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Nutritional (mineral content and antioxidant capacity) and organoleptic characterization of lettuce grown in soil amended with blood meal and its hydrolyzate

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ABSTRACT

Hidden hunger is recognized as a global public health issue affecting especially at-risk population in developing countries. Food fortification is an effective tool to combat this problem. In this study, we investigated the re-use of blood meal (BM) and hydrolyzed blood meal (HBM) on Fe-Zn-Cu-Mn-Se levels, antioxidant capacity (AOC) and organoleptic characteristics of baby-lettuces. The experiment involved baby-lettuces' cultivation on amended soils with BM and HBM in comparison to urea as control. Baby-lettuces without cultivation were also analyzed. Zn content significantly increased with both intervention treatments, while Mn and Se decreased ($p <$ 0.001). Fe diminished with HBM application ($p = 0.003$). Baby-lettuces had significantly higher content for all minerals. AOC was mainly liberated during the *in vitro* fermentation process. BM and HBM impact positively on Zn content, without altering organoleptic perception, and total AOC measured by ABTS method in lettuce. Baby-lettuces are important sources for Fe-Cu-Mn in diet.

1. Introduction

Micronutrient deficiency, called hidden hunger, is a prevalent problem, affecting more than one-third-of the world's population, especially in developing countries. It is due to insufficient intake or absorption difficulties of minerals and vitamins, and leads to health problems and even death (Zou et al., 2019). Particularly, the deficiency of microelements with antioxidant capacity (AOC) such as Fe, Zn, Cu, Mn and Se derives in increased oxidative stress, dysfunctionalities at hormonal, immune, nervous and reproductive system levels.

Since foods of agricultural origin represent an important part of the human diet, the relationship between the nutrient content of soil, crops and the nutritional status of a population are closely related (Zou et al., 2019). Both Fe and Zn are found especially in low amounts in highly consumed foods in developing countries, which implies one of the main causes of deficiency (Kumar et al., 2022). Therefore, biofortification is an effective and economical method to combat deficiency diseases such as anemia and other situations of malnutrition, especially in subjects with limited access to a varied and healthy diet (Liberal et al., 2020; Kaur et al., 2022) and an alternative to other types of nutritional interventions such as supplementation or direct food fortification (Burgos et al., 2023). Specifically, biofortification involves the improvement of the nutritional quality of crops by the indirect addition of nutrients through different biotechnological, conventional or agronomic practices (Kaur et al., 2022).

Among the different types of fortification, agronomic biofortification, both with organic amendments and inorganic salts, has yielded good results in essential elements such as Fe, Zn, Cu, V, Co, I, Se, and Mn (Garg et al., 2018; Cervera-Mata et al., 2019). Even the biofortification of minerals such as Zn and Se can reduce harmful contents

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of toxic metals in crops, especially Cd and Pb (Tang et al., 2020). Social acceptance of biofortified foods is steadily increasing because it is now understood that organoleptic characteristics are not altered with the addition of micronutrients. This makes this practice an efficient and sustainable tool to increase the concentrations and bioavailability of nutrients in foods (Izydorczyk et al., 2021).

Sustainable agriculture seeks an efficient use of natural resources and the reduction of dependence on synthetic fertilizers used in Spanish agriculture (Ministerio de Agricultura Pesca y Alimentación, 2024), through the reuse of agro-industrial by-products such as blood meal (BM), obtained from the processing of animal blood (Álvarez-Castillo et al., 2021; Ronga et al., 2021). Despite research on the agricultural use of BM as an organic fertilizer (Roy et al., 2016; Ginting, 2020; Carucci et al., 2021; Martins et al., 2021; Bergstrand, 2022; Kulesza et al., 2022), evidence of its use for biofortification in essential antioxidant elements such as Fe and influence on others (Zn, Mn, Cu and Se) and on the antioxidant capacity of crops obtained with its use is lacking.

The use of the hydrolysis product of BM (HBM) as a source of protein and Fe has also been studied with possible applications in food (da Silva Bambirra Alves et al., 2021). Hydrolysis of BM favors protein solubilization, a process that could influence the assimilation of heme-iron, constituting a good option for its biofortification in food (Berraquero-García et al., 2022). Additionally, enzymatic hydrolysis allows the release of peptide sequences with possible antioxidant or antimicrobial biological activities (da Silva Bambirra Alves et al., 2021; Berraquero-García et al., 2022). For the application of blood meal to the soil, it is necessary to evaluate the costs of hydrolyzing this byproduct.

Consumers, in addition to sustainable foods, are looking for healthier foods and new textures and flavors, which in recent years has led to a new trend towards the consumption of baby vegetables. It has been indicated that these have a higher content of micronutrients and antioxidant compounds compared to mature vegetables (Collado-González et al., 2022).

There is little evidence on the modification of the nutritional composition of lettuce-baby, after application of organic biofortifiers such as BM and HBM to the soil, in bioclimatic chamber cultures, as alternatives to urea commonly used as nitrogen fertilizer in agriculture. Therefore, the objective of the study is to evaluate the application of both by-products as organic soil amendments to assess the possible biofortification in Fe and influence on the levels of other antioxidant minerals (Zn-Cu-Mn-Se) and on the AOC in lettuce-baby. In addition, we intend to determine the influence on the biomass and organoleptic characteristics of lettuce.

2. Materials and methods

2.1. *Lactuca sativa* baby lettuces

Baby-lettuces (*Lactuca sativa* variety longifolia) acquired at the Saliplant nursery (Granada, Spain) after 30 days of sowing were used. They were chosen because they are a leafy vegetable highly consumed in the West, and because of their short growth cycle (Cervera-Mata et al., 2021).

2.2. Blood meal and hydrolyzed blood meal

The BM used (PROTESAN®, Deham, Iowa, United States) stands out for its N (15.2 %), Fe (0.25 %) and Mn (30 mg/kg) content (Table S1). HBM was obtained by enzymatic hydrolysis of BM, whose enzyme and hydrolysis conditions were previously described (Berraquero-García et al., 2022). The PTN enzyme (Pancreatic Trypsin Novo, EC 3.4.21.4) was chosen for its high degree of iron-heme release (Novozymes®, Bagsværd, Denmark). The reaction was carried out in a jacketed reactor using 168.5 g of BM (150 g of protein) with an enzyme-substrate ratio of 10 %, at 50 °C and pH 8, in a thermostated bath and pH control with an automatic titrator adding 2 N NaOH. Hydrolysis lasted 4 h, reaching a degree of hydrolysis of 13.4 %, determined by the Adler-Nissen method (1979), with subsequent enzymatic deactivation, vacuum filtration and lyophilization.

The mineral characterization of the bioproducts (BH and HBM; Table S2) was performed by prior mineralization and analysis by the inductively coupled plasma mass spectrometry (ICP-MS) technique subsequently described. Inorganic urea (100 % rich, GUINAMA, Valencia, Spain) was used as a control, as a nitrogenous agronomic fertilizer (46 % N) in food crops (Blatt, 1991).

2.3. Soil

Soil from the Vega de Granada (37°14′7.1″N, 3°45′40.7″W), from the arable portion of the land (0–20 cm) with previously described characteristics was used.

2.4. Experimental design

The trial consisted of applying BM, HBM and urea as fertilizers on lettuce-baby soils with 12 microcosms per treatment ($n = 36$), which resulted in the experimental groups of lettuces studied (BM-grown: BM-lettuce; HBM-grown: HBM-lettuce; urea-grown: control-lettuce). A group of uncultivated baby-lettuces ($n = 12$) was also considered.

The dose of bioproducts used corresponded to the contribution of 7.5 mg Fe/kg of soil, necessary for biofortification. To homogenize the doses, the N supply provided by the BM was calculated (0.17 g N/400 g soil). Thus, for every 400 g of soil, 0.36 g of urea, 1.2 g of BM or 1.05 g of HBM were applied. After homogenization of the soil with each treatment, transfer to pots and transplantation of baby-lettuces, they were incubated in a bioclimatic chamber under controlled conditions for the 40 days of the trial. Irrigation was manual and maintained constant between field capacity and the wilting point of the plant (Cervera-Mata et al., 2021).

2.5. Treatment of lettuces samples

After harvest, lettuce was weighed (fresh weight), and dried at 65 °C 24 h until constant weight (dry weight), and eight samples ($n = 8$) from each experiment were randomly assigned to mineral and AOC analysis, and four ($n = 4$) to organoleptic characterization. Between 4–6 g of fresh lettuce were used in the analysis of AOC, after labelling and freezing (−80 °C) until determination. In the remaining quantity, Fe, Zn, Se, Cu and Mn were measured after drying (60 °C, 24 h) in an oven (SL-SIMPLE, Pol-Eko, Poland) and subsequent pulverization in a grinder (Comelec, model Mc 1261, Valencia, Spain).

2.6. Mineralization and analysis of Fe, Zn, Cu, Mn and Se

0.25 g of dried sample was weighed into borosilicate tubes and 3 mL of 69 % HNO_3 (TarceSELECT, Honeywell, Fluka, France) was added. Mineralization was performed in the microwave digester (Multiwave 5000 with Rotor 24HVT50, Anton Parr GmbH, Graz, Austria), using the procedure previously described (García-Conde et al., 2023, 2024). The mineralized samples were diluted to 40 mL with reagent-grade Milli-Q water to obtain the analytical solution (4.14 % acidity in HNO_3) in which the studied elements were analyzed by ICP-MS technique (Agilent 8900 Triple Quadrupole ICP-MS/MS, Agilent Technologies Inc., Santa Clara, CA, USA). The samples were analyzed in triplicate.

The determination of Fe, Zn, Mn, Cu and Se was performed by linear calibration curves by dilution from their standard dilutions of 1000 mg/L in 1 % HNO_3 (ppm; Merck; Darmstadt, Germany). The internal standard used in the measurement was "Internal Standard Kit (^{72}Ge, ^{193}Ir, ^{103}Rh, ^{45}Sc; ISC Science, batch 20,210,712)". The limits of detection (LOD) for Fe, Zn, Cu, Mn and Se were 0.11, 0.42, 0.76, 1.15, and 0.065 μg/L, respectively. In the certified reference standard for these elements "Bovine muscle powder no 8414″, concentrations of 70.5 ±

1.9, 145 ± 2. 5, 2.82 ± 0.07, 0.38 ± 0.05 and 0.077 ± 0.043 μg/g for certified levels of 71.2 ± 9.2, 142 ± 14, 2.84 ± 0.45, 0.37 ± 0.09 and 0.076 ± 0.010 μg/g, respectively. The recovery percentages (Cervera-Mata et al., 2019), ranged from 97.8 to 101.6 % and the coefficients of variation had a mean value of 2.83 %.

2.7. *In vitro digestion and fermentation method*

Lettuce samples were subjected to *in vitro* gastrointestinal digestion/fermentation in triplicate, according to previously described protocols (Pérez-Burillo et al., 2018, 2021). Table S3 includes the description of the different salts included in simulated saliva, simulated gastric fluid and simulated intestinal fluid. The first step included the oral phase, in which lettuces (5 g), previously homogenized, were added to falcon tubes, together with 5 mL of simulated salivary fluid (1:1 w/v, including α-amylase). Second, in the gastric phase 10 mL of simulated gastric fluid was added (including pepsin). Finally, the intestinal phase was carried out, in which 20 mL of simulated intestinal fluid was added (including pancreatin and bile salts), simulating the content of intestinal juices. Once the intestinal phase was finished, the reactions were stopped with ice and then centrifuged for 10 min at 6000 *rpm*. The resulting solid pellet served as an *in vitro* fermentation substrate, and represents the undigested portion entering the large intestine. The supernatant, which represents the fraction available for absorption in the small intestine, was stored in 1 mL tubes at −80 °C until analysis.

In vitro fermentation was carried out using a pull of fecal samples from five healthy donors (mean Body Mass Index = 21.3, aged 20–65 years, not taken antibiotics for three months prior to the assay and not diagnosed of chronic gastrointestinal disorders or any other chronic disease or special diet) to reduce inter-individual variability. The informed consent document was signed by all fecal donors. That form included all of the information of the study as well as the exclusion and inclusion criteria. The study was conducted according to the guidelines of the Declaration of Helsinki. It was approved by the Ethics Committee of the University of Granada (protocol code 1080/CEIH/2020). After *in vitro* fermentation, the supernatant, representing the fraction available for absorption in the large intestine, was stored at −80 °C until analysis. The solid pellet, representing the portion that was not fermented, was properly disposed of.

2.8. *Antioxidant tests used for the evaluation of the AOC*

The AOC of the different lettuce groups (obtained after *in vitro* digestion, *in vitro* fermentation and evaluation of total AOC (Pastoriza et al., 2011) was calculated for fresh weight (f. w.). FRAP, DPPH, ABTS and Folin-Ciocalteu (FC) methods described in detail previously (Navajas-Porras et al., 2024a) were used. Results were expressed as Trolox equivalent AOC *versus* reducing capacity ($TEAC_{FRAP}$), *versus* DPPH radicals ($TEAC_{DPPH}$) and *versus* ABTS radicals ($TEAC_{ABTS}$) (mmol Trolox equivalents/kg lettuce). The FC method measured total phenolic content and was expressed as mg gallic acid equivalents (GAE)/kg lettuce. A FLUOstar Omega microplate reader (BMG Labtech, Ortenberg, Germany) was used for all 4 methods.

2.9. *Organoleptic evaluation*

The tasting panel was specifically formed for this research study with voluntary participants who wished to take part in the lettuce tasting. In our case, only 7 people offered to participate, but they were sufficient to meet the stated objectives.

The organoleptic assays were carried out on the day of harvest, and consisted of performing sensory tests of preference and differential, with the assignment of a three-digit code to each sample and preparation of a tasting card designed for this purpose. First, as indicated in UNE-EN ISO 5495 (2009), the preferential evaluation was carried out by asking the participants to indicate the number of the preferred sample, between the pairs control and BM-lettuce, and control and HBM-lettuce. To establish preferences, at a significance level of 95 % (alpha equal to 0.05), a minimum of 28 preferences of any of the products evaluated with forced judgment is required (Casal del Rey Barreiro & del Castillo García, 2005). Second, in the differential evaluation, three sets were established (urea control, BM-lettuce and HBM-lettuce) with their possible six combinations to reach a minimum of 22 correct responses of difference and thus obtain statistical significance of 99 % (alpha equal to 0.1) necessary with forced judgment. In each set, two equal samples and one different sample were compared (UNE-EN ISO 4120, 2022). The organoleptic evaluation was carried out with 7 participants, the minimum number necessary to achieve 42 responses in triangular tests with an alpha risk of 0.1. Each participant evaluated the 6 possible combinations between the compared samples. This test is considered suitable to establish whether there are differences between samples that have undergone different processing and there are no perceptible differences in their appearance. Therefore, when comparing the treatments, sheets of similar size were chosen in each case so as not to bias the choice.

2.10. *Statistical analysis*

The SPSS program (version 28.0) was used for the statistical treatment of the data, with a 95 % confidence level ($p < 0.05$). The existence of homogeneity of variances was previously tested using Levene's test ($p > 0.05$) and the normal distribution of the results ($p > 0.05$) with the Kolmogorov-Smirnov test. Student's *t*-test was used in the analysis of variance (ANOVA) for parametric variables and the Kruskal-Wallis test for nonparametric variables, specifically using the Mann-Whitney test when comparing between two independent samples.

3. Results and discussion

3.1. *Analysis of biomass*

After harvest, lettuce weights were recorded as 16.2 ± 1.66, 16.9 ± 1.37 and 19.2 ± 1.63 g for control-lettuce, HBM-lettuce or BM-lettuce, respectively, with the weight of the latter group being higher ($p < 0.01$; Fig. 1). We also found that the mean moisture content of BM-lettuce was significantly ($p < 0.01$) higher (92.1 ± 0.005 %) than that of control-lettuce (91.5 ± 0.004 %). Therefore, the use of BM as an organic amendment increased biomass by 18.51 % and water content by 0.66 % with respect to the control group. Other researchers have indicated that organic productions generate crops with less water, and similarly to our work, higher dry matter content than the conventional system (Rosen & Allan, 2007). Lara-Ramos et al. (2023) also analyzed the dry and fresh weight of lettuce after the application of Zn-functionalized coffee grounds biochar, finding no variation between fresh and dry weight with respect to the control. The difference in the findings of our study may be due to the different amount of nitrogen provided by the different amendments, being of the order of 3 % N (Lara-Ramos et al., 2023), and 15 % N for the BM in the present study. The addition of BM with high amounts of nitrogen can affect plant growth, impacting both fresh and dry weight. Nitrogen is the nutrient most closely associated with plant yield in global crop production. Nitrogen is a limiting factor and the one that most significantly stimulates plant growth (Stoytcheva & Zlatev, 2013). Therefore, in the present work, the higher application of N to the soil creates differences in both fresh and dry weight compared to the control sample. Nitrogen is deeply involved in plant growth through photosynthesis, as plant leaves contain chloroplasts (Ma et al., 2023).

Despite the high applied dose of N (1020 kg/ha) in the different treatments, there was a higher yield in BM-lettuce. This result coincides with that observed by Polat et al. (2010), following tomato production with BM plus other organic compounds *vs.* conventional fertilization. These findings contradict claims that the use of organic fertilizers or fortificants decreases plant yield (Rosen & Allan, 2007; Cervera et al.,

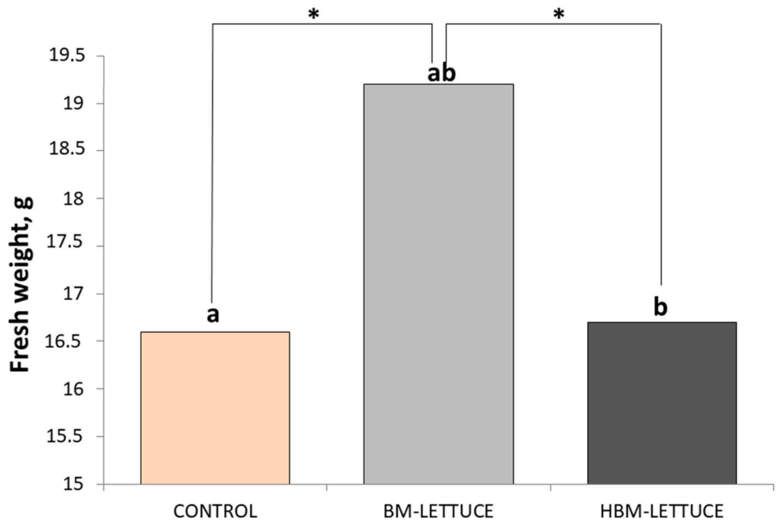

Fig. 1. Comparison of fresh weight values in control samples (lettuce grown with urea) *vs.* lettuce grown with BM (BM-lettuce) and with hydrolyzed BM (HBM-lettuce). The presence of equal letters above the columns denotes the existence of statistically significant differences with respect to the control group at significance levels of $p < 0.05$ (*).

2019; Thavarajah et al., 2022; Jeon et al., 2024).

Zandvakili et al. (2019) also observed that the use of N-rich organic fertilizers at low application rates increased lettuce growth. The difference could be related to possible atmospheric losses of N (N_2O) (Ciavatta et al., 2008; Toonsiri et al., 2016) after fertilization with organic products. It has been shown that N_2O emission increases linearly or exponentially with the amount of N added to the soil as the N available for nitrification and denitrification processes increases (Toonsiri et al., 2016).

3.2. Fe, Zn, Cu, Mn and Se levels

As for Fe, a significant decrease was observed in the HBM-lettuces (5.85 mg/kg) compared to the control (16.1 mg/kg; $p = 0.003$; Fig. 2a). Paradoxically, the use of BM as an organic soil amendment as an alternative to urea does not significantly modify the levels of Fe present ($p > 0.05$). Possibly, Fe as a facilitated heme-group may have been mobilized by soil microorganisms as a factor in their growth, limiting its bioavailability to lettuce, an aspect to be studied in future research. Rosen & Allan (2007) reported that the use of organic compost could stimulate the development of chelate-producing microorganisms affecting the uptake of micronutrients (Fe) by the plant. Mondini et al. (2008) observed that after application of BM, defatted and non-fatted animal bone meal (200 and 400 kg N/ha, respectively), and incubation for 14 days (15–20 °C), microbial content and activity, and nitrogen content (NH_4^+ and NO_3^-) in the soil increased according to the dose applied.

Giordano et al. (2019) increased Fe content in lettuce grown in bioclimatic chambers with soilless hydroponic system at pH 5.8 after application of inorganic fertilizers (Fe-EDDHA-ethylenediamine-N, N'-bis(2-hydroxy-phenylacetic acid). They determined that the addition of 1.02 and 2.02 mM Fe in the nutrient solution resulted in a significant increase in Fe content (20.5 % and 53.7 %, respectively). In contrast, they found a reduction of up to 25 % in crop yield proportionally to the increase in Fe added (Giordano et al., 2019). However, in alkaline soils, such as those used in our study, it has been documented that Fe is rapidly converted into non-absorbable forms for roots, by precipitation, adsorption and oxidation phenomena (Buturi et al., 2021). This could, to some extent, also justify our finding on the non-influence of BM use on Fe content in lettuce compared to the control group.

Yunta et al. (2013) found that the addition of a BM-based compound, obtained after alkaline digestion and precipitation of the heme-group in an acid medium, prevented chlorosis diseases in plants by facilitating the maintenance of Fe bound to organic compounds. Consequently, the form of application of the mineral and the pH of the medium could be crucial for its assimilation by the plant, so future research on Fe

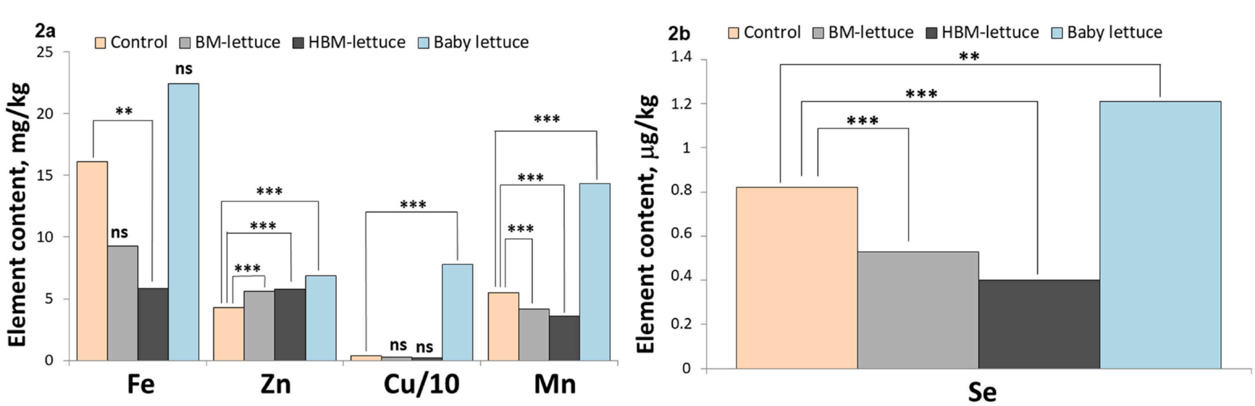

Fig. 2. Comparison of the levels of antioxidant minerals determined in the control samples (lettuces grown with urea) with respect to the other experimental groups (lettuce grown with blood meal: BM-lettuce; or with hydrolyzed blood meal: HBM-lettuce; and baby lettuce not grown (baby-lettuce). (2a) Fe, Zn, Cu and Mn (mg/kg). (2b) Se (µg/kg). Statistically significant differences were found with respect to the control group with significance levels of $p < 0.01$ (**) and $p < 0.001$ (***). No significant differences were found with respect to the control group (ns).

biofortification is necessary.

Cervera-Mata et al. (2021) found that the addition of coffee grounds functionalized with Fe and Zn (chelated) increased their total content (28–30 and 177–416 %, respectively). Buturi et al. (2022) when applying Fe at doses of 1.02 and 2.02 mmol/l not only increased Fe, but also Zn at the highest dose, and Mn at the lowest dose. Similarly, we have observed that Fe is significantly correlated with Mn ($r = 0.557$; $p < 0.001$) and Se ($r = 0.746$; $p < 0.001$) in lettuce. Therefore, there are interactions between different elements, where the dose of the mineral seems to be a determinant of the content of others in the plant.

Ciavatta et al. (2008) observed that direct application of BM to the soil decreased Fe and Mg content thirty days after incubation. It is possible that there are also interactions in the soil with the microbial population that affect the bioavailability of these minerals, resulting in lower amounts in plants grown in the soil.

A statistically significant decrease in Fe concentrations was observed in HBM-lettuce *vs.* BM-lettuce (Table 1). Consequently, the hydrolysis followed leads to a decrease in available and assimilable Fe in lettuce. Zn significantly increased (31.9 and 35.9 %) in BM-lettuce and HBM-lettuce compared to controls, respectively ($p < 0.001$; Fig. 2a; Table 1). This increase in lettuce may be a way to help counteract low levels of Zn intake as an element of so-called hidden hunger in vulnerable groups in developing countries.

Jeon et al. (2024) determined the levels of Fe, Zn, Mn and Mg after applying coffee grounds and organic waste in amounts of 7.5, 15 and 30 mg N/ha and urea as a control. The lower doses enriched Fe in lettuce, and decreased Zn and Mn. This result is contrary to what was found in our study for Fe and Zn, and similar for Mn. However, Jeon et al. (2024) do not indicate the content of these minerals in the amendments used, so their composition and origin may be related to the differences found. In contrast, Olszyk et al. (2020) observed a decrease in Zn concentrations in lettuce after the application of different organic amendments (pine chip biochar, poultry litter, pig solids and switchgrass *Panicum virgatum*).

In line with our findings, Mounirou et al. (2023) after the application of other bioproducts as amendments (goat manure and its biochar), observed a significant increase in Zn content and a decrease in Cu. Also Kumar et al. (2022) observed Zn increases of ≈5 % in maize crops after application of 7.5 and 10 kg/ha of Zn *vs.* no application. In the present trial, by adding considerably lower amounts (142 and 75 g/ha of Zn) with the application of BM and HBM, higher percentage increases in Zn were also achieved (32 and 36 %, respectively). It is therefore possible that BM and HBM are very efficient organic soil amendments as Zn biofortifiers in lettuce. After considering the size of the usual consumption ration in Spain Mercasa, (2023), the % coverage of the reference dietary intakes for the Spanish population (Cuervo et al., 2010), considering all experimental groups, is remarkable with values of 8.91 and 12.0 %, for men and women, respectively (Table 2).

For Cu, the addition of BM and HBM did not influence its content in lettuce, compared to the control (Fig. 2a; Table 1). The high content in the uncultivated baby-lettuces was significantly higher than in the other groups (Table 1).

In BM-lettuce and HBM-lettuce, Mn and Se concentrations were significantly lower than in the control group ($p \leq 0.001$; Fig. 2a and b, respectively). In addition, Se was significantly decreased in HBM-lettuces with respect to BM-lettuces ($p < 0.05$; Table 1). Thus, hydrolysis of BM negatively influences the bioavailability of Se and Fe, as previously indicated in BM-lettuces and HBM-lettuces. Buturi et al. (2021) observed increases of Se in lettuce with foliar application of Na_2SeO_4 or by fertigation of the same inorganic complex.

The elements analyzed in the present study act as antioxidant agents as cofactors of the enzymes catalase (the Fe), superoxide dismutase (SOD; Zn and Cu in the cytoplasmic SOD1 isoform, and Mn in the mitochondrial SOD2 isoform), and of the glutathione peroxidase, thioredoxin reductase and several Selenoprotein families (for Se) (Zulaikhah, 2017). However, in the lettuce samples analyzed the activity of the antioxidant enzymes and proteins referred to were not determined, so that it is not known the specific way in which such activity would influence the lettuce, and could affect the results obtained. However, this would be an interesting aspect to be addressed in future studies.

As can be seen in Table 2, the consumption of the typical Spanish lettuce ration (150 g; SENC, 2004) provides >9 % of the reference dietary intake (Cuervo et al., 2010) for Cu and Mn, and for Fe in men and Zn in women, for the four experimental lettuce groups studied.

The content of all anitoxidant minerals studied (Fe, Zn, Cu, Mn and Se) was significantly higher in baby-lettuce than in BM-lettuce and HBM-lettuce (Table 1), as well as in the control group (except for Fe; Fig. 2a and 2b; Table 1). Our results align with and reinforce the statement that baby-leaf lettuces present higher levels of minerals, strengthening their high nutritional value (Collado-González et al., 2022), as also indicated for other leafy baby greens for Fe and Mn (Lenzi et al., 2019). Furthermore according to Regulation (EU) No 1169/2011 and considering the results found, baby-lettuce can be considered significant sources of Fe, Cu and Mn by containing per 100 g (f. w.), at least 15 % of the dietary reference intakes values (Table 2). This result is covered to a greater extent when considering the typical ration size in Spain SENC, (2004); Table 2.

3.3. AOC measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods

The AOC of the samples evaluated by FRAP (Fig. 3a), DDPH (Fig. 3b), ABTS (Fig. 3c) and Folin-Ciocalteu (Fig. 3d) methods distinguishes between digestion and fermentation liquids after subjecting the lettuce to the previously optimised *in vitro* digestion/fermentation method (Pérez-Burillo et al., 2018, 2021). AOC values are significantly higher in fermentation liquids ($p < 0.001$; Fig. 3a, b, c and d), increasing when digestion solids are put in contact with the faecal inoculum pool, for the 4 methods employed (Fig. 4), which coincides with that previously found also in lettuce (Navajas-Porras et al., 2020). This result is contrary to that found in cucumbers where the highest AOC is released after *in vitro* digestion (Navajas-Porras et al., 2024b).

For the FRAP method, BM-lettuce and HBM-lettuce showed similar total $TEAC_{FRAP}$ values to the control ($p > 0.05$; Fig. 3a). Differently, Buturi et al. (2022) found higher total $TEAC_{FRAP}$ values for Romaine lettuce fortified with Fe (2.02 mmol/L) compared to the control (without addition of the mineral). However, in line with our findings, Navajas-Porras et al. (2020) found similar $TEAC_{FRAP}$ values for Fe-fortified Romaine lettuce (2.02 mmol/L) compared to the control (without addition of the mineral). On the other hand, in the digestion liquids, as was also seen for the DPPH method (Fig. 3a), the $TEAC_{FRAP}$ in the HBM-lettuce was significantly higher than in the control group (Fig. 3a). In the fermentation liquids, the $TEAC_{FRAP}$ in the BM-lettuce

Table 1
Mean Fe, Zn, Cu, Mn and Se contents (± standard deviation, SD; fresh weight) in experimental groups of lettuce samples analyzed (lettuces cultivated in soils amended with blood meal (BM), with hydrolyzed blood meal (HBM) or urea (control group) and baby-lettuces).

Element	Control group	BM-lettuce	HBM-lettuce	Baby-lettuce
Fe (mg/kg)	16.1 ± 10.7^{a}	9.26 ± 3.09^{bc}	5.85 ± 1.48^{abd}	22.4 ± 1.11^{cd}
Zn (mg/kg)	4.26 ± 0.26^{abc}	5.62 ± 0.39^{ad}	5.79 ± 0.73^{be}	6.87 ± 3.13^{cde}
Cu (mg/kg)	3.65 ± 1.73^{a}	2.55 ± 1.32^{b}	2.27 ± 1.53^{c}	77.6 ± 3.37^{abc}
Mn (mg/kg)	5.46 ± 0.6^{abc}	4.18 ± 0.64^{ad}	3.61 ± 0.66^{be}	14.3 ± 0.79^{cde}
Se (μg/kg)	0.82 ± 0.24^{abc}	0.53 ± 0.12^{ade}	0.40 ± 0.14^{bdf}	1.21 ± 0.21^{cef}

[#]Rows labelled with the same superscript lowercase letters for element values in lettuce samples for different experimental groups denotes the existence of statistically significant differences ($p < 0.05$).

Table 2
Daily intake and from a serving of lettuce commonly consumed in Spain of antioxidant minerals and percent coverage of dietary reference intakes (DRIs) of healthy adult Spanish men and women (20–49 years old), and daily intakes and from a serving of lettuce of polyphenols, and daily intakes of total polyphenols (determined by the Folin-Ciocalteu method) and antioxidant capacity (determined by the $TEAC_{FRAP}$, $TEAC_{DPPH}$ and $TEAC_{FRAP}$ methods) from the different experimental groups.

Compound	Control group	BM-lettuce	HBM-lettuce	Baby-lettuce
Fe				
Daily intake (mg)[a]	0.159	0.091	0.058	0.221
DRI (mg/day; Men/Women)[b]	(9.5/18)	(9.5/18)	(9.5/18)	(9.5/18)
%DRI (Men/Women)	(1.67/0.88)	(0.96/0.51)	(0.61/0.32)	(2.32/1.23)
Intake/serving (mg/serving)[c]	2.41	1.39	0.88	3.36
%DRI/serving (Men/Women)	(25.4/13.4)	(14.6/7.72)	(9.24/4.88)	(35.4/18.7)
Zn				
Daily intake (mg)	0.042	0.055	0.057	0.068
DRI (mg/dia; Men/Women)[b]	(9.5/7)	(9.5/7)	(9.5/78)	(9.5/18)
%DRI (Men/Women)	(0.44/0.60)	(0.58/0.79)	(0.60/0.82)	(0.71/0.97)
Dietary intake/serving (mg/serving)[c]	0.64	0.84	0.870	1.04
%IDR/serving (Men/Women)	(6.72/9.13)	(8.88/12.0)	(9.15/12.4)	(10.9/14.7)
Cu				
Daily intake (mg)[a]	0.036	0.025	0.022	0.765
DRI (mg/day; Men/Women)[b]	(1.1/1.1)	(1.1/1.1)	(1.1/1.1)	(1.1/1.1)
%DRI (Men/Women)	(3.27/3.27)	(2.29/2.29)	(2.03/2.03)	(69.6/69.6)
Dietary intake/serving (mg/serving)[c]	0.55	0.38	0.34	11.64
%IDR/serving (Men/Women)	(49.8/49.84)	(34.8/34.8)	(31.0/31.0)	(1058/1058)
Mn				
Daily intake (mg)[a]	0.054	0.041	0.036	0.141
DRI (mg/day; Men/Women)[b]	(2.3/1.79)	(2.3/1.8)	(2.3/1.8)	(2.3/1.8)
%DRI (Men/Women)	(2.34/2.99)	(1.79/2.29)	(1.55/1.98)	(6.13/7.83)
Dietary intake/serving (mg/serving)[c]	0.829	0.63	0.54	2.14
%DRI/serving (Men/Women)	(35.6/45.5)	(27.3/34.8)	(23.6/30.1)	(93.3/119)
Se				
Daily intake (μg)[a]	0.008	0.005	0.004	0.012
DRI (μg/day; Men/Women)[b]	(55/55)	(55/55)	(55/55)	(55/55)
%DRI (Men/Women)	(0.015/0.015)	(0.010/0.010)	(0.007/0.007)	(0.022/0.022)
Dietary intake/serving (mg/serving)[c]	0.123	0.079	0.060	0.181
%DRI/serving (Men/Women)	(0.223/0.223)	(0.144/0.144)	(0.109/0.109)	(0.330/0.330)
Total polyphenols				
Daily intake (mg)[d]	25.2	23.5	25.2	25.0
Intake (typical serving size, mg)[c]	383	358	384	380
$TEAC_{FRAP}$				
Daily intake (mg)[e]	0.230	0.238	0.238	0.246
Dietary intake/serving (mg/serving)[c]	3.50	3.62	3.61	3.74
$TEAC_{DPPH}$				
Daily intake (mg)[e]	0.188	0.206	0.199	0.205
Dietary intake/serving (mg/serving)[c]	2.86	3.14	3.03	3.12
$TEAC_{ABTSP}$				
Daily intake (mg)[e]	2.04	2.13	2.16	1.93
Dietary intake/serving (mg/serving)[c]	31.1	32.5	32.8	29.4

[a] Calculated by multiplying the average amount present in the different experimental groups by the daily consumption of lettuce by the Spanish population at home (Mercasa, 2023).
[b] Dietary reference intakes for Spanish adult population (Cuervo et al., 2010).
[c] Dietary intake calculated from consumption of a typical serving size in Spain (SENC, 2004).
[d] Calculated by transforming mmol of gallic acid/kg to g of gallic acid/kg of lettuce in the different experimental groups and multiplying by the daily consumption of lettuce by the Spanish population at home (Mercasa, 2023).
[e] Calculated by transforming the mmol Trolox equivalents/kg to g of antioxidants of lettuce in the different experimental groups and multiplying by the daily consumption of lettuce by the Spanish population at home (Mercasa, 2023).

was significantly higher than in the control group (Fig. 3a).

The total $TEAC_{DPPH}$ values are similar in all 4 sample groups considered ($p > 0.05$; Fig. 3b). In the digestion liquids, the $TEAC_{DPPH}$ in the control lettuce and BM-lettuce is significantly lower than that found in the HBM-lettuce ($p < 0.01$; Table 3). The prior hydrolysis of BM could leave bioactive compounds with antioxidant capacity determined in the intestinal digestion available.

The total $TEAC_{ABTS}$ values of BM-lettuce and HBM-lettuce were significantly higher than that of the control group ($p = 0.009$ and $p = 0.004$; respectively; Fig. 3c). This increase in total $TEAC_{ABTS}$ occurs at the expense of the $TEAC_{ABTS}$ established in the fermentation liquids, as there are identical significant differences to those indicated in the total $TEAC_{ABTS}$ (Fig. 3c).

Using the Folin-Ciocalteu method, related to the content of phenolic compounds, it was observed that the total $TEAC_{FC}$ in the control lettuces and the HBM-lettuces were significantly higher than in the BM-lettuces ($p = 0.017$ and $p = 0.015$, respectively; Table 3). Such differences in total $TEAC_{FC}$ are established in the digestion liquids, where with respect to the control group $TEAC_{FC}$ decreases and increases with respect to BM-lettuce and HBM-lettuce, respectively (Fig. 3d; Table 3). In general, the total $TEAC_{FC}$ values determined in our study in the different groups considered are slightly lower (Table 2) than those found, also in lettuces commonly consumed by the same method (FC: 19,165 ± 247 mmol gallic acid/kg; Navajas-Porras et al., 2020). However, the referred study does not indicate the variety of lettuce used in the assay, which is an important factor in both the content and type of phenolic compounds assessed by the Folin-Ciocalteu method (Shi et al., 2022). In contrast, Pannico et al. (2020) found in baby vegetables (microgreens), that after application of 16 μM Se in soilless cultures, the total content of phenonyl compounds increased (21 and 95 %) in coriander (*Coriandrum sativum*) and tatsoi lettuce (*Brassica rapa*) microgreens, respectively. Also Jeon et al. (2024) found that the application of dehydrated food waste increased the content of antioxidant bioactive compounds, especially anthocyanins and phenolic compounds in leafy vegetables. It would therefore be interesting to develop future studies in order to identify which specific phenolic compounds appear in control lettuces and in BM-lettuce and HBM-lettuce, and at the expense of which group decrease in total $TEAC_{FC}$ in BM-lettuce compared to control lettuces.

Table 4 shows that the correlations of the TEAC with the levels of minerals analysed, contrary to what was expected, all the statistically significant ones are negative, such as those established in the digestion liquids between Fe and the values of $TEAC_{FRAP}$ and $TEAC_{FC}$, and Se with those of $TEAC_{FRAP}$ and $TEAC_{DPPH}$. Also the values of total $TEAC_{ABTS}$ and that of the fermentation liquids are negatively and significantly correlated with Fe, Mn and Se ($p < 0.05$).

In the baby-lettuces, as uncultivated and non-biofortified sprouts (Fig. 3), the $TEAC_{FRAP}$, $TEAC_{DPPH}$ and $TEAC_{FC}$ values in the digestion liquids were significantly higher than those of the control lettuces, and those of $TEAC_{DPPH}$ and $TEAC_{FC}$ with respect to the BM-lettuce ($p < 0.05$; Table 2). However, fermentation led to a different behaviour in the baby-lettuces, as $TEAC_{ABTS}$ and $TEAC_{FC}$ decreased with respect to the other experimental groups studied (Table 3). Finally, the total $TEAC_{FRAP}$

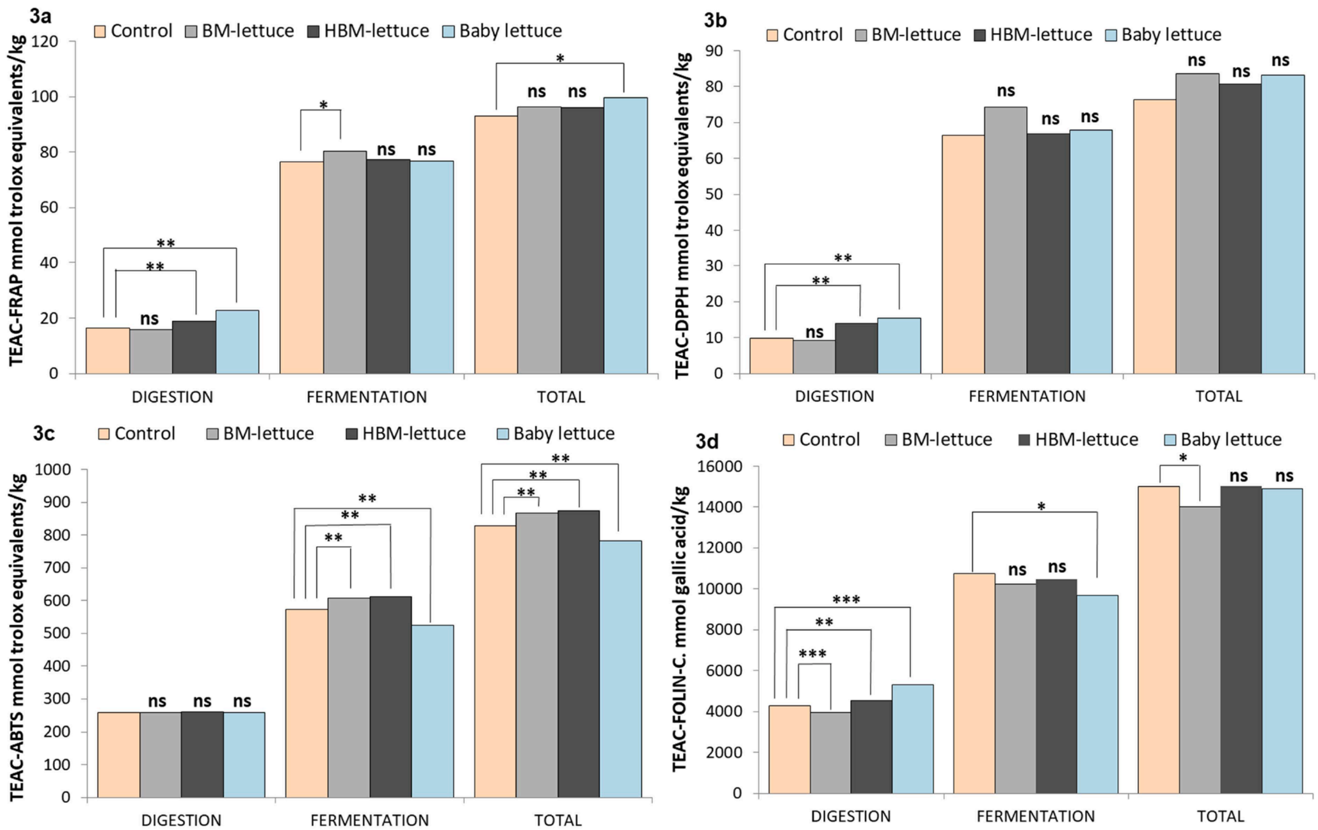

Fig. 3. Comparison of antioxidant capacity (AOC) values (TEAC) assessed by FRAP (a), DPPH (b), ABTS (c) and Folin-Ciocalteu (d), determined in control samples (lettuce grown with urea) *vs.* to the remaining experimental groups (lettuce grown with blood meal: BM-lettuce; or with hydrolyzed blood meal: HBM-lettuce; and baby lettuce not grown (baby-lettuce) after undergoing *in vitro* digestion, *in vitro* fermentation and total *in vitro* digestion/fermentation. Statistically significant differences were found with respect to the control group with significance levels of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). No significant differences were found with respect to the control group (ns).

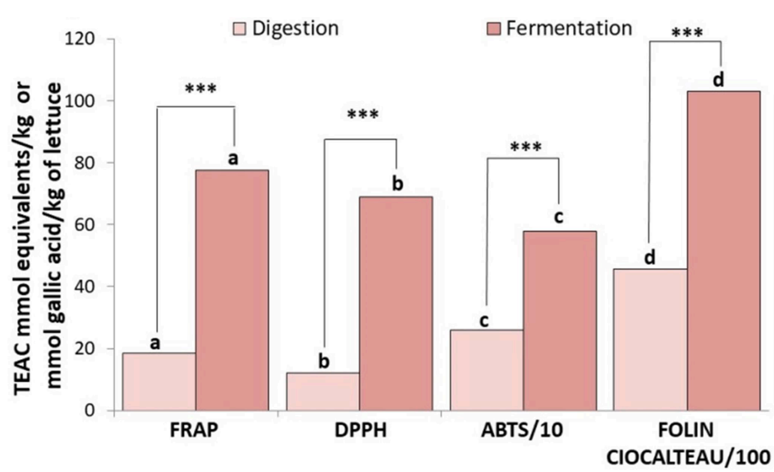

Fig. 4. Comparison of antioxidant capacity values determined in digestion liquids *vs.* fermentation liquids for all lettuce samples. Statistically significant differences were found with respect to the control group with significance levels of $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

values in the baby-lettuces increased significantly with respect to the control group. As for the total $TEAC_{ABTS}$ values, they decreased significantly ($p < 0.05$) in the baby-lettuces with respect to the other groups (control, BM-lettuce, HBM-lettuce), at the expense of the fermentation process, as the same behaviour was observed. It should be noted that the significantly higher levels of minerals studied in baby-lettuces (above all Cu, and to a lesser extent Fe; Table 1) compared to the controls, BM-lettuce and HBM-lettuce, are possibly related to the decrease in total antioxidant capacity (total $TEAC_{ABTS}$) detected in these baby-lettuces. In this sense, it is known that free Cu and Fe, unlike when they act as co-factors of antioxidant enzymes (superoxide dismutase and catalase, respectively) are prooxidants. This result contradicts what has been indicated about the use of baby-leaf lettuce in food because it has an increased antioxidant capacity (Collado-González et al., 2022). Since the methods used to evaluate the antioxidant capacity are carried out in different reaction media (aqueous, organic, acidic or basic), the disparate results obtained for the measurement of TEAC by the 4 methods used in this study in the evaluation of AOC would be justified.

Table 3
Mean antioxidant capacity (AOC) (± standard deviation, SD; fresh weight) of the different experimental lettuce groups (obtained after *in vitro* digestion, *in vitro* fermentation and total *in vitro* digestion/fermentation procedure) expressed as the Trolox equivalent AOC referred to reducing capacity, against DPPH and against ABTS radicals ($TEAC_{FRAP}$, $TEAC_{ABTS}$ and $TEAC_{DPPH}$, respectively; mmol Trolox equivalents/kg, fresh weight), and the total phenolic content (Folin-Ciocalteu; mmol gallic acid/kg, fresh weight). Experimental groups: lettuces cultivated in soils amended with blood meal (BM-lettuce), with hydrolyzed blood meal (HBM-lettuce) or urea (control group), and baby-lettuces.

Element	Control group	BM-lettuce	HBM-	Baby lettuce
$TEAC_{FRAP}$				
Digestion	16.5 ± 0.74[ab]	15.9 ± 0.88[c]	19.0 ± 0.03[acd]	22.9 ± 1.01[bd]
Fermentation	76.6 ± 2.25[a]	80.4 ± 3.43[a]	77.2 ± 3.70	76.7 ± 3.64
Total	93.1 ± 3.03[a]	96.3 ± 4.30	96.1 ± 3.69	99.6 ± 4.70[a]
$TEAC_{DPPH}$				
Digestion	9.82 ± 0.40[ab]	9.17 ± 0.90[cd]	13.9 ± 0.27[ac]	15.4 ± 1.32[bd]
Fermentation	66.5 ± 6.56	74.4 ± 10.9	66.9 ± 6.66	67.8 ± 14.5
Total	76.3 ± 6.69	83.6 ± 10.2	80.8 ± 6.49	83.2 ± 15.0
$TEAC_{ABTS}$				
Digestion	258 ± 1.08	259 ± 2.75	261 ± 4.06	258 ± 1.19
Fermentation	572 ± 20.0[abc]	607 ± 6.65[ad]	613 ± 9.85[be]	526 ± 3.76[cde]
Total	829 ± 20.3[abc]	866 ± 7.78[ad]	874 ± 12.3[be]	783 ± 5.10[cde]
Folin-Ciocalteu				
Digestion	4290 ± 61.6[abc]	3935 ± 163[ade]	4556 ± 142[bdf]	5313 ± 309[cef]
Fermentation	10,735 ± 798[a]	10,238 ± 402[b]	10,484 ± 447[c]	9693 ± 218[abc]
Total	15,025 ± 839[a]	14,017 ± 293[abc]	15,040 ± 476[b]	14,887 ± 280[c]

[#]Rows labelled with the same superscript lowercase letters for AOC values for different experimental groups of lettuce samples denotes the existence of statistically significant differences ($p < 0.05$).

Vegetables like lettuces have been reported to be a major source of dietary active antioxidant species that protects the body against several oxidative stress-induced (Carlsen et al., 2010). For this reason, efforts have been made to evaluate the total antioxidant capacity of foods (Apak et al., 2007). To do this, studies must comply the following main points (Siddeeg et al., 2021):

1. Include water-soluble (hydrophilic) and lipid-soluble (lipophilic) antioxidants.
2. Combine different analytical methods following the two main antioxidant mechanisms: A hydrogen atom transfer (HAT) method, which donates a hydrogen ion from a stable molecule thus allowing the antioxidant to scavenge the radical species; A single electron transfer (SET) method, which depends on the potential of the antioxidant to reduce certain molecules and compounds by transferring an electron.

In order to fully comply with these recommendations, in this study hydrophilic and lipophilic antioxidant compounds (released after *in vitro* digestion and further trasnformation by bacteria after fermentation) are released and solubilized in the liquids obtained after *in vitro* digestion and fermentation thanks to the enymatic activity and the emulsion properties of bile stalts. In addition, one HAT method (ABTS) and three SET methods (DPPH, FRAP and total phenols by Folin-Ciocalteu) were used to cover different antioxidant mechanisms, solvents (water for FRAP and total phenols, methanol for DPPH and water:ethanol for ABTS) and pH (acidic for FRAP and basic for total phenols).

Additionally, correlations between phenols determined by the Folin–Ciocalteau method and antioxidant activity measured by the remaining methods ($TEAC_{FRAP}$, $TEAC_{DPPH}$ and $TEAC_{ABTS}$) have been performed (Table 5). Only for digestion liquids (between Total polyphenols *vs.* $TEAC_{FRAP}$ and $TEAC_{DPPH}$) and fermentation liquids (between Total polyphenols *vs.* $TEAC_{ABTS}$) linear significant correlations were found ($p < 0.05$; Table 5).

The phenolic compounds provided in the daily diet considering the average concentration determined and the average daily amount of lettuce consumed in Spain (9.86 g/day; Mercasa, 2023), and from a typical serving of lettuce in Spain (150–200 g; SEN, 2004) are shown in Table 2. The mean content, considering all experimental groups, of phenolic compounds per serving (376 mg/serving) is higher than that established in previous studies on vegetables (53 mg/serving; Saura-Calixto & Goñi, 2006). However, the antioxidant substances associated with the mean AOC determined by FRAP and ABTS methods are considerably lower in the present study compared to that of Saura-Calixto &Goñi (2006) (3.62 *vs.* 189 and 31.2 *vs.* 123.2 mg/serving), respectively.

A strong point of the present study lies in the existence of limited scientific evidence that analyses the AOC of vegetables, after the application of BM or HBM as organic soil amendments, as an alternative to the use of a traditional fertiliser such as urea. It also means the

Table 4
Linear correlation coefficients and significance levels between Fe, Zn, Cu and Mn (mg/kg; fresh weight), and Se (μg/kg; fresh weight) concentrations and partial (in digestion and fermentation liquids) and total antioxidant capacity (AOC) determined by different methods (FRAP, DDPH and ABTS: mmol Trolox equivalents/kg; and Folin-Ciocalteu; mmol gallic acid/k; fresh weight) in all lettuce samples.

AOC	Fe		Zn		Cu		Mn		Se	
	r	p[a]	r	p[a]	r	p[a]	r	p[a]	r	p[a]
$TEAC_{FRAP}$										
Digestion	−0.570	0.033	-	ns	-	ns	-	ns	−0.587	0.027
Fermentation	-	ns	-	ns	-	ns	-	ns	-	ns
Total	-	ns	-	ns	-	ns	-	ns	-	ns
$TEAC_{DPPH}$										
Digestion	-	ns	-	ns	-	ns	-	ns	−0.621	0.018
Fermentation	-	ns	-	ns	-	ns	-	ns	-	ns
Total	-	ns	-	ns	-	ns	-	ns	-	ns
$TEAC_{ABTS}$										
Digestion	-	ns	-	ns	-	ns	-	ns	-	ns
Fermentation	−0.522	0.032	-	ns	-	ns	−0.623	0.008	−0.664	0.004
Total	−0.520	0.033	-	ns	-	ns	−0.613	0.009	−0.484	0.049
Folin-Ciocalteu										
Digestion	−0.623	0.017	-	ns	-	ns	-	ns	-	ns
Fermentation	-	ns	-	ns	-	ns	-	ns	-	ns
Total	-	ns	-	ns	-	ns	-	ns	-	ns

[a] ns: not significant.

Table 5
Linear correlation coefficients and significance levels between partial (in digestion and fermentation liquids) and total antioxidant capacity (AOC) determined by the Folin-Ciocalteu method (mmol gallic acid/kg; fresh weight) and remaining methods (FRAP, DDPH and ABTS: mmol Trolox equivalents/kg; fresh weight) in all lettuce samples.

Total polyphenols	$TEAC_{FRAP}$		$TEAC_{DPPH}$		$TEAC_{ABTS}$	
	r	p[a]	r	p[a]	r	p[a]
Digestion	0.884	<0.001	0.856	< 0.001	–	ns
Fermentation	–	ns	–	ns	0.771	0.020
Total	–	ns	–	ns	–	ns

[a] ns: not significant.

possible reuse of an industrial waste with a high biological oxygen demand, with a high capacity to pollute the environment, thus facilitating environmental sustainability and the development of a more sustainable agriculture (Navajas-Porras et al., 2024b). In addition, the same can be evaluated after a process of *in vitro* digestion and fermentation, to which the lettuces are subjected, allowing a more approximate evaluation of the final antioxidant capacity released after the *in vitro* digestion/fermentation processes.

3.4. Organoleptic characterization

In the organoleptic evaluation of lettuces compared to the control, it was observed that 50 % of the members of the tasting panel preferred the BM-lettuce and 43 % the HBM-lettuce, but without statistically significant differences ($p > 0.05$). Therefore, there is no preference between the control lettuces and the BM-lettuce, nor with respect to the HBM-lettuce (Fig. 5a). This figure shows that none of the preferences (dotted line) reached the minimum number of responses necessary to consider that there is a statistical preference for one product or the other. Similarly, the differential organoleptic evaluation showed that there were no significant differences between the control lettuces and the BM-lettuce and HBM-lettuce (Fig. 5b). Lettuce is a vegetable that is highly perishable, making a good appearance of the product essential for consumer acceptance. It has been demonstrated that the various treatments tested do not affect the appearance of the lettuce leaves or any of its other organoleptic characteristics. Therefore, this is a positive result that could promote the incorporation of this food into the diet and its acceptance by some consumers.

In the same line, Blatt (1991) found no differences in taste when applying BM together with meat meal in beetroot cultivation. Similarly, Zhao et al. (2007) in sensory evaluation of lettuce grown organically (pre-sowing compost, bovine manure and alfalfa + irrigation during cultivation with fish emulsion), and conventionally (pre-sowing NPK plus irrigation with calcium nitrate), found that the level of liking, flavor intensity and perceived bitterness did not differ significantly.

4. Conclusions

BM as an organic amendment increases the biomass of lettuce, which makes this by-product of the meat industry a good option as a nitrogen fertilizer in its cultivation, and an alternative to urea as a traditional fertilizer. The use of BM and HBM increases the Zn content in lettuce by 30 and 36 % compared to the control, respectively, so they could be used in biofortification in communities with a nutritional deficiency in this element. Contrary to what was hypothesized due to the high levels of Fe-heme present in the blood, the use of BM as an organic amendment to the cultivation soil does not influence its content in the lettuce. This finding, which could be related to possible interactions with the soil microbiome, which would use it as a growth factor, limiting its use by the plant, an aspect that should be specifically addressed in future studies. The use of BM reduces the levels of Mn and Se, compared to traditional fertilizer. Baby-lettuces have very high levels of antioxidant minerals, especially Cu, which reinforces their nutritional value as an important source of Fe, Cu and Mn in the diet. Only the total $TEAC_{ABTS}$ and $TEAC_{FC}$ of the BM-lettuce was higher and lower, respectively, than that of the control group. The compounds related to antioxidant capacity in all groups studied and for the 4 antioxidant assessment methods used, are mainly released at the fermentation stage. Further studies are needed to determine the specific lettuce components released in the fermentation process that are associated with the increased antioxidant capacity observed in this process. Total and fermentation stage $TEAC_{ABTS}$ decrease with increasing levels of Fe, Mn and Se in lettuce. The use of BM and HBM does not influence the organoleptic characteristics of the lettuce, an aspect of crucial importance due to the lack of influence on its consumption. It should be noted that the process of hydrolyzing blood meal is costly, and a prior cost-benefit assessment must be conducted before its application.

Declarations

The study was conducted according to the guidelines of the Declaration of Helsinki. It was approved by the Ethics Committee of the University of Granada (protocol code 1080/CEIH/2020)

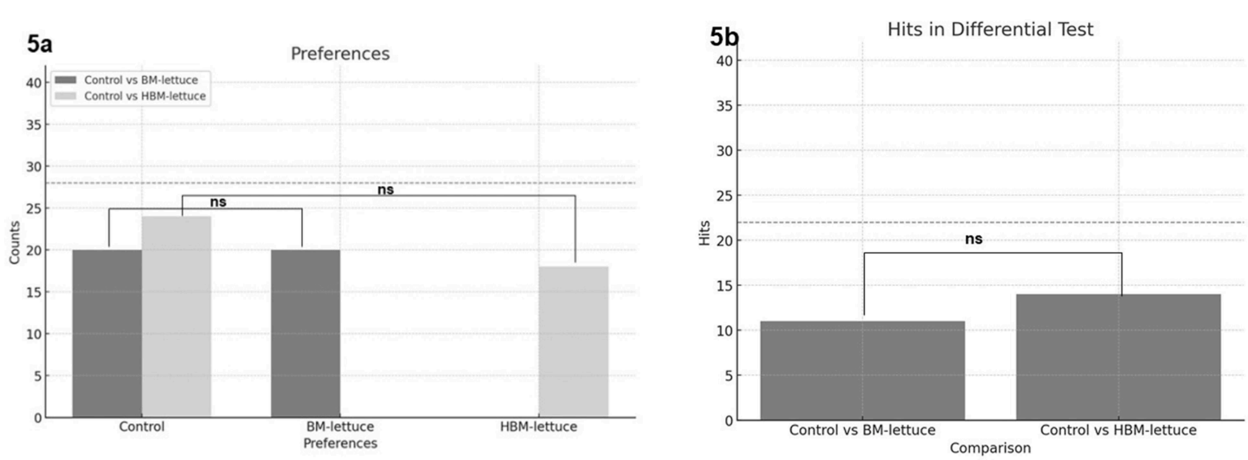

Fig. 5. Organoleptic characterization of the control lettuce group with respect to the other experimental groups (lettuce grown with blood meal: BM-lettuce; or with hydrolyzed blood meal: HBM-lettuce) determined by preferential evaluation (5a) and by differential evaluation (5b). No significant differences were found with respect to the control group (ns).

Ethical statement

This research word does not require any ethical approval.

CRediT authorship contribution statement

Victoria Fernández-Tucci: Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Ana Cervera-Mata:** Validation, Methodology, Investigation. **Alejandro Fernández-Arteaga:** Project administration, Methodology, Investigation, Funding acquisition. **José Javier Quesada-Granados:** Writing – review & editing, Visualization, Supervision, Project administration, Investigation, Conceptualization. **María del Carmen Almécija-Rodríguez:** Methodology, Investigation. **Adriana Delgado-Osorio:** Methodology, Investigation. **Miguel Navarro-Moreno:** Investigation, Data curation. **Silvia Pastoriza:** Writing – original draft, Conceptualization. **Gabriel Delgado:** Validation, Methodology, Investigation. **Miguel Navarro-Alarcón:** Writing – original draft, Validation, Methodology, Formal analysis. **José Ángel Rufián-Henares:** Validation, Methodology, Investigation.

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.afres.2024.100663.

Data availability

Data will be made available on request.

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