Nutritional, antioxidant and sensory properties of functional beef burgers formulated with chia seeds and goji puree, before and after *in vitro* digestion

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Abstract

The addition of chia seeds and goji puree (2.5 and/or 5%) was evaluated in terms of their effects on the fatty acid profile, lipid peroxidation, total phenols and antioxidant capacity of cooked beef burgers. In comparison to control burgers, polyunsaturated fatty acids doubled or tripled in samples containing chia seeds; polyphenols and antioxidant capacities (ORAC, ABTS, DPPH) increased up to 70% and malondialdehyde values were reduced up to 50% in burgers formulated with both ingredients.

Polyphenols, antioxidant capacity and lipid peroxidation were also assessed after *in vitro* digestion. A marked increase of polyphenol bioaccessibility and antioxidant capacity was observed for all samples, but also malondialdehyde values were increased after digestion, especially in samples containing 5% chia seeds.

Finally, hedonistic tests were conducted on young (18-30 years), adult (31-60 years) and elderly (>60 years) subjects and the burgers resulted acceptable by all groups, appointing to their potential application as functional burgers.

Keywords: Functional beef burgers; chia seeds; goji puree; natural antioxidants; lipid peroxidation; *in vitro* digestion.

1. Introduction

Meat represents a good source of proteins with high biological value and various micronutrients, including vitamin B12, zinc, phosphorus and iron (De Smet & Vossen, 2016). Nevertheless, in the past two decades, the consumption of red meat and meat products has been stigmatized due to their saturated fat, cholesterol and salt contents, which are considered as the main risk factors for several chronic diseases (De Smet & Vossen, 2016).

Despite this, the most recent findings suggest that dietary policies should encourage the intake of vegetables or foods rich in polyunsaturated fatty acids, whose consumption is lower than the optimal levels, more than reducing the consumption of red meat or processed meat. This might have a greater health effect than dietary policies only targeting sugar and fat (Afshin et al., 2019).

In Italy, the average annual consumption of beef meat has fallen from 24.9 (2007) to 17.1 (2017) kg per capita. However, in 2018, an increase in red meat consumption (+5%) was registered, with consumers paying more attention to the quality, genuineness and possible health benefits of meat (ISMEA, 2018).

Several strategies have been proposed to improve the quality of meat, not only at the level of breeding, but also during processing, to develop functional or nutraceutical meat products and provide adequate responses to consumer requirements (Decker & Park, 2010; Olmedilla-Alonso, Jimenez-Colmenero, & Sanchez-Muniz, 2013). Such strategies include the addition of natural antioxidants, able to prevent lipid peroxidation, which causes the deterioration of meat quality (Falowo, Fayemi, & Muchenje, 2014; Kumar, Yadav, Ahmad, & Narsaiah, 2015; Ribeiro et al., 2019) or vegetable oils or oilseeds, able to improve the fatty acid profile of meat (Decker & Park, 2010). Because of their promising functional properties, chia seeds and goji berries have gained attention as potential healthy ingredients for developing new functional foods.

The popularity of chia seeds (*Salvia hispanica* L.) has grown rapidly in the last few years because of their health-promoting activities, which include cardio-protective, antioxidant, anticancer and antimicrobial effects (Muñoz, Cobos, Diaz, & Aguilera, 2013; Ullah et al., 2016). In particular, chia seeds have high lipid, polyphenol and fiber contents and represent the source with the highest concentration of ω-3 alpha-linolenic acid (ALA) and ω-6 linoleic acid (Ayerza & Coates, 2011).

Goji berries (*Lycium barbarum* and *Lycium chinense* fruits) are commonly consumed in their dried form. Recently, they have become increasingly popular as “superfruits” because of their potential health-promoting properties, including antioxidant, hypoglycemic, lipid-lowering, immunostimulatory and anticancer effects (Chang, Alasalvar, & Shahidi, 2018; Kulczyński & Gramza-Michałowska, 2016; Ma et al., 2019). The main bioactive compounds in goji berries are water-soluble and highly branched polysaccharides; carotenoids, which confer the orange-red color to the berries; and phenolic compounds and flavonoids, which have a very high antioxidant capacity (Kulczyński & Gramza-Michałowska, 2016).

Although goji berries and chia seeds have been widely recognized as potentially healthy foods, few studies have reported their application in meat products (Aco, Aco, & Elena, 2018; Bulambaeva, Vlahova-Vangelova, Dragoev, Balev, & Uzakov, 2014; Pintado, Herrero, Jiménez-Colmenero, Pasqualin Cavalheiro, & Ruiz-Capillas, 2018; Pintado, Herrero, Jiménez-Colmenero, & Ruiz-Capillas, 2016; Souza et al., 2015; Zaki, 2018). To the best of our knowledge, no studies have investigated the combination of goji berries, in a puree form, and chia seeds, used as sources of natural antioxidants and polyunsaturated fatty acids to formulate beef burgers. Hence, the aim of the present study was to the evaluate the effects of the addition of chia seeds and/or goji puree, used alone or in combination at 2.5 and 5%, on the physicochemical and nutritional properties of cooked beef burgers. An overall screening of the antioxidant properties of the realized burgers was also performed by measuring their total phenol content, antioxidant capacities (ORAC, ABTS and DPPH assays) and malondialdehyde (MDA, a secondary product of lipid peroxidation) levels.

In order to be able to confer health benefits, bioactive compounds must resist food processing and be bioavailable, i.e. be released from the food matrix and be bioaccessible in the gastrointestinal tract (Angelino et al., 2017; Espín, García-Conesa, & Tomás-Barberán, 2007; Rein et al., 2013). We therefore applied an *in vitro* digestion model, characterized by an oral, gastric and intestinal phase, to evaluate the bioaccessibility of antioxidants and their ability to counteract lipid peroxidation in digested cooked burgers.

Finally, as the addition of ingredients potentially able to improve the nutritional quality of products may also alter their sensory properties, a hedonic test of our products was performed among different groups of consumers (young, adults and elderly subjects), in order to evaluate the overall liking of the burgers.

2. Materials and methods

2.1. Materials

Beef and vegetable dietary fibers (fibers 84 %; botanical origin: carrot, bamboo, potato, *Plantago spp.*) were supplied by the Company Baldi srl (Jesi, AN, Italy). Goji puree, made from fresh goji berries grown in Italy, was supplied by Rete di Imprese "Likion" per la Filiera del Goji Italiano®, (Villa San Giovanni, RC, Italy). Chia seeds were purchased from a local market. The average nutritional values of the goji puree and chia seeds, along with their moisture and pH values are reported in Table S1.

AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride), α-amylase, BHT (2,6-di-tert-butyl-4-methylphenol), bile, BSA (bovine serum albumin), DPPH (2,2-diphenyl-1-picrylhydrazyl), fluorescein as the percentage of total fatty acids determined. The atherogenicity (AI) and thrombogenicity (TI) indexes were calculated as reported in the literature (Ulbricht & Southgate, 1991).

2.5. *In vitro* digestion

The *in vitro* digestion (oral, gastric and intestinal phases) was performed on cooked burgers, in triplicate, following the procedure reported in the literature (Ninfali, Mari, Meli, Roselli, & Antonini, 2019) with slight modifications (Table S2). The final suspension derived from the intestinal phase (≈ 35 mL) was used "as-is" for the malondialdehyde (MDA) measurement or centrifuged at 15,000 rpm at +4 °C for 30 min and filtered using 0.45 µm filters for the polyphenol, ORAC, ABTS and DPPH assays. A blank sample was prepared with the digestive juices only.

2.6. Extraction and determinations of total phenols (TPs)

The extraction of TPs from goji puree, pulverized chia seeds and cooked burgers was performed in triplicate, as previously reported by Wu, Duckett, Neel, Fontenot, and Clapham (2008). TPs were assayed using the Folin-Ciocalteu method, as previously reported by Singleton, Orthofer, and Lamuela-Raventós (1999). A calibration curve was prepared using gallic acid (from 2 to 10 µg/mL) as a standard and values were expressed as mg of gallic acid equivalents (GAE)/100 g product.

2.7. Antioxidant assays (ORAC, DPPH, ABTS)

Three assays were performed to examine the antioxidant capacity of TPs in all samples: the oxygen radical absorbance capacity (ORAC), the DPPH and the ABTS radical scavenging activity assays. Each assay was performed in triplicate.

The ORAC method was performed as previously reported by Ninfali et al. (2009). The DPPH and ABTS assays were performed as reported by Brand-Williams, Cuvelier, and Berset (1995) and Ferri, Gianotti, and Tassoni (2013), respectively. A calibration curve was prepared for each assay (ORAC, DPPH, ABTS) with Trolox (from 2 to 20 µM) used as a standard and values expressed as µmol of Trolox equivalents (TE)/100 g product.

2.8. Measurement of malondialdehyde (MDA)

The extraction and quantification of malondialdehyde (MDA) in cooked burgers, before and after digestion, was performed in triplicate, following the procedure reported by Jung, Nam, and Jo (2016), with slight modifications. Briefly, 4.5 g of digested and non-digested cooked burgers were homogenized with 30 ml of 7.5% TCA solution and 150 µL of 7.2% BHT in ethanol using an Ultra-Turrax® at 16,000 rpm for 1 min. The homogenate was centrifuged at 10,000 rpm for 15 min, filtered with 20-25 µm filters and used as the MDA extract. For its quantification, 1 mL of MDA extract was mixed with 1 mL of 20 mM TBA in screw-cap tubes. The tubes were heated in a boiling water bath at

90 °C for 30 min and cooled in ice. Absorbance was measured at 532 nm using the UV–vis spectrophotometer. A calibration curve was prepared using TEP (1-32 μM) and results were expressed as mg of MDA/100 g product.

2.9. Sensory evaluation

Sensory tests were conducted on three different groups of subjects: young (18-30 years; n=56; M=48%), adult (31-60 years; n=38, M=74%) and elderly (>60 years; n=33, M=39%). The young subjects were recruited at the University of Gastronomic Sciences (Bra, CN, Italy), the adults were recruited at the Baldi srl Company and the elderly subjects joined the test at the retirement home "Casa di riposo – Residenza protetta Hermes di proprietà della Fondazione Opere Laiche Lauretane e Casa Hermes" (Loreto, AN, Italy). The sensory study was approved by the Ethics Committee of the University of Gastronomic Sciences. Written informed consent was collected from participants prior to the test.

Four samples (CTRL, $G_{2.5}$, $C_{2.5}$, $G_{2.5}$+$C_{2.5}$) were evaluated. Cooked burgers were divided into approximately 20 g portion and served in transparent plastic containers sealed with a lid. Samples were codified with three-digit random codes for young and adult subjects and with two-digit random codes for elderly subjects. The serving order was randomized and balanced across assessors.

Subjects were required to observe, smell and taste all the samples and give a judgment of liking considering appearance, odor, taste, flavor, texture and overall liking. Liking was evaluated on a nine-point hedonic scale ranging from 1 = extremely dislike to 9 = extremely like (Peryam & Pilgrim, 1957). A 30-sec break was required among samples combined with a rinsing procedure with water. Plain bread was given to the elderly subjects, while non-salted crackers were given to the young and adult groups as palate cleansers.

Participants were asked to complete a short questionnaire, which included questions regarding the frequency of consumption of red/white meat, consumption of cured meats, preferred type of meat (red, white or cured meat). Considering the frequency of meat consumption, subjects were classified in three clusters: occasional (once a week or less), frequent (2-3 times per week) and very frequent (at least 4 times per week). The tests lasted approximately from 10 min (young) to over 40 min (elderly). Data were collected with an automated procedure (FIZZ Acquisition software, version 2.51C, Biosystèmes, Courtenon, France) for the youngest, while paper sheets were used for the adults and elderly subjects.

2.10. Statistical analysis

The statistical significance (P < 0.05) of the effect of sample formulation was tested by one-way analysis of variance (ANOVA) using the SPSS® 17.0 statistical package program (IBM, Chicago, IL, USA). The entire experiment was performed in triplicate on three different days and no statistically significant differences were found between replicates. Differences between means for formulations were compared using Bonferroni's test.

The effect of the subject group (young, adult, elderly) on liking for the four prototypes was assessed adopting two-way mixed ANOVA models (fixed factor: sample; random factor: group) separately for appearance, odor, taste, flavor, texture and overall liking. Two-ANOVA mixed models (fixed factor: sample; random factor: assessor) were separately applied within each group and on the whole population to assess the effect of the sample on liking for each sensory modality, followed by Fisher's LSD test to estimate significant differences among mean values ($P < 0.05$). The effect of the frequency of consumption on the liking of samples was assessed with two-way mixed ANOVA models (fixed factor: frequency of consumption; random factor: assessor). Sensory analyses were conducted with XLStat 2019.1.1, Addinsoft, Boston, USA.

3. Results and discussion

3.1. Proximate composition, pH and cooking loss

Table 2 shows the proximate composition, pH and cooking loss (CL) of burgers made with different percentages of goji puree and chia seeds, used alone or in combination.

The moisture content ranged from 64.7 to 60.7% (Table 2). The addition of 5% chia seeds significantly decreased the moisture content of C_5 and G_5+C_5 burgers due to the increase of dry matter in the formulations. In fact, the chia seeds used in our experiments were characterized by only 5.5% moisture, a significantly lower value than that of goji puree (76.8%) (Table S1).

The fat content ranged from 11.8 to 14.7% (Table 2), with the highest level found in C_5 burgers due to the presence of chia seeds, which were characterized by a fat content of 35% (Table S1). The addition of up to 5% goji puree and chia seeds did not influence the protein content, which showed an average value of 22.8% with no significant differences among samples (Table 2).

The pH values ranged from 5.9 to 5.7 (Table 2). The addition of 5% goji puree led to a greater decrease in the pH values of G_5 and G_5+C_5 burgers due to its acidic nature (pH = 4.8) compared to chia seeds (Table S1).

CL varied from 26.1 to 16.7% (Table 2). Burgers containing chia seeds showed smaller losses than the other samples due to the high water retention and emulsifying capacities of the mucilaginous compounds produced by glucuronic acid and neutral sugars, which constitute the soluble fiber of chia seeds (de Melo et al., 2015).

3.2. Fatty acid composition

Table 3 shows the fatty acid composition of cooked burgers and their nutritionally significant ratios. In all samples, the main fatty acids were monounsaturated (MUFA), followed by saturated (SFA) and polyunsaturated (PUFA). Oleic acid (C18:1) predominated among MUFA, whereas palmitic acid (C16:0) was the main acid among SFA.

The addition of chia seeds enhanced the nutritional quality of the beef burgers (Table 3), resulting in:

i) an increase in total PUFA content, which ranged from 2.9% (CTRL) to 6.9% (mean value of $C_{2.5}$ and $G_{2.5}+C_{2.5}$) and 10.5% (mean value of C_5 and G_5+C_5), due to the high levels of α-linolenic acid (C18:3) present in the chia seeds;

ii) an improved PUFA/SFA ratio, which increased from 0.06 (CTRL) to 0.26 (G_5+C_5). Considering the medium-high fat content of the beef used to formulate the burgers, compared to studies in the literature, the improved PUFA/SFA ratio should be considered of interest, even though it is half the recommended ratio of 0.4 (Wood et al., 2004);

iii) an improved ω-6/ω-3 ratio, decreasing from 5.67 (CTRL) to 0.65 (G_5+C_5). This ratio plays a very important role in human nutrition, more so than the ratio reported in the previous point. According to nutritional guidelines, the ω-6/ω-3 ratio should not be higher than 4 (Simopoulos, 2002);

iv) a reduction of atherogenicity (AI) and thrombogenicity (TI) indexes, ranging from 0.60 and 1.56 (CTRL) to 0.51 and 0.87 (C_5), respectively. These indexes take into account the different effects of fatty acids on cardiovascular risk: food products with low values of AI and TI can inhibit the aggregation of platelets and decrease the levels of esterified fatty acids, cholesterol and phospholipids, thereby lowering the risk of micro- and macro-coronary disease (Ulbricht & Southgate, 1991).

The improved lipid profile obtained by adding chia seeds to beef burgers can play an important role in the prevention and treatment of cardiovascular diseases, hypertension, diabetes, arthritis and other autoimmune disorders (Cifuni, Napolitano, Riviezzi, Braghieri, & Girolami, 2004).

In addition, the fatty acid profile of our burgers formulated with chia seeds made it possible to satisfy EU Regulation n° 116/2010 regarding the claims “source of omega-3 fatty acids” and “high omega-3 fatty acids”, which can be used if the product contains at least 0.3 g or 0.6 g α-linolenic acid (ALA) per 100 g, respectively. According to our results, $C_{2.5}$ and $G_{2.5}+C_{2.5}$ contain an average amount of 0.49 g ALA/100 g; C_5 and G_5+C_5 contain about 0.91 g ALA/100 g. This fatty acid contributes to the maintenance of normal blood cholesterol levels and this beneficial effect is obtained with a daily intake of 2 g of ALA (EU Reg. 432/2012).

3.3. Polyphenol contents and antioxidant profiles of cooked beef burgers before and after *in vitro* digestion

In this study, we first evaluated the antioxidant profiles of the goji puree and chia seeds used in the formulation of beef burgers (Table 4). Both plant-based ingredients were characterized by similar total phenols, which could be ascribed to their pool of antioxidant molecules belonging to the same family, i.e. carotenoids, chlorogenic and caffeic acids, quercetin, and kaempferol (Kulczyński & Gramza-Michałowska, 2016; Ullah et al., 2016). Nevertheless, the food matrix of each product influenced its antioxidant capacity in different ways, with the goji berry puree showing higher ORAC and ABTS values and the chia seeds showing higher DPPH values (Table 4).

Moreover, meat itself contains hydrophilic and lipophilic antioxidants, which moderately contribute to its antioxidant capacity. Among these, the most efficient antioxidant compounds are dipeptides such as carnosine and anserine, and other substances such as L-carnitine, glutathione, taurine and creatine (Liu, Xing, Fu, Zhou, & Zhang, 2016; M Antonini et al., 2002). Before digestion, the polyphenol content of our CTRL burger was 20.9 mg/100g (Fig. 1A, blank bar). This value increased to 25 and 29 mg/100g when the goji puree and chia seeds were added to the formulation at 2.5% ($G_{2.5}$ and $C_{2.5}$) and 5% (G_5 and C_5), respectively. The combination of the two ingredients at 5% (G_5+C_5) showed the highest polyphenol level (34 mg/100 g), thus suggesting that the antioxidant molecules that characterize goji berries and chia seeds have a synergistic effect (Fig. 1A, blank bars). To the best of our knowledge, in literature there are not studies regarding the possible synergistic effect of these two healthy foods, although interactions between phytochemicals from fruits and vegetables have been recently reviewed (Phan, Paterson, Bucknall, & Arcot, 2018). The two main methods used to determine the types of interaction of binary mixtures of phytochemicals, i.e. isobologram and combination index, reported in this review, could be used in future studies to test the possible interaction between phytochemicals of chia seeds and goji puree.

Polyphenol bioaccessibility is an important parameter that represents the amount of polyphenols which are released from the food matrix, strongly influenced by the physicochemical properties of the food matrix and by the technological processes used in food production, as well as by the physiological condition of the individual (Angelino et al., 2017). After digestion, the polyphenol content showed a ten-fold increase in most of the analyzed burgers (Fig 1A, grey bars) due to the further solubilization of polyphenols in digestive fluids (Kim & Hur, 2018; Pešić et al., 2019). Digestive enzymes are able to transform phenolic compounds into different structural forms possessing alterated chemical properties. The increase in the total phenolic content may be attributed to an acidic hydrolysis of phenolic glycosides during gastric digestion, with a higher antioxidant activity displayed by aglycone phenolics than their glycoside forms (Lee, Lee, Chung, & Hur, 2016). Moreover, the change of pH from an acidic to the alkaline environment, during intestinal digestion, may improve the antioxidant capacity of phenolics through the deprotonation of the hydroxyl moieties present on their aromatic ring (Kim & Hur, 2018).

Nevertheless, the polyphenol pattern of digested burgers (Fig 1A, grey bars) was not the same as that of non-digested burgers (Fig 1A, blank bars). The best results were found in $G_{2.5}$ and G_5 digested burgers (average value of 245 mg/100g), while C_5 digested burgers showed the lowest polyphenol bioaccessibility (188 mg/100 g). All other digested samples did not show a statistically significant difference compared to digested CTRL burgers (214 mg/100 g).

The antioxidant capacity of the burgers was measured before and after *in vitro* digestion using three different methods: ORAC (Fig 1B), ABTS (Fig 1C) and DPPH (Fig 1D). These methods were chosen in order to evaluate different aspects of the chemical mechanisms of action (Serpen, Gökmen, & Fogliano, 2012). In both cases (digested or non-digested), we obtained the following results: ORAC > ABTS > DPPH.

Before digestion, we found a good correspondence between antioxidant capacities and polyphenol patterns, especially for the ORAC and ABTS assays (Fig. 1A, B, C, blank bars). CTRL burgers showed interesting antioxidant capacity values, specifically 1104 (ORAC), 132 (ABTS) and 51 (DPPH) μmol_{TE}/100 g. The addition of goji puree and chia seeds in G_5+C_5 burgers increased the antioxidant capacities of our burgers up to 1902 (ORAC), 236 (ABTS) and 132 (DPPH) μmol_{TE}/100 g.

After digestion, the antioxidant capacities of cooked burgers were all higher than non-digested samples, thus reflecting a higher polyphenol bioaccessibility (Fig. 1A, B, C, D grey bars). The ORAC and DPPH methods revealed a higher antioxidant capacity when goji puree and chia seeds where added, respectively, thus highlighting the different ability of polyphenols to scavenge free radicals.

3.4. Lipid peroxidation in cooked beef burgers before and after the *in vitro* digestion

Unsaturated fatty acids in meat are susceptible to oxidation leading to a deterioration in quality, which may include color changes, off-flavors and odors. Several authors have demonstrated that lipid peroxidation in meat products could be prevented by the addition of protective compounds such as polyphenols (Gorelik, Ligumsky, Kohen, & Kanner, 2008), fibers (Hur, Lim, Park, & Joo, 2009), minerals and vitamins (Pierre et al., 2013).

We therefore investigated lipid peroxidation by measuring MDA formation on cooked burgers before and after *in vitro* digestion (Figure 2).

Before digestion (Fig. 2, blank bars), CTRL burgers showed the highest MDA value (0.66 mg_{MDA}/100 g). The addition of goji puree at 2.5% ($G_{2.5}$) or 5% (G_5) resulted in a gradual statistically significant MDA decrease (0.48 and 0.35 mg_{MDA}/100 g, respectively), notwithstanding the possible interference of sugars and red pigments contained in this plant-based additive (Jung et al., 2016). The addition of chia seeds also provided a significant reduction in MDA levels compared to CTRL burgers, though not in a dose-dependent manner (0.30 mg_{MDA}/100 g for both $C_{2.5}$ and C_5). The protective effect against lipid peroxidation is attributable to the high polyphenol and fiber content of goji puree (Kulczyński & Gramza-Michałowska, 2016; Zhou et al., 2017) and chia seeds (Alfredo, Gabriel, Luis, & David, 2009; Ullah et al., 2016).

After digestion (Fig. 2, grey bars), lipid peroxidation significantly increased in all samples (for example, from 0.66 in non-digested to 2.13 mg_{MDA}/100 g in digested CTRL burgers) because of the oxidative processes occurring during digestion described in the literature (Kim & Hur, 2018; Martínez, Nieto, Castillo, & Ros, 2014). The addition of goji puree and chia seeds provided different results regarding protection against lipid peroxidation, with the former showing good results when used at higher concentrations (G_5 = 1.46 mg_{MDA}/100 g) and the latter when used at lower concentrations ($C_{2.5}$ = 1.57 mg_{MDA}/100 g).

It is well known that high-fat beef products are more susceptible to lipid peroxidation due to their content of heme-Fe, which catalyzes the production of ROS and the oxidation of unsaturated fatty acids. Beef burgers containing 5% chia seeds, used alone (C_5) or in combination with goji puree (G_5+C_5), showed an important increase in MDA levels after *in vitro* digestion, thus suggesting a pro-oxidative effect of the seeds. Indeed, chia seeds contain high levels of unsaturated fatty acids, especially α-linolenic acid (Muñoz et al., 2013), which is probably made more susceptible to lipid peroxidation by the *in vitro* digestion process. Moreover, the C_5 burger was the sample that showed the lowest polyphenol bioaccessibility after digestion (Fig. 1A, grey bar), thus suggesting poor antioxidant protection against oxidation caused by the digestion process.

3.5. Sensory acceptability

Considering the latest results regarding lipid peroxidation, we decided to perform the sensory analysis on only four out of seven burgers (CTRL, $G_{2.5}$, $C_{2.5}$, $G_{2.5}$+$C_{2.5}$).

A significant effect of the subject group ($P < 0.0001$) was found on liking for all the sensory modalities. The elderly always showed significantly higher mean liking scores for all sensory modalities than adults or young people. The liking of the elderly was also significantly higher than adults for odor, taste, texture and overall liking. Therefore, in general, liking decreased as follows: elderly > adults > young. Acceptability (score = 5) was attained in all groups for all samples.

The mean liking values obtained for the four burgers by all assessors and the three related groups (young, adult, elderly) are shown in Table 5.

Considering the whole population, samples only significantly affected liking as regards appearance, with $G_{2.5}$ and CTRL showing the highest mean value.

Within the groups, the effect of the sample on liking was different. In the young group, the sample had a significant ($P < 0.01$) effect on liking as regards appearance, taste, flavor, texture and overall liking. Liking scores were generally modest in this group. For all sensory modalities, young people liked the $G_{2.5}$ sample at the same level as the control sample (CTRL). In adults, the effect of the sample was only observed on liking for flavor, with $C_{2.5}$ proving to be the most preferred sample. In the elderly, no significant effects of the sample on liking were observed for any of the sensory modalities. However, liking scores were generally higher than 6 (moderately liked); therefore, all samples showed a good performance regardless of the formulation.

Results regarding the frequency of meat consumption (as reported in Materials and Methods section) showed the following distribution in the three classes: 13% occasional meat-eaters, 38% frequent meat-eaters, and 49% very frequent meat-eaters. The frequency of consumption of meat had a significant effect ($P \leq 0.05$) on appearance, odor, taste, flavor, and texture but not on overall liking. In all cases, liking increased as the frequency of consumption increased: very frequent > frequent > occasional. Mean values of all sensory modalities were significantly higher for the very frequent consumers than for the occasional consumers. Significant different mean values between very frequent and frequent consumers were observed only in terms of liking as regards flavor.

The high liking score results obtained from the hedonic tests were extremely encouraging. This is not always the case in functionalized meat products, as observed in beef patties formulated with flaxseeds, where an increase in the plant-based ingredient led to lower liking scores (Elif Bilek & Turhan, 2009).

Moreover, the most positive results were found among the elderly, who could be considered as a particularly desirable consumer target for the developed burgers. In fact, elderly people often do not cover their protein needs. This situation is especially worrisome in retirement communities, where the majority of elderly residents do not meet their caloric and protein requirements and the institutions have to face the elderly's beliefs that 'At my age, I no longer need to eat so much meat' (Sulmont-Rossé, and Van Wymelbeke, 2019). The tests involving elderly subjects were conducted in this type of setting (retirement home); hence, these types of meats could have promising applications in the near future.

Conclusions

The addition of goji berry puree and chia seeds affected the physical-chemical, nutritional and sensory properties of cooked beef burgers in different ways. Chia seeds make it possible to label burgers with EU health claims regarding fatty acids. Both plant-based ingredients ameliorated polyphenol content and total antioxidant capacity of beef burgers, before and after digestion, thus suggesting a better bioaccessibility of antioxidant molecules and a possible greater bioavailability. The addition of antioxidants to the diet, especially if they are consumed at the same time as meat products or in the same meat preparation, could be a good strategy to counteract the lipid peroxidation that usually occurs in cooked meat products. Nevertheless, the concentration of the vegetables added to the burgers should be carefully dosed in order to avoid a pro-oxidative effect. Finally, sensory acceptability was attained in all groups for all the samples, with the most interesting results obtained in the elderly target (<60 years). This finding, together with all the improved nutritional qualities and antioxidant capacities, suggest that these enhanced burgers could become a valid meal alternative for human nutrition.

Declarations of interest

None.

Acknowledgments

The authors wish to express their gratitude to the Company Baldi srl and professor Paolino Ninfali for their support in the study and to Dr. Timothy Bloom for his linguistic revision of the manuscript.

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Table 1. Beef burger formulations.

Ingredients (%)	**CTRL**	**$G_{2.5}$**	**$C_{2.5}$**	**G_5**	**C_5**	**$G_{2.5}$+$C_{2.5}$**	**G_5+C_5**
Ground beef meat	85	85	85	85	85	85	85
Chia seeds (C)	0	0	2.5	0	5	2.5	5
Goji puree (G)	0	2.5	0	5	0	2.5	5
Vegetable dietary fibre	2	2	2	2	2	2	2
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Water	12.5	10	10	7.5	7.5	7.5	2.5
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 2. Proximate composition, pH and cooking loss of beef burgers.

	Samples[*]						
	CTRL	**$G_{2.5}$**	**$C_{2.5}$**	**G_5**	**C_5**	**$G_{2.5}$+$C_{2.5}$**	**G_5+C_5**
Proximate analysis							
Moisture (%)	64.7 ± 0.5^a	64.3 ± 0.3^a	64.1 ± 0.1^a	63.0 ± 0.5^a	61.9 ± 0.3^b	63.1 ± 0.5^a	60.7 ± 0.5^b
Fat (%)	12.3 ± 0.1^c	12.0 ± 0.1^c	13.8 ± 0.1^b	11.8 ± 0.1^c	14.7 ± 0.2^a	13.8 ± 0.1^b	14.0 ± 0.2^b
Protein (%)	23.5 ± 0.5^a	23.2 ± 0.3^a	22.7 ± 0.2^a	23.3 ± 0.3^a	22.4 ± 0.2^a	22.3 ± 0.3^a	22.0 ± 0.4^a
pH	5.9 ± 0.0^a	5.8 ± 0.0^b	5.8 ± 0.0^b	5.7 ± 0.0^c	5.8 ± 0.0^b	5.8 ± 0.0^b	5.7 ± 0.0^c
CL (%)	26.1 ± 1.2^a	24.2 ± 1.0^a	21.2 ± 0.6^b	24.5 ± 0.8^a	16.7 ± 0.8^c	19.8 ± 0.8^b	18.7 ± 0.3^b

[*] For sample formulations see Table 1. CL, cooking loss. Values are reported as mean ± standard error.

^{a,b}Different letters in the same row indicate statistically significant differences ($P < 0.05$).

Table 3. Fatty acid profiles and nutritional significance ratios on beef burgers.

Parameters	**Samples**[*]						
	CTRL	**$G_{2.5}$**	**$C_{2.5}$**	**G_5**	**C_5**	**$G_{2.5}$+$C_{2.5}$**	**G_5+C_5**
C14:0	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	2.9 ± 0.1	3.1 ± 0.1	2.9 ± 0.1
C 16:0	27.3 ± 0.5	26.8 ± 0.5	25.5 ± 0.5	26.8 ± 0.5	25.0 ± 0.5	25.6 ± 0.5	24.4 ± 0.5
C 18:0	15.1 ± 0.4	15.9 ± 0.4	15.0 ± 0.4	15.8 ± 0.4	14.7 ± 0.4	15.0 ± 0.4	14.0 ± 0.4
Total SFA	**45.5 ± 0.7^{a}**	**45.9 ± 0.7^{a}**	**43.5 ± 0.7^{b}**	**45.8 ± 0.7^{a}**	**42.6 ± 0.7^{b}**	**43.7 ± 0.7^{b}**	**41.2 ± 0.7^{b}**
C14:1	1.1 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
C16:1	4.0 ± 0.2	3.9 ± 0.2	3.8 ± 0.2	3.9 ± 0.2	3.5 ± 0.1	3.8 ± 0.2	3.6 ± 0.2
C18:1	47.9 ± 0.8	46.7 ± 0.8	45.0 ± 0.7	46.7 ± 0.8	43.0 ± 0.7	45.0 ± 0.7	43.5 ± 0.7
Total MUFA	**52.9 ± 0.8^{a}**	**51.6 ± 0.8^{a}**	**49.8 ± 0.8^{ab}**	**51.6 ± 0.8^{a}**	**47.5 ± 0.7^{b}**	**49.8 ± 0.8^{ab}**	**48.1 ± 0.7^{b}**
C18:2 (ω-6)	2.4 ± 0.1	2.4 ± 0.1	3.4 ± 0.1	2.4 ± 0.1	4.1 ± 0.2	3.3 ± 0.1	4.3 ± 0.2
C18:3 (ω-3)	0.4 ± 0.0	0.4 ± 0.0	3.6 ± 0.2	0.4 ± 0.0	6.1 ± 0.2	3.4 ± 0.1	6.6 ± 0.3
Total PUFA	**2.9 ± 0.1^{c}**	**2.8 ± 0.1^{c}**	**7.0 ± 0.2^{b}**	**2.8 ± 0.1^{c}**	**10.1 ± 0.3^{a}**	**6.7 ± 0.2^{b}**	**10.9 ± 0.3^{a}**
PUFA/SFA	0.06	0.06	0.16	0.06	0.24	0.15	0.26
ω-6/ω-3	5.67	6.34	0.93	5.90	0.67	0.96	0.65
AI	0.60	0.61	0.56	0.61	0.53	0.56	0.51
TI	1.56	1.62	1.13	1.61	0.94	1.15	0.87

[*] For sample formulations see Table 1. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; AI, atherogenicity index; TI, thrombogenicity index. Fatty acid profiles (percentages of total fat) are reported as average values ± standard error. ^{a,b}Different letters in the same row indicate statistically significant differences ($P < 0.05$).

Table 4. Antioxidant profiles of goji puree and chia seeds.

	Goji puree	**Chia seeds**
TPs (mg$_{GAE}$/100 g)	153 ± 5^{a}	151 ± 2^{a}
ORAC (µmol$_{TE}$/100 g)	9950 ± 29^{a}	6500 ± 23^{b}
ABTS (µmol$_{TE}$/100 g)	2059 ± 101^{a}	1227 ± 55^{b}
DPPH (µmol$_{TE}$/100 g)	213 ± 13^{b}	659 ± 8^{a}

TPs, total phenols. Values are reported as mean ± standard error. ^{a,b}Different letters in the same row indicate statistically significant differences ($P < 0.05$).

Table 5. Overall liking and liking for appearance, odor, taste, flavor and texture obtained by the totality of the subjects and the three groups of consumers (young, adults, elderly) for burger samples.

Group	Samples*	Appearance	Odor	Taste	Flavor	Texture	Overall
All (n=127)	CTRL	6.0 ± 0.1^{a}	5.6 ± 0.1^{a}	5.9 ± 0.1^{a}	5.8 ± 0.1^{ab}	6.2 ± 0.1^{a}	6.0 ± 0.1^{ab}
	$G_{2.5}+C_{2.5}$	5.6 ± 0.1^{b}	5.4 ± 0.1^{a}	5.7 ± 0.1^{a}	5.6 ± 0.1^{b}	5.9 ± 0.1^{a}	5.8 ± 0.1^{b}
	$G_{2.5}$	6.0 ± 0.1^{a}	5.7 ± 0.1^{a}	6.1 ± 0.1^{a}	6.0 ± 0.1^{a}	6.2 ± 0.1^{a}	6.1 ± 0.1^{a}
	$C_{2.5}$	5.5 ± 0.1^{b}	5.6 ± 0.1^{a}	5.8 ± 0.1^{a}	5.8 ± 0.1^{ab}	6.0 ± 0.1^{a}	5.9 ± 0.1^{ab}
Young (n=56)	CTRL	5.8 ± 0.1^{a}	5.3 ± 0.1^{a}	6.0 ± 0.1^{a}	5.7 ± 0.1^{ab}	5.9 ± 0.2^{a}	5.8 ± 0.1^{a}
	$G_{2.5}+C_{2.5}$	4.6 ± 0.1^{b}	4.8 ± 0.1^{b}	5.3 ± 0.1^{b}	5.1 ± 0.1^{c}	5.2 ± 0.2^{bc}	5.1 ± 0.1^{c}
	$G_{2.5}$	5.8 ± 0.1^{a}	5.3 ± 0.1^{a}	6.0 ± 0.1^{a}	5.8 ± 0.1^{a}	5.7 ± 0.2^{ab}	5.8 ± 0.1^{a}
	$C_{2.5}$	4.5 ± 0.1^{b}	4.9 ± 0.1^{ab}	5.1 ± 0.1^{b}	5.1 ± 0.1^{bc}	5.0 ± 0.2^{c}	5.1 ± 0.1^{b}
Adult (n=38)	CTRL	6.1 ± 0.1^{a}	5.6 ± 0.1^{a}	5.6 ± 0.1^{b}	5.7 ± 0.1^{b}	6.2 ± 0.1^{ab}	5.9 ± 0.1^{b}
	$G_{2.5}+C_{2.5}$	6.2 ± 0.1^{a}	5.5 ± 0.1^{a}	5.8 ± 0.1^{ab}	5.9 ± 0.1^{b}	6.1 ± 0.1^{b}	6.0 ± 0.1^{ab}
	$G_{2.5}$	5.8 ± 0.1^{a}	5.5 ± 0.1^{a}	5.6 ± 0.1^{b}	5.8 ± 0.1^{b}	6.2 ± 0.1^{ab}	6.0 ± 0.1^{ab}
	$C_{2.5}$	6.2 ± 0.1^{a}	5.8 ± 0.1^{a}	6.2 ± 0.1^{a}	6.4 ± 0.1^{a}	6.6 ± 0.1^{a}	6.4 ± 0.1^{a}
Elderly (n=33)	CTRL	6.1 ± 0.1^{b}	6.1 ± 0.2^{b}	6.2 ± 0.2^{a}	6.2 ± 0.2^{a}	6.8 ± 0.1^{a}	6.3 ± 0.1^{a}
	$G_{2.5}+C_{2.5}$	6.6 ± 0.1^{a}	6.4 ± 0.2^{a}	6.5 ± 0.2^{a}	6.2 ± 0.2^{a}	6.8 ± 0.1^{a}	6.7 ± 0.1^{a}
	$G_{2.5}$	6.6 ± 0.1^{a}	6.6 ± 0.2^{a}	6.8 ± 0.2^{a}	6.8 ± 0.2^{a}	7.0 ± 0.1^{a}	6.8 ± 0.1^{a}
	$C_{2.5}$	6.4 ± 0.1^{ab}	6.4 ± 0.2^{ab}	6.5 ± 0.2^{a}	6.1 ± 0.2^{a}	6.8 ± 0.1^{a}	6.7 ± 0.1^{a}

*For sample formulations see Table 1. Values are reported as mean ± standard error. Different letters among the same sub-column indicate statistically significant differences ($P < 0.05$).

Table S1. Average nutritional values of goji puree and chia seeds used in beef burger formulations.

	Goji puree	**Chia seeds**
Nutritional values[*]		
Energy (kJ and kcal/100 g)	337 / 80	1992 / 482
Fat (g/100 g)	0.91	35
of which saturates (g/100 g)	0.43	3.5
Carbohydrate (g/100 g)	13	5.3
of which sugars (g/100 g)	9.5	0.55
Dietary fiber (g/100 g)	5.4	29
Protein (g/100 g)	2.2	21
Salt (g/100 g)	0.13	0.05
Moisture (%)	76.8 ± 0.9^{a}	5.5 ± 0.4^{b}
pH	4.8 ± 0.0^{b}	7.8 ± 0.0^{a}

[*] Average nutritional values as reported by the manufacturers on the label. Values of moisture and pH are reported as mean ± standard error. [a,b] Different letters in the same row indicate statistically significant differences ($P < 0.05$).

Table S2. Three-stage *in vitro* digestion procedure (A) and composition of digestive juices (B).

A) Digestion phases

	Sample	Extraction mixture	Time (min)	Temperature (°C)
Oral	Cooked beef burgers (5 g)	6 ml of salivary solution (pH = 6.8)	5	37
Gastric	Suspension after the oral phase	12 ml of gastric solution (pH = 1.5)	120	37
Intestinal	Suspension after the gastric phase	12 ml of duodenal + 6 ml of biliar + 1 M bicarbonate solutions (pH = 8.2)	120	37

B) Constituents and composition of 1L of salivary, gastric, duodenal and biliar juices

	Salivary	**Gastric**	**Duodenal**	**Biliar**
Organic and inorganic components	1.7 ml NaCl (175.3 g/L) 8.0 ml urea (25.0 g/L) 15 mg uric acid	6.5 ml HCl (37 %) 18.0 ml $CaCl_2$ (22.2 g/L)	6.3 ml KCl (89.6 g/L) 9.0 ml $CaCl_2$ (22.2 g/L)	68.5 ml $NaHCO_3$ (84.7 g/L) 10.0 ml $CaCl_2$ (22.2 g/L)
Enzymes	290 mg α-amilasi 25 mg mucin	2.5 g pepsin 3 g mucin 1 g BSA	1.5 g lipase 9 g pancreatin 1 g BSA	30 g bile 1.8 g BSA

After mixing all ingredients (organic and inorganic components and enzymes), the volume was increased to 1 L with distilled water. If necessary, the pH of the juices was adjusted to the appropriate value.

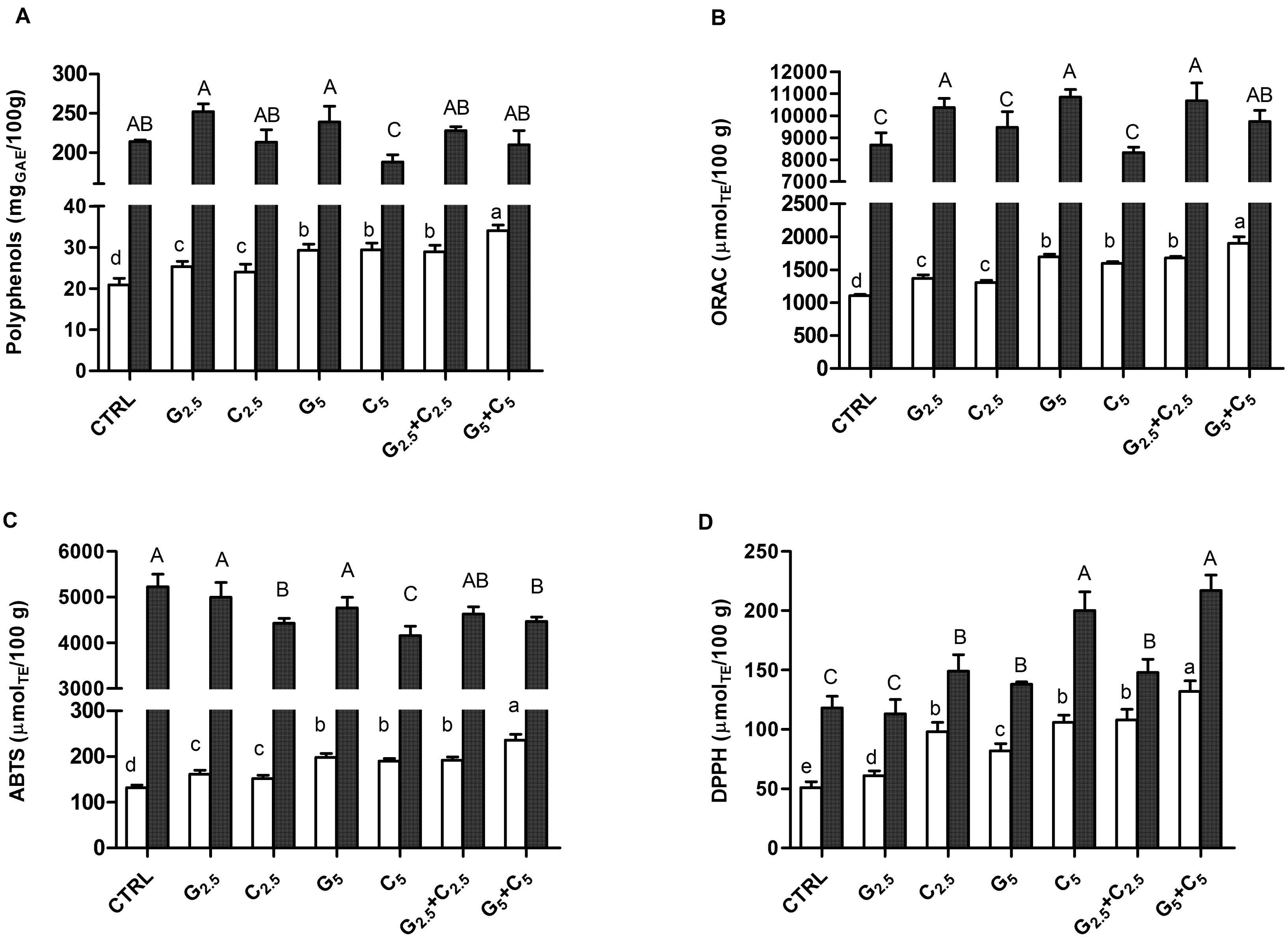
Figure captions

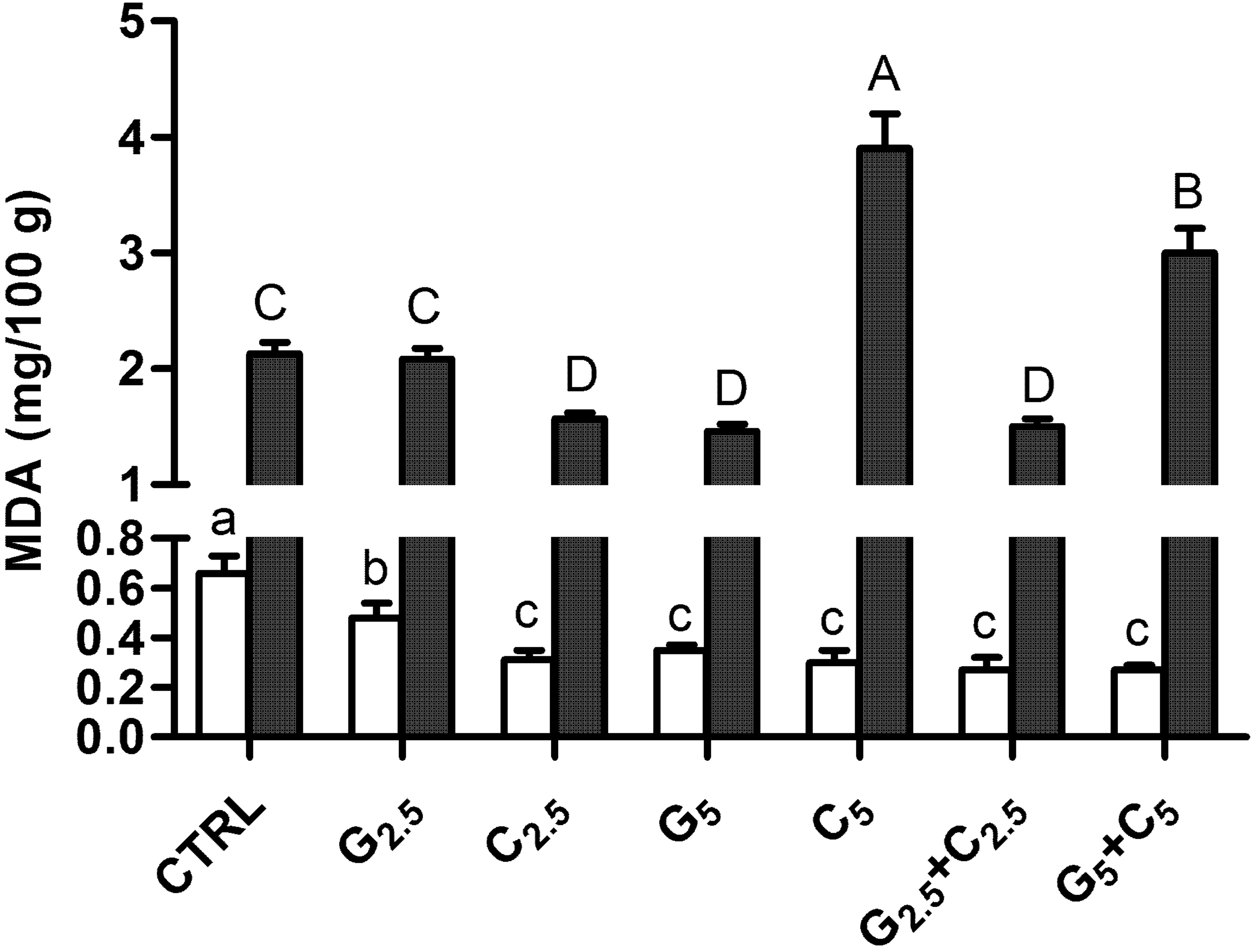
Figure 1. Polyphenols (A), ORAC (B), ABTS (C), DPPH (D) of cooked beef burgers before (blank bars) and after (grey bars) *in vitro* digestion process.

For sample formulations see Table 1. Values are reported as mean ± standard error. Different letters indicate statistically significant differences among non-digested (a, b) or digested (A, B) samples ($P < 0.05$).

Figure 2. Lipid peroxidation of cooked beef burgers before (blank bars) and after (grey bars) *in vitro* digestion process.

For sample formulations see Table 1. Values are reported as mean ± standard error. Different letters indicate statistically significant differences among non-digested (a, b) or digested (A, B) samples ($P < 0.05$).


A
Polyphenols (mg$_{GAE}$/100g)
300
250
200
40
30
20
10
0
AB
A
AB
A
C
AB
AB
d
c
c
b
b
b
a
CTRL
$G_{2.5}$
$C_{2.5}$
G_5
C_5
$G_{2.5}$+$C_{2.5}$
G_5+C_5
B
ORAC (μmol$_{TE}$/100 g)
12000
11000
10000
9000
8000
7000
2500
2000
1500
1000
500
0
C
A
C
A
C
A
AB
d
c
c
b
b
b
a
CTRL
$G_{2.5}$
$C_{2.5}$
G_5
C_5
$G_{2.5}$+$C_{2.5}$
G_5+C_5
C
ABTS (μmol$_{TE}$/100 g)
6000
5000
4000
3000
300
200
100
0
A
A
B
A
C
AB
B
d
c
c
b
b
b
a
CTRL
$G_{2.5}$
$C_{2.5}$
G_5
C_5
$G_{2.5}$+$C_{2.5}$
G_5+C_5
D
DPPH (μmol$_{TE}$/100 g)
250
200
150
100
50
0
e
C
d
C
b
B
c
B
b
A
b
B
a
A
CTRL
$G_{2.5}$
$C_{2.5}$
G_5
C_5
$G_{2.5}$+$C_{2.5}$
G_5+C_5



MDA (mg/100 g)
5
4
3
2
1
0.8
0.6
0.4
0.2
0.0
C
C
D
D
A
D
B
a
b
c
c
c
c
c
CTRL
$G_{2.5}$
$C_{2.5}$
G_5
C_5
$G_{2.5}+C_{2.5}$
G_5+C_5