrients, proteins and lipids, was also calculated (Fig. 1B). In these cases, differences between beef and plant-based burgers were not so clear. The highest protein digestibility values were found for beef burgers and burgers produced using low moisture extrusion (79–84 %), whereas those produced with high moisture extrusion showed lower values, ranging from 67 % to 73 %, both for soy and pea sources. Xie et al. (2022) reported higher protein digestibility in beef meat than in their respective analogue (formulated with protein from soybean, rice, pea and mug bean). Still, extrusion technology has been reported to increase digestibility as compared to non-extruded plant proteins, as high temperature and pressure denature proteins thereby exposing more enzymatic cleavage sites. Therefore, the digestibility of plant protein is improved with this technology, as compared to non-extruded plant protein (Fu et al., 2024). Wang et al. (2024) found different effects of high moisture and low moisture extrusion in the protein digestibility of three plant protein products: pea-soy and soy protein products had higher digestibility values with low moisture extrusion, and peanut-pea products had higher digestibility values with high moisture extrusion. They concluded that the extrusion process can affect protein digestion, either positively or negatively, depending on the type of plant protein used. In a recent work, analyzing the effect of high moisture extrusion in different isolated pulse proteins, it was found that the different protein subunit composition (globulins subunits 7S and 11S) can give rise to different textural properties and behavior under in vitro digestion (Zang et al., 2025). They found that 7S/11S ratio was positively correlated with the melt viscosity and negatively correlated with in vitro digestibility. They also found that extruded soy showed lower protein digestibility than extruded pea, chickpea and mug, possibly due to the higher dense structure and lower susceptibility to gastric enzymes of the extruded soy. In this work, no differences were detected due to the type of protein neither the cooking technology. Luo et al. (2018) analyzing beef meat did not observe differences in protein digestibility due to the cooking technologies applied (oven or microwave).

The variability in the lipid digestibility was quite broad, with no clear effect from any of the analyzed factors (type of protein, type of extrusion or cooking method). Keuleyan et al. (2025) analyzing the digestibility of emulsions formulated with different protein ingredients observed that lipid bioaccessibility varied depending on the protein source. Other works have reported, in pork samples, higher lipid digestibility values for microwave cooking compared to pan frying (Hur et al., 2014), but our results did not show a clear pattern in this parameter. In general, the lowest values were found in beef burgers (25–27 g FFA/ 100 g of fat) compared to plant-based burgers (27–57 g FFA/ 100 g of fat), although only in half of the cases the differences were statistically significant. In the literature there are controversial results. Zhou et al. (2023) showed better lipid bioaccessibility in plant-based burgers than in beef burgers (28 % and 9 %, respectively). They argued that the compact food matrix in beef products makes more difficult the action of lipase when comparing with the less linked structure of vegan products. On the contrary, Zhou et al. (2021)

Table 3
Fatty acid profile (g FA/100 g total FA) of burgers: before digestion (cooked samples) and after digestion (bioaccessible fraction).

		LS		LP		HS		HP		BB	
		PF	Mw	PF	Mw	PF	Mw	PF	Mw	PF	Mw
SFA	Before digestion	18.8 ± 0.4^{AB}	19.6 ± 1.8^{B}	18.3 ± 0.2^{AB}	18.5 ± 0.1^{AB}	18.2 ± 0.2^{A}	18.6 ± 0.1^{AB}	18.3 ± 0.2^{AB}	18.2 ± 0.7^{A}	46.0 ± 0.8^{C}	46.3 ± 0.6^{C}
	After digestion	21.5 ± 0.2^{a}	24.2 ± 0.5^{a}	23.2 ± 0.3^{a}	24.9 ± 3.7^{a}	31.1 ± 0.7^{b}	25.6 ± 1.4^{a}	23.6 ± 0.1^{a}	25.1 ± 0.2^{a}	48.3 ± 0.2^{c}	50.0 ± 0.3^{c}
	Student t-test	***	*	***	**	***	***	***	***	**	***
MUFA	Before digestion	67.1 ± 0.6^{E}	66.3 ± 1.4^{DE}	64.4 ± 0.3^{C}	64.4 ± 0.1^{C}	67.5 ± 0.1^{E}	67.3 ± 0.1^{E}	62.5 ± 0.8^{B}	64.6 ± 1.9^{CD}	45.1 ± 0.6^{A}	45.3 ± 0.8^{A}
	After digestion	61.4 ± 0.4^{d}	57.4 ± 2.8^{cd}	53.3 ± 2.3^{cd}	50.4 ± 2.7^{bc}	41.9 ± 1.2^{ab}	54.2 ± 6.8^{cd}	51.2 ± 0.2^{bce}	53.9 ± 0.0^{cd}	39.1 ± 0.5^{a}	36.5 ± 0.9^{a}
	Student t-test	***	***	***	***	***	**	***	***	***	***
PUFA	Before digestion	14.1 ± 0.2^{B}	13.8 ± 0.3^{B}	16.5 ± 0.7^{C}	16.4 ± 0.0^{C}	13.8 ± 0.1^{B}	13.7 ± 0.1^{B}	18.6 ± 1.0^{D}	16.7 ± 2.5^{C}	4.0 ± 0.3^{A}	4.0 ± 0.1^{A}
	After digestion	16.9 ± 0.1^{b}	17.8 ± 1.8^{b}	22.2 ± 1.9^{bc}	22.7 ± 1.5^{bc}	25.5 ± 1.2^{c}	19.2 ± 4.2^{bc}	23.5 ± 0.1^{bc}	20.9 ± 0.1^{bc}	8.0 ± 0.5^{a}	9.5 ± 0.2^{a}
	Student t-test	***	**	***	***	***	**	**	ns	***	***
TRANS	Before digestion	0.1 ± 0.1^{A}	0.3 ± 0.1^{AB}	0.7 ± 0.2^{B}	0.6 ± 0.1^{B}	0.6 ± 0.1^{AB}	0.4 ± 0.1^{AB}	0.6 ± 0.0^{AB}	0.5 ± 0.1^{AB}	4.8 ± 0.3^{C}	4.3 ± 0.7^{C}
	After digestion	0.2 ± 0.1^{a}	0.6 ± 0.4^{a}	1.3 ± 0.1^{a}	1.4 ± 0.3^{a}	1.1 ± 0.0^{a}	0.9 ± 1.1^{a}	1.8 ± 0.0^{a}	0.1 ± 0.0^{a}	4.4 ± 0.2^{b}	4.0 ± 0.9^{b}
	Student t-test	ns	ns	**	***	***	ns	***	**	ns	ns

LS: burger with low moisture extruded soy protein; LP: burger with low moisture extruded pea protein; HS: burger with high moisture extruded soy protein; HP: burger with high moisture extruded pea protein; BB: beef burger; PF: pan frying; Mw: microwave; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. For each parameter an ANOVA and Tukey post hoc test were applied to analyze differences among the different samples. Different capital letters in each row show significant differences ($p < 0.05$) among samples before digestion. Different small letters in each row show significant differences ($p < 0.05$) among samples after digestion. Student t-test was done for each parameter, within each type of burger, to assess differences between before and after digestion. p: significance. Not significant (ns): $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.001$.

comparing the in vitro digestibility of PBMA and conventional beef burgers observed that the former showed lower lipid digestibility, hypothesizing that it was because of the fiber presence in the formulations of analogues, which avoided the pancreatic lipase activity over the fat globules. Finally, Ryu et al. (2024) found higher lipid and protein digestibility in chicken meat than in their plant-based analogues, explaining that it was due to the use of texturizing agents.

Fatty acids profile and lipid oxidation

As it has been stated previously, cooking did not notably affect the fatty acid profile of any of the samples. In addition, the fatty acids were measured after in vitro digestion in the micellar fraction, which is the bioaccessible fraction. Table 3 shows the fatty acid profiles in both cooked and digested samples.

Plant-based cooked burgers exhibited similar fatty acid profiles with no significant differences due to the type of protein, the extrusion process or cooking technology applied. They showed higher MUFAs (62.5–67.5 %) and PUFAs (13.7–18.7 %) content than beef burgers (45 and 4 % of MUFA and PUFA content, respectively). Conversely, they contained much lower SFAs (among 18.2–19.6 %) compared to beef burger (46 %). Trans fatty acids were found in significant amounts only in beef burgers (4.5 %).

After digestion, some changes in the total fatty acid profiles of the micellar fraction were noticed, with significant increases in the percentages of SFAs and PUFAs and a decrease of MUFAs. These changes are difficult to explain as although it is true that MUFAs are more frequently in sn-2 position of the triglyceride (Michalski et al., 2013), which is less susceptible to be attacked by pancreatic lipase (Park and Park, 2022), in this case total fatty acids and not FFAs were determined. So, the only explanation would be that the distribution of fatty acids between the pellet and the bioaccessible phases were someway selective. As a consequence of these changes, polyunsaturated/saturated (P/S) ratios increased in all cases in the micellar fraction, especially in beef burgers. Nevertheless, the final values were much lower in digested beef burgers (P/S ratios 0.16–0.19) than in plant-based ones (0.70–1.01).

The oxidation status of the lipids that can be absorbed by the organism is an important aspect to be evaluated considering the implication of oxidized lipids in the onset of many diseases. Thiobarbituric acid reactive substances (TBARs) values (Fig. 2) showed that, in all samples, the digestion process increased the amount of secondary lipid oxidation products. This was expected, due to the pro-oxidant conditions on the digestion tract, such as low pH of gastric fluid, the incorporation of oxygen due to the mastication, reactive species of food bolus, and the high concentration of fatty acids present in the medium due to lipolysis (Nieva-Echevarría et al., 2020). This fact has been found in many previous works (Hur et al., 2015; Kim and Hur, 2018; Sobral et al., 2020; Trujillo-Mayol et al., 2022). It is worth noting that plant-based burgers formulated with low moisture extruded protein showed the highest oxidation degree, both in cooked samples and after the digestion process, and the highest increases during digestion. The type of protein did not affect the lipid oxidation status in cooked samples; however, after the in vitro digestion, higher values were generally observed in burgers based on pea protein. The potential effect of the slight differences in the plant-based burgers composition due to the initial formulations, was also assessed by expressing the results of TBARs on a dry matter basis and also on a fat basis (see supplementary material figure S2). Similar results to those shown in Fig. 2 were obtained, indicating no effect of the different formulations.

Protein oxidation and peptides

Protein oxidation in cooked samples and in the micellar phases after digestion was assessed by measuring the amount of carbonyls (nmol carbonyls/ mg protein) (Fig. 3). Cooked samples showed a great variability among them in the carbonyls amount (12.7–41.5 nmol

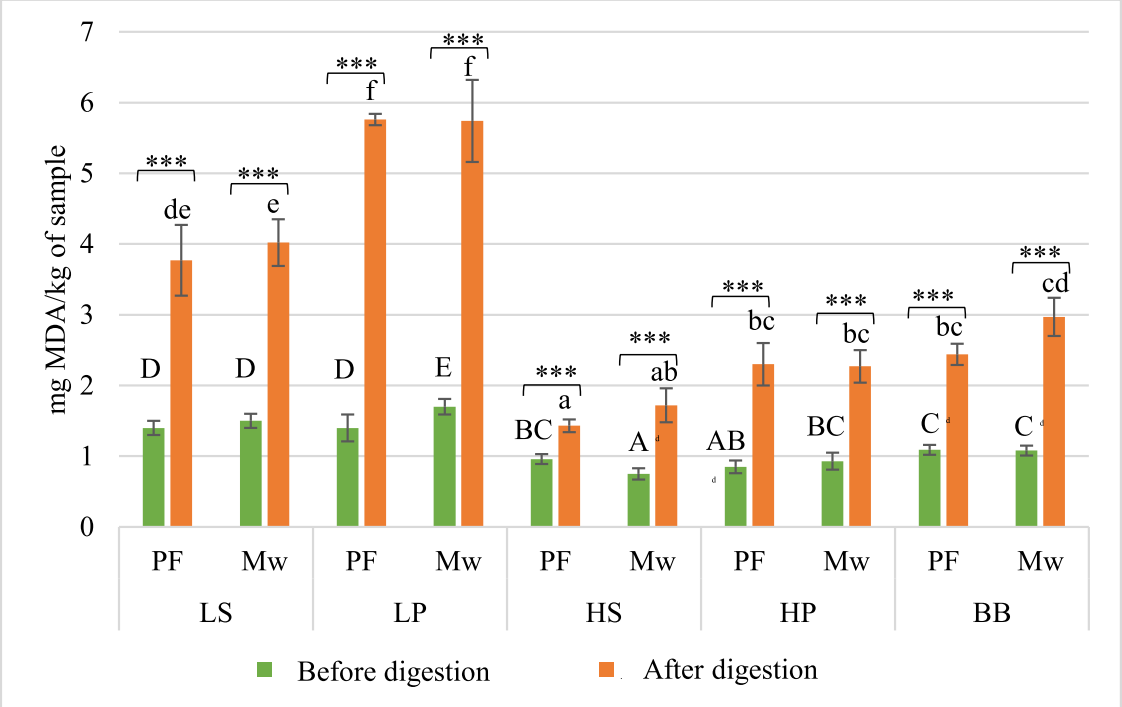

Fig. 2. Lipid oxidation (mg MDA/ kg of sample) of burgers: before digestion (cooked samples) and after digestion (bioaccessible fraction). LS: burger with low moisture extruded soy protein; LP: burger with low moisture extruded pea protein; HS: burger with high moisture extruded soy protein; HP: burger with high moisture extruded pea protein; BB: beef burger; PF: pan-frying; MW: microwave. ANOVA and Tukey post hoc test were applied to analyze differences among samples before digestion (capital letters) and after digestion (small letters) (different letters indicate significant differences, $p < 0.05$). Student t-test was done, within each type of burger, to assess differences between before and after digestion. p: significance. ***: $p < 0.001$.

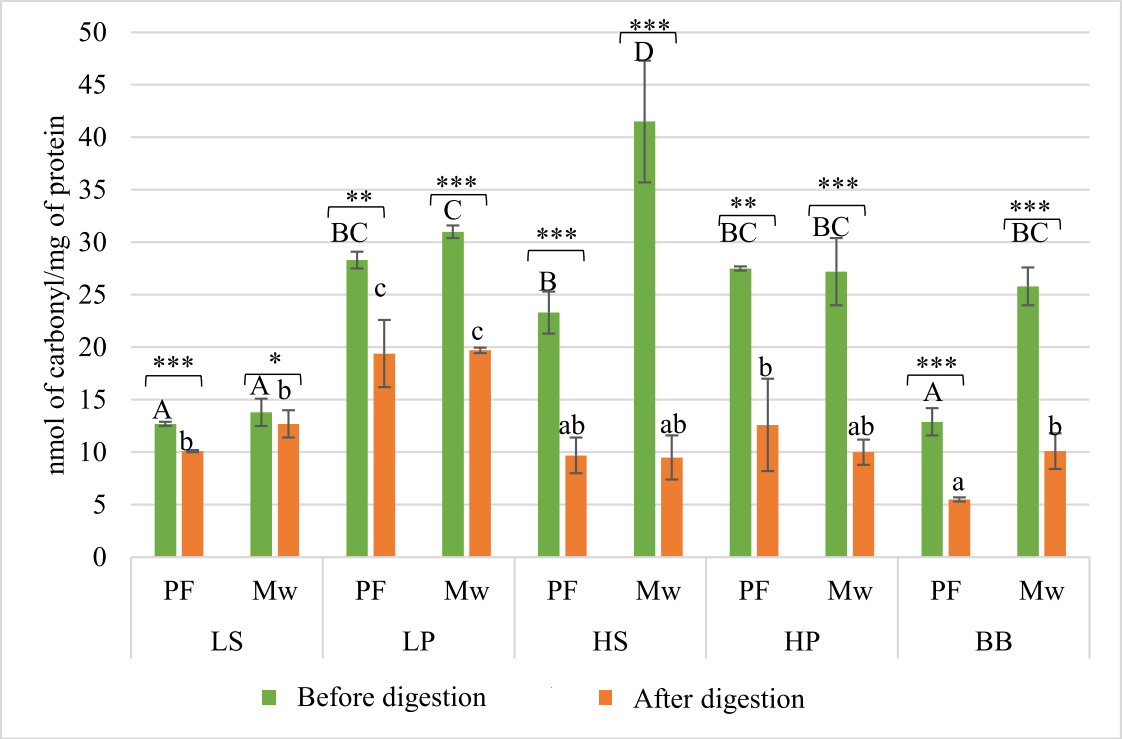

Fig. 3. Protein oxidation (nmol of carbonyl/ mg of protein) of burgers: before digestion (cooked samples) and after digestion (bioaccessible fraction). LS: burger with low moisture extruded soy protein; LP: burger with low moisture extruded pea protein; HS: burger with high moisture extruded soy protein; HP: burger with high moisture extruded pea protein; BB: beef burger; PF: pan-frying; MW: microwave. ANOVA and Tukey post hoc test were applied to analyze differences among samples before digestion (capital letters) and after digestion (small letters) (different letters indicate significant differences, $p < 0.05$). Student t-test was done, within each type of burger, to assess differences between before and after digestion. p: significance. *: $p < 0.05$; **: $p < 0.01$ and ***: $p < 0.001$.

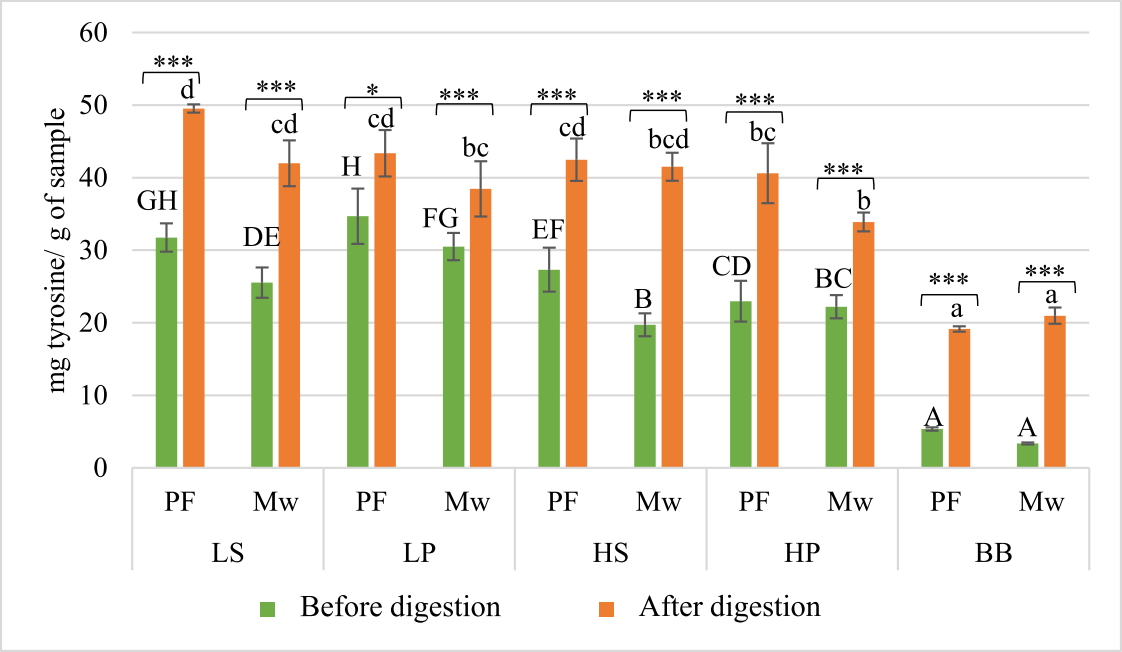

Fig. 4. TCA soluble peptides (mg tyrosine/ g of sample) of burgers: before digestion (cooked samples) and after digestion (bioaccessible fraction). LS: burger with low moisture extruded soy protein; LP: burger with low moisture extruded pea protein; HS: burger with high moisture extruded soy protein; HP: burger with high moisture extruded pea protein; BB: beef burger; PF: pan frying; MW: microwave. ANOVA and Tukey post hoc test were applied to analyze differences among samples before digestion (capital letters) and after digestion (small letters) (different letters indicate significant differences, $p < 0.05$). Student t-test was done, within each type of burger, to assess differences between before and after digestion. p: significance. *: $p < 0.05$ and ***: $p < 0.001$.

carbonyls/ mg protein) without a clear effect of the type of protein. Only soy protein burgers showed significant differences between low moisture and high moisture extrusion (12–13 and 23–41 nmol carbonyls/ mg protein, respectively). It is known that cooking promotes protein oxidation (Traore et al., 2012). In this work, microwaving resulted in higher protein oxidation intensity than pan frying in beef and in high moisture extruded soy burgers, with no differences in the rest of the samples. Hu et al. (2018) found that frying and roasting fish gave rise to higher levels of carbonyls compared to microwaving. They explain this stronger effect by the longer heating time involved.

After the digestion process, the amount of oxidized proteins in all samples was lower in the micellar fraction (5.5–19.7 nmol carbonyl/ mg of protein) than in their respective cooked samples. These results seem to indicate that the oxidized proteins were degraded during digestion, or that they remained in the non-absorbable fraction (pellet), not being available for their absorption. In the literature, different results have been reported regarding the effect of digestion in protein oxidation status of foodstuffs. Some authors pointed out a higher protein oxidation after the digestion processes (Sobral et al., 2020; Trujillo-Mayol et al., 2022). However, Rysman et al. (2016) showed no differences between before and after digestion samples in pork and beef meat.

One of the consequences of both the cooking and the digestion

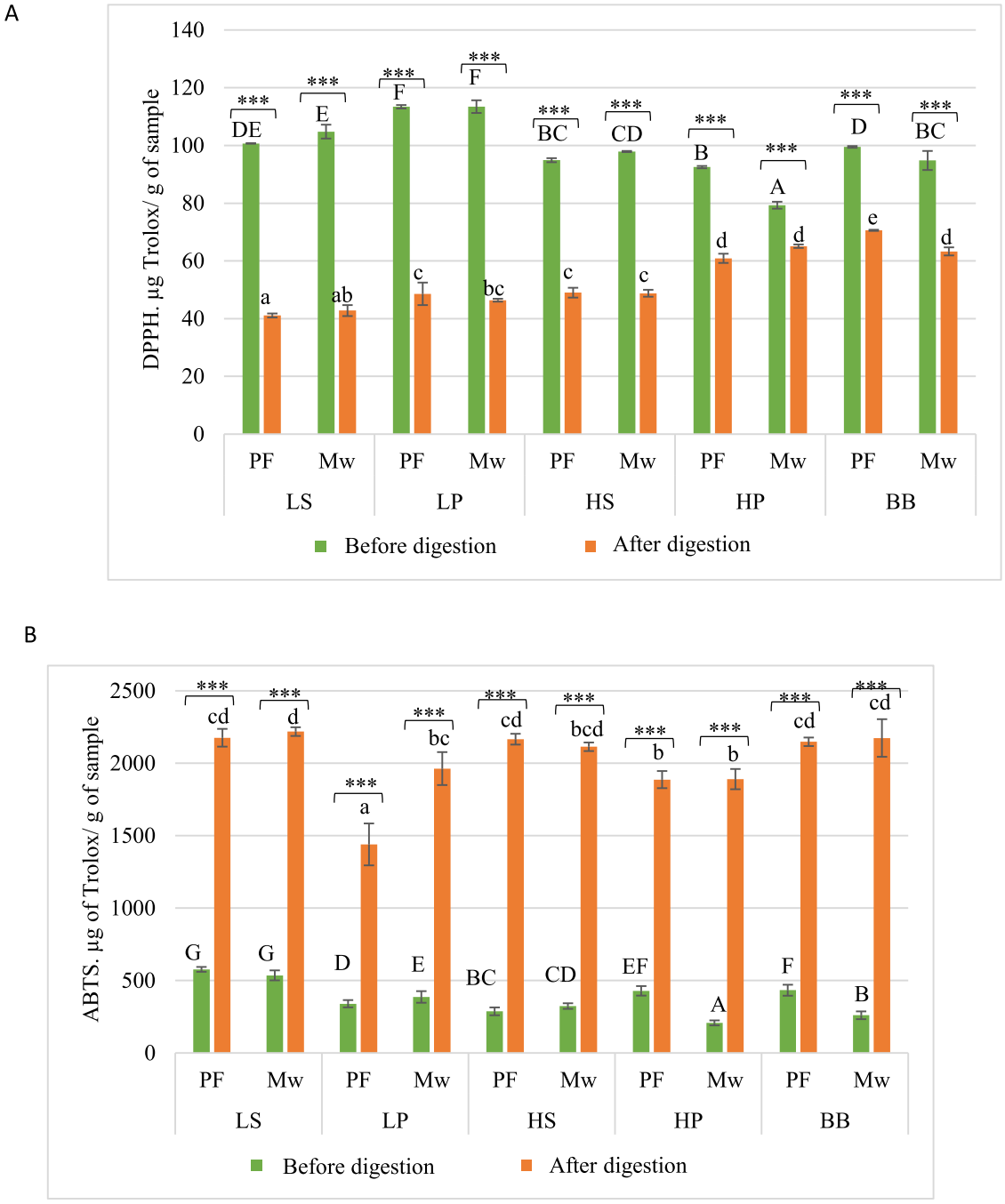

Fig. 5. A) DPPH (µg Trolox/ g of sample) and B) ABTS (µg Trolox/ g of sample) of burgers: before digestion (cooked samples) and after digestion (bioaccessible fraction). LS: burger with low moisture extruded soy protein; LP: burger with low moisture extruded pea protein; HS: burger with high moisture extruded soy protein; HP: burger with high moisture extruded pea protein; BB: beef burger; PF: pan frying; MW: microwave. ANOVA and Tukey post hoc test were applied to analyze differences among samples before digestion (capital letters) and after digestion (small letters) (different letters indicate significant differences, $p < 0.05$). Student t-test was done, within each type of burger, to assess differences between before and after digestion. p: significance. ***: $p < 0.001$.

processes of foods is the partial protein hydrolysis, giving rise to peptides that, in some cases, could have beneficial health effects and therefore could be considered as bioactive compounds (Grassi et al., 2023; Lafarga and Hayes, 2014; Wang et al., 2023). Since the extrusion process uses high temperature/pression to change the proteins structure, higher protein degradation was expected in cooked plant-based burgers compared to beef burgers. Indeed, the amount of peptides was 4–11-times higher in plant-based than in beef burgers (Fig. 4).

During in vitro digestion, all samples increased their peptides amount, according with the protein breakdown occurring during this process. Cutroneoet al. (2024) found that the release of soluble proteins and peptides after in vitro digestion of meat analogues was similar than in their corresponding meat products. In this work the increases were much higher in beef burgers (250–500 %) than in plant-based burgers (13–110 %). However, the overall peptide content in the micellar phase was significantly lower in beef burgers than in plant-based burgers, as occurred in cooked samples. Taking into account that all burgers had the same protein content in the initial formulation, the much higher amount of peptides found in plant-based burgers, both after cooking and after the in vitro digestion, could explain potential beneficial effects of these type of products. Moreover, it has to be noted that no significant differences were found in general in the total final amount of peptides among the different plant-based samples. Similar results were obtained when expressing the values on dry matter basis (see supplementary material Figure S3). However, a deep study about the nature of these bioactive peptides and their potential functions should be carried out.

Antioxidant capacity

In addition to peptides, there are other bioactive compounds with antioxidant activity, especially in vegetal ingredients. So, the antioxidant capacity of cooked samples was determined by two methods, DPPH (Fig. 5A) and ABTS (Fig. 5B). Additionally, the effect of the digestion process on antioxidant capacity was evaluated by measuring these parameters in digested samples.

As the developed formulations showed slight differences in the amount of added spices (1.6 % in LS, 1.6 % in LP, 2 % in HS, and 1.7 % in HP), a correlation analysis was performed between the antioxidant capacity (DPPH and ABTS) and the spices content. The results were no significant ($p = 0.8170$ for DPPH and $p = 0.7276$ for ABTS), suggesting no relationship between these two variables in the tested formulations.

In cooked samples, values for DPPH were much lower than those for ABTS (79–113 and 208–578 μg Trolox/ g of sample, respectively). These great differences between the two parameters had also been reported in other works (Antonini et al. 2020) and could be explained because DPPH radical is only soluble in organic solvents so it reacts with lipophilic antioxidant compounds, whereas ABTS radical is soluble in both organic and aqueous media and it may react with hydrophobic and hydrophilic antioxidant compounds (Sadeer et al., 2020). No clear effect of the type of protein or the cooking technology was observed on any of these parameters. Some statistically significant differences were found among samples with different type of extrusion, especially for DPPH; however, most of these differences disappeared when the results were expressed on dry matter basis (see supplementary material figure S4).

The effect of the digestion process on the antioxidant capacity of foods is not clear. Some of the antioxidant compounds present in the food could be degraded during digestion and others could be released or even activated. In our study, high increases in ABTS were observed in all samples (277–810 %) and, at the same time, significant decreases were observed for DPPH (17–59 %). Similar differences between both assays were also observed in other studies (Gallego et al., 2020; Kim and Hur, 2018; Xiao et al., 2020). The protein hydrolysis and the consequent increment of peptides observed in every sample during digestion would explain the increases in ABTS. Gallego et al. (2021) also found significant increases in the ABTS after digestion of different legume pastes, which were attributed to the release of peptides and amino acids more than to free phenolics. They explained that these peptides increase the medium's hydrophilicity, making it more difficult for the DPPH radical to react with them. No relevant effects due to the type of protein, type of extrusion or cooking method were observed on the antioxidant capacity of samples after digestion.

Conclusion

The results showed that the plant-based burgers, formulated to match the protein content of beef burgers, suffered lower cooking losses than the beef counterparts, especially when pan frying was applied. The type of extrusion applied to plant proteins (low or high moisture extrusion) seemed to affect the intensity of the changes in protein and lipid fractions much more strongly than the protein source (soy or pea). Plant-based burgers with low moisture extrusion showed the highest lipid oxidation, both before and after in vitro digestion, and also the highest protein digestibility. In vitro digestion caused, in all types of burgers, an increase of lipid oxidation status and a decrease of protein oxidation status in the micellar phase. In relation to the antioxidant activities, no clear effect was found due to the type of protein or cooking method applied.

These results contributed to increase the knowledge about the real nutritional value of plant-based products, considering also the effect of the digestion process. Nevertheless, there are still other future aspects to be explored, especially regarding long-term safety and epidemiological studies.

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Ethical statement

No animal or human studies were performed in this work.

CRediT authorship contribution statement

Itziar Ariz-Hernandez: Writing – review & editing, Writing – original draft, Methodology, Data curation. **Iciar Astiasaran:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Diana Ansorena:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, 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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100712.

Data availability

Data will be made available on request.

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