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Total antioxidant capacity of meat and meat products consumed in a reference 'Spanish standard diet'

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Summary The antioxidant capacity (AC) of meat, meat products and the comparison with fish, vegetable products, milk and a balanced and healthy diet was determined using the ORACFL assay. The hydrophilic ORACFL (H-ORACFL) of hake and sardine was 596 ± 133 and 641 ± 128 μmol Trolox Equivalents (TE) per 100 g, respectively. The highest H-ORACFL value was found in cured meat samples, where Iberian cured ham (4890 ± 443 μmol TE per 100 g), whereas the lowest level of 797 ± 68 μmol TE per 100 g was found in Frankfurt sausages. Products like mortadela with olives, sobrasada and salami showed intermediate values ranging between 1107 ± 123 μmol TE per 100 g and 1011 ± 63 μmol TE per 100 g. Iberian cured ham presents the higher AC of all meat products studied, and this value being higher than that provided by red wine (3135 ± 312 μmol TE per 100 g). The AC of orange juice was lower than meat products, with the exception of Frankfurt sausages. Finally, the estimated total antioxidant capacity (TAC) of the reference standard diet was 29 006 μmol TE per intake whole diet per day, and meat representing 10.51% per intake per day of the TAC of the whole diet.

Keywords Antioxidant, antioxidant capacity, meat product.

Introduction

In recent years, a greater emphasis has been placed on the link between oxidative stress, the pathogenesis of disease and the prevention of these with a human diet rich in natural antioxidants. The ability of antioxidants to scavenge free radicals in the human body and thereby decrease the amount of free radical damage to biological molecules (lipids and DNA) may be one of their protective mechanisms. Some studies have evaluated the associations between foods and chronic diseases, and a consensus about the role of nutritional factors in the aetiology of these diseases has emerged. In this sense, diets rich in fruit and vegetables are believed to decrease the risk factors for chronic diseases (Esmaillzadeh *et al*., 2006; Stea *et al*., 2008) such as coronary heart disease and cancer (Castellini *et al*., 2002a).

The Mediterranean diet is a healthy and balanced diet characterised by a high intake of vegetables, legumes, paprika, garlic, fruits and nuts, cereals and olive oil, a moderately high intake of fish, a low-to-moderate intake of dairy products (mostly in the form of cheese or yogurt), a low intake of meat and poultry and a moderate intake of ethanol, primarily in the form of wine (Willett *et al*., 1995). Ecologic evidence suggesting the beneficial health effects of the Mediterranean diet has emerged from the classic studies of Keys (Keys, 1980).

Although meat is not highly consumed in the Mediterranean diet, its presence is necessary as a source of proteins, minerals and oligoelements. In addition to this, meats possess natural hydrophilic and lipophilic antioxidants, and limited research has been conducted to investigate the antioxidant activity of meat and several meat products (studies in beef: Descalzo *et al*., 2007; Wu *et al*., 2008; chicken: Sacchetti *et al*., 2008; pork and fish: Serpen *et al*., 2012). The balance between endogenous or exogenous antioxidant and prooxidant substances determines the oxidative stability of the meat. Furthermore, the composition of endogenous antioxidants can vary among meat of different species and among animals of a single species (Descalzo & Sancho, 2008). It also depends on the diet of the animal and the thermal treatments during cooking (Hernandez *et al*., 2004; Tornberg, 2005).

The hydrophilic antioxidants of meat may be endogenous (peptides, uric acid, polyamines, ascorbate; antioxidant enzymes like superoxide dismutase, catalase

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and glutathione peroxidase; and minerals such as selenium and zinc) or exogenous (nitrites, phenols and ascorbate). Between the most important endogenous antioxidants, the dipeptides, such as carnosine (b-alanyl-L-histidine) and anserine (Nb-alanyl-1-methyl-L-histidine), were reported as effective hydrophilic antioxidants, and carnosine has been shown be the principal antioxidant in meat (Antonini *et al.*, 2002). The antioxidant action of carnosine is due both to its radical-scavenging activity and to its metal-chelating properties (Chan & Decker, 1994). On the other hand, the lipophilic antioxidants of meat are α-tocopherol, carotenoids and ubiquinone, the latter being an endogenous antioxidant and the first being endogenous and exogenous (Decker & Xu, 1998).

Nevertheless, the contribution of lipophilic antioxidants to total antioxidant activity is much lower than is the contribution of hydrophilic compounds (Sacchetti *et al.*, 2008). In addition, the activity of natural endogenous antioxidants presents in meat, and new antioxidants compounds generated during processing can be evaluated by a hydrophilic ORACFL assay.

Given the growing scientific evidence associating an antioxidant-rich diet with a lower development of disease, an analysis of the contribution of meat and meat products to the antioxidant capacity of the diet and a comparison of the antioxidant activity of meat with the antioxidant activity of vegetable products is necessary. For this study, a hydrophilic ORACFL assay (H-ORACFL) was used, as it is one of the most important and referenced methods in the scientific literature to quantify the antioxidant capacity of plasma and other biological and food samples (Prior *et al.*, 2003).

The objectives of this research were as follows: (i) evaluate the AC of meat and meat products from different animal species, (ii) evaluate the effect of heat treatment and cooking on the AC of meat, (iii) compare the AC provided by different vegetable products, fish and animal products and (iv) estimate the TAC of a healthy diet and balanced diet (based on the Mediterranean diet).

Materials and methods

Chemicals and apparatus

Absolute p.a ethanol was purchased from Panreac (Barcelona, Spain). 2,2′-Azobis (2-amidino-propane) dihydrochloride (AAPH) was purchased from Acros (Fair Lawn, NJ, USA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Fluorescein (Na salt) (FL) were obtained from Sigma (St. Louis, MO, USA).

Diet

The calculation and development of a balanced and healthy diet (Mediterranean diet) used the DietSource® 122 3.0 program for assessment and nutritional support of an inpatient and an outpatient (Nestlé Healthcare Nutrition S.A., Vevey, Canton of Vaud, Switzerland). The diet, that is, a reference Spanish and standard diet, was developed based on the nutritional recommendations of macro- and micronutrients established by FAO/WHO (Salas-Salvadó *et al.*, 2008) and according to the energy requirements of a 70 kg male (30–59 years old) established by FAO/WHO/UNA (2001) (2350 Kcal day^{-1}). Data on dietary intake of a reference Spanish standard diet (consistent with the Spanish National Nutrition Survey) were obtained through 24-h food recalls. The reference Spanish standard diet (based on the average intake of the completed records) was divided into six different intakes per day and contained foods typically consumed by the Spanish consumers: cereals, bread, tubercules and legumes as a source of carbohydrates; five pieces of fruit; vegetables; milk; olive oil as source of fat; fish, chicken and pork meat as a source of proteins; and nonalcoholic and alcoholic beverages (orange juice and red wine). The whole diet provided 2335 Kcal and showed the following macronutrient profile: proteins (15%), lipids (30%) and carbohydrates (CH) (55%), with a total dietary fibre of 38.4 g (Table S2). Related to CH, the concentration of simple sugars was lower than 10%. On the other hand, the content of saturated fatty acids was not above 10%. Polyunsaturated fatty acids ranged from 3% to 7%. The concentrations of cholesterol and salt were lower than 300 mg and 6 g day^{-1}, respectively.

Food sampling

The food samples were obtained from a local supermarket and included four categories: raw meat and fish (chicken breast, turkey breast, pork ham, chicken meat, pork meat, turkey meat, hake and sardine), meat products (Iberian cured ham, cured ham, chorizo, spiced sausage, imperial, salami, sobrasada, mortadella of pork with olives, turkey mortadella and Frankfurt sausages), vegetable products (commercial orange juices, breeding red wine (2007), purple garlic and smoked paprika) and packed UHT semi-skimmed milk (cow) (Tetra brix). All the samples were kept in refrigeration at a controlled temperature of 4 °C until their analysis.

Thermal treatments of meat and fish samples

The pork ham was boiled, the baked ham and roast chicken were cooked in an oven (until reach internal

temperature of 71.5 °C), and pork hamburgers were cooked on the grill. The boiling process of the pork ham was carried out as follows: 0.5 kg of pork ham was finely minced with a meat grinder (Mincer, dpa 151; Moulinex, Caen, France). About 418 g of minced meat was put into plastic bags and was mixed with 82 g of brine (brine composition: 96.75% of water, 1.25% of lactic acid and 2% of salt). After mixing, the plastics bags were refrigerated for 12 h to stabilise the mixture. Next, the mixture was stuffed into 50-mL falcon tubes (Deltalab S.L, Barcelona, Spain). Once these tubes were half closed, the air was removed by a centrifugal process: 4 min, 3500 ***g*** and 4 °C (centrífuge Kubota 2010; Kubota Corporation, Tokyo, Japan). Following this, the falcon tubes were closed correctly. The tubes containing the meat sample were put into an industrial cooking pot and were cooked for 35 min at 72 °C, which is time enough to reach a temperature of 72 °C in the interior of the meat piece. Once cooked, the sample was immediately chilled in a bath of ice. The hamburgers were prepared as follows: meat and back fat were obtained from pork. Twenty grams salt per kilogram was added. The meat was minced (5 mm) using a P3298 cutter (Braher International, San Sebastian, Spain) and mixed for 5 min using a RM-60 mixer (Mainca, Granollers, Spain). The meat temperature during processing did not exceed 12 °C. Patties were formed using a burger machine (100 g per patty) (Juan Martinez Perez Ltd., Murcia, Spain), to give average dimensions of 10 cm diameter and 1.5 cm thickness. The chemical composition (fat and moisture content, %) of the ground fat and lean sources were analysed using a HFT-2000 fat analyser (Data Support Co. Inc., Encino, CA, USA; accuracy, ±0.5%). The fat concentration was used to calculate mixture proportions of the fat and lean sources to obtain meat samples with the target fat concentration (15%). The hamburgers were treated as follows: the burgers were grilled in a preheated (180–200 °C) frying pan until the interior of the meat reached a temperature of 72 °C. To measure the temperature of the sample, a contact thermometer was used (TTX 110; PCE Ibérica S.L., Tobarra, Spain).

The turkey sausages were prepared as follows: the day before the experiment, ground lean and fat samples were stored at 4 °C overnight. The preweighed amounts of lean and fat (15%), salt, crushed ice and water were mixed. Treatment formulations were adapted from the study of Feng *et al.* (2003). Raw material mixtures were chopped using a 9-kg capacity bowl chopper (CM-14; Mainca, St. Louis, MO, USA). Knife and bowl speeds of 3000 and 10 rpm, respectively, were used. The mixture was chopped to an emulsion temperature of 15 °C (~9 min). The raw emulsion was immediately stuffed into 27 mm diameter frankfurter cellulose casings using a hand stuffer. Frankfurters were then manually tied into 12 cm sausages, weighed and cooked for 90 min in an Alkar smokehouse (450 U; Alkar- RapidPak Inc., Lodi, WI, USA) to an internal temperature of 71 °C.

Regarding the fish, two of the most consumed spices were chosen to make the comparison: hake and sardine. The fish was boiled as follows: clean and boneless fillets of hake and sardines were vacuum packed. Following this, the bags with fish were put into an industrial cooking pot and cooked 35 min at 72 °C. After cooking, the fish samples were quickly cooled in an ice bath. The cooked meat and fish samples were kept in refrigeration at a controlled temperature of 4 °C until the analysis.

Sample extraction

The sample extraction was modified based on the methodology described by Wu *et al.* (2008). Two grams of finely minced solid samples, including raw or cooked meat and fish samples, meat products, garlic and smoked paprika, were weighed in falcon tubes of 50 mL, 18.5 mL of 25% ethanol (v/v) added and were flushed with N_2 to prevent oxidation. The tubes were placed upside-down on a magnetic shaker (TENZO M-500, Barcelona, Spain), for 1 h at 680 rpm at room temperature and were centrifuged for 4 min at 3500 ***g*** and 4 °C. The precipitate obtained was eliminated, and the resulting solution was filtered with hydrophilic 0.45-μm nylon filters (Whatman, Clifton, NJ, USA). The filtered samples were used for H-ORACFL. For the liquid vegetable products (commercial orange juices and wine), 5 mL samples was put into 15-mL falcon tubes. These tubes were subsequently centrifuged for 20 min at 3500 ***g*** and 4 °C. The solution above was used for the H-ORACFL assay. Milk was centrifuged for 20 min at 11 rpm and 4 °C. The upper layer obtained was filtered with hydrophilic nylon filters of 0.2 μm (Whatman). The filtered milk was used for H-ORACFL.

Hydrophilic ORACFL assay

The H-ORACFL assay was modified based on the methodology described by Held (2005).

Filtered samples and centrifuged liquid vegetable products were diluted 50, 100, 200, 500 or 1000 times with phosphate buffer (pH 7.4) for H-ORACFL analysis. These mentioned dilutions were prepared according to the antioxidant activity of each analysed sample. An aliquot (20 μL) of the diluted sample or blank (phosphate buffer) or Trolox calibration solutions (6.25, 12.5, 25, 50 μM) was added to a well in 96-well bottom reading microplate (Costar, New York, USA). 200 μL of fluorescein work solution at 0.095 μM was added to each well of plate, and then,

the microplate was incubated at 37 °C for 15 min before an aliquot of 20 μL AAPH solutions (79.6 mM) was added to each well as peroxyl radicals generator to start reaction. The microplate reader (Multi-Detection Microplate Reader (Synergy™ HT); Biotek Instruments, Winooski, VT, USA) was programmed to read the fluorescence with an excitation wavelength of 485 nm and an emission wavelength of 528 nm at 1-min interval for 1:30 h using software GEN 5™ (Held, 2005).

The final H-ORACFL values were calculated by using a linear regression model ($Y = aX + b$) between Trolox concentration (μM) and the net area under the fluorescein decay curve. Data are expressed as micromoles of Trolox Equivalents (μmol TE) per 100 g of fresh weight of sample. The area under curve (AUC) was calculated using the next equation:

$$\mathrm{AUC} = 1 + (R_2/R_1) + (R_3/R_1) + \ldots(R_{91}/R_1)$$

where R_1 = the initial fluorescence reading, R_{91} = the last fluorescence reading at the 91st min. The net AUC was obtained by subtracting the AUC of the blank from that of a sample.

Statistical analysis

Each sample extraction was repeated twice, and ORAC analysis was conducted in duplicate. The analytical data were given as means ± SD. A probability of $P < 0.05$ was adopted as the criterion for establishing significant differences. The effect of heat treatment on the AC of meat from different animal species was evaluated by one parametrical independent samples Student's *t*-tests for assuming a significance level of 95% ($P < 0.05$). Two nonparametrical independent samples (H of Kruskal–Wallis and U of Mann–Whitney) tests were used to compare the AC of meat products, vegetable products and raw and cooked fish, assuming a significance level of 95% ($P < 0.05$). The statistic programme used was Statistical Package for the Social Sciences (SPSS) version 15.0 for windows (SPSS Inc, Chicago, IL, USA).

Results and discussion

Effects of heat treatments on AC of meat and meat products

The AC of raw and cooked meat samples determined by the H-ORACFL is presented in Fig. S1. The AC of raw and cooked meat samples was in the range of 1873 ± 216 μmol TE per 100 g to 887 ± 119 μmol TE per 100 g provided by the chicken breast and baked ham of pork, respectively. According to the comparison of ORAC values of the raw meat based on samples by animal species, the AC of chicken was significantly higher than that of pork and turkey ($P < 0.05$). On the other hand, turkey (raw breast and turkey sausages) and pork (raw ham, boiled ham) samples did not show statistically significant differences between them ($P > 0.05$). Figure S1 shows the influence of thermal treatment on the AC of meat samples measured using the H-ORACFL. While the AC of baked ham, grilled hamburger and roast chicken significantly decreased ($P < 0.05$) with the thermal treatment, the AC of boiled ham and turkey sausages was not affected by cooking. In the same way, Serpen *et al.* (2012) showed that upon increasing the heating time of meat samples, TAC levels decreased. These results are due to the heating of proteins and their resulting behaviour. Heating can alter the structure of proteins, resulting in modifications of their physical properties and modifying the ability of antioxidant amino acids to scavenge free radicals (Elias *et al.*, 2007).

In addition, the blood heteroprotein haemoglobin (Hb) is present in vertebrate and some invertebrate animals . Elias *et al.* (2008) studied the effect of heating in the antioxidant activity of Hb by using the ORAC assay and showed that native oxyhaemoglobin (OxyHb) when heated at 45 °C had a higher AC than OxyHb heated at 68 °C, which in turn was higher than that heated at 90 °C. Similarly, methaemoglobin (MetHb) heated at 90 °C presented lower AC than native and the other MetHb treatments (45 and 68 °C). The reduction of the AC of the Hb by the effect of heating may be due to a lower exposure of amino acids with radical-scavenging capacity. This decrease in the AC of the Hb by thermal treatment can be another reason for the lower AC shown by meat thermally treated.

AC of meat and meat products

The AC values of meat products are presented in Fig. S2. In general, the highest H-ORACFL value was found in cured meat samples, where Iberian cured ham showed a value of 4890 ± 443 μmol TE per 100 g, whereas the lowest level of 797 ± 68 μmol TE per 100 g was found in Frankfurt sausages. Products like mortadela with olives, sobrasada and salami showed intermediate values ranging between 1107 ± 123 μmol TE per 100 g and 1011 ± 63 μmol TE per 100 g. The antioxidant capacity of meat can be due to different antioxidant compounds, such as some proteins and peptides (Elias *et al.*, 2007, 2008). Specifically, amino acids and dipeptides, mainly carnosine and anserine, and other substances such as L-carnitine, glutathione, taurine and creatine present in meat may act as antioxidants (Antonini *et al.*, 2002; Wu *et al.*, 2005), mainly due to the fact that carnosine and anserine contribute to the inactivation of free radicals (Kohen *et al.*, 1988; Decker *et al.*, 1992) and

lipid oxidation catalysts (Saiga *et al*., 2003) and may also counteract the glycation of proteins. On the other hand, these peptides have shown to be very resistant to heat treatment and proteolysis by muscular proteolytic enzymes (Toldrá, 2010). Other amino acids, such as histamine, tyramine, methionine and cysteine, also possess antioxidant activity (Marcuse, 1960, 1962; Yamashoji *et al*., 1979). Depending on the amino acid sequence, the peptides can have various activities, including mineral binding, as well as immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic and antihypertensive actions (Kitts & Weiler, 2003; Kovacs-Nolan *et al*., 2005; Hartmann & Meisel, 2007). The differences found between different samples of meat has been shown previously; indeed, the presence of antioxidants may differ between the meat of different animal species (Descalzo & Sancho, 2008). Moreover, the anatomical location of the meat sample can also influence antioxidant activity. Sacchetti *et al*. (2008) showed that chicken breast had higher AC than chicken thigh because thighs contain more fat, and fat-soluble meat antioxidants have a much lower AC than the water soluble constituents. In addition, levels of antioxidants and the oxidative status in animal meat could be altered by diet, genotype, and diet during breeding (Morrissey *et al*., 1998; Castellini *et al*., 2002a, 2002b). The antioxidant capacity shown in a cured meat product is due to the fact that during processing, such as curing, fermentation, ageing and enzymatic hydrolysis, functional compounds, especially peptides, can be generated from meat and meat products (Erdmann *et al*., 2008; Zhang & Zhou, 2010). During these processes, many biochemical changes, such as proteolysis, lipolysis and oxidation, can occur in meat products, especially in dry-cured meat products, and in the degradation of ribonucleotides, which play a key role in the typical aromatic volatile compounds' development. Generally, proteolysis includes three main steps during curing: the degradation of major myofibrillar proteins, the generation of polypeptides as substrates for peptidases to produce small peptides, and the production of free amino acids (Toldrá, 2006). The large release of peptides and amino acids in meat during technological processing may explain the sharp increase in antioxidant capacity experienced by some meat products. The AC levels shown in chorizo and sobrasada are also due to the fact that these meat products are elaborated with different spices; among the spices used to elaborate chorizo, should be noted paprika (30 g paprika per kg meat in the chorizo elaboration), which has high AC levels (19 213 $\pm$ 1171). The elevated AC provided by smoked paprika could be another reason for the high H-ORACFL shown in these meat products. Sobrasada showed a lower H-ORACFL than chorizo. Taking into account that cure increases the AC of meat products, and sobrasada's shorter curing period could be the reason for the lower AC; moreover, sobrasada presents higher fat and lower protein contents. Mortadela with olives, turkey mortadela and salami are also prepared with different spices and vegetables; among these ingredients, the presence of garlic is responsible for the AC of the meat products, showing a value of 6780 μmol TE per 100 g. The combination of the AC of some proteins and peptides together with the AC provided by these vegetable products could explain the higher AC shown by some of these meat products.

AC of fish: effects of heat treatment

Figure S3 presents the influence of thermal treatment on the AC of sardine and hake using the hydrophilic ORACFL. The H-ORACFL ranged between 641 $\pm$ 128 μmol TE per 100 g and 367 $\pm$ 66 μmol TE per 100 g provided by raw sardine and cooked hake, respectively. No differences were found between fish species. Concretely, the differences in H-ORACFL data between the raw sardine and hake were not statistically significant ($P > 0.05$). Similar to the results shown in the meat samples, thermal treatment moderately reduced the AC in fish ($P < 0.001$); this diminution was around 40%. Therefore, the heating process reduced the AC of meat and fish in the oxygen radical-scavenging assay.

It is known that some thermal treatments can cause thermoxidation of different components of muscle foods producing a consumption of antioxidant substances and a decrease of AC levels. This can be related to the accumulation of oxidised proteins and the loss in functionality of active meat and fish peptides. In addition to this, the degradation of endogenous antioxidants such as vitamin E, vitamin C, carotenoids, ubiquinols, polyphenols and cellular thiols can be caused by heating. Fish is a source of structurally bioactive compounds. Scientific studies have shown that some peptides isolated from different fish species possess antioxidant and antimicrobial properties (Najafian & Babji, 2012). Erdmann *et al*. (2006) demonstrated in an in vitro study how a bioactive dipeptide Methionine-tyrosine (Met-Tyr), derived from sardine muscle, encourages expression of the antioxidant defence proteins hemeoxygenase (HO-1) and ferritin in endothelial cells. Arbeloa *et al*. (2010) assessed the AC of a natural antioxidant gadusol (3,5,6-trihydroxy-5-hydroxymethyl-2-methoxycyclohex- 2-en-1-one) in some species from the Argentina Sea: argentine hake (*Merluccius hubbsi*), Brazilians and perch (*Pinguipes brasilianus*) and argentinians and perch (*Pseudopercis semifasciata*). The antioxidant capacity of this metabolite was measured by ORACFL and ABTS

assays. The results showed that gadusol is a significant breaker of the chain reactions carried by peroxyl radicals. Moreover, its ability to reduce radicals was comparable with that of ascorbic acid. Gadusol is biosynthesised through bacteria, algae, plants and fungi. Fish probably accumulate this substance by diet acquisition or by symbiotic or bacterial association (Shick & Dunlap, 2002). In the comparison of the results obtained from meat and fish samples, the value of AC in fish was lower than in meat and meat products. Apart from a lower content of blood in the fish sample, the loss of endogenous antioxidant compounds that may inhibit unsaturated lipid oxidation could be one of the reasons for the low radical scavenging antioxidant capacity of fish compared with turkey, pork and chicken.

AC of different vegetable products and milk typically consumed in a reference Spanish standard diet

Table S1 shows the H-ORACFL of different vegetable products and milk. H-ORACFL ranged from 764 ± 34 μmol TE per 100 g (orange juice) to 19 213 ± 1171 μmol TE per 100 g (smoked paprika).

Processed fruit products, such as fruit beverages, constitute the primary source of naturally occurring antioxidants in the human diet (Cilla *et al*., 2011). Orange juice is a rich source of vitamin C and carotenoids (Rodríguez-Amaya, 1997). Carotenoids have diverse biological functions, such as antioxidant activity, provitamin A activity and macula protection (Van den Berg *et al*., 2000). In addition, orange juice is a major source of antioxidant flavanones (mainly hesperidin) in the diet of developed countries (Gil-Izquierdo *et al*., 2001). The AC shown by orange juice (Table S1) was very similar to that provided by the USDA (2010) (726 μmol TE per 100 g). If we compare the AC of the orange juice with the AC of raw and cooked meat (Fig. S1) and meat products (Fig. S2), it can be observed that orange juice has the lowest AC of all the mentioned products with the exception of Frankfurt sausages, which exhibit a similar AC to that provided by orange juice.

Abundant studies have shown the antioxidant properties of some spices (Dang *et al*., 2001; Lee & Shibamoto, 2002; Shobana & Naidu, 2002).

In the case of smoked paprika, the AC presented by plant products such as spices (smoked paprika), is attributed to the large presence of essential oils with antioxidant properties. Moreover, the main hydrophilic antioxidants of spices are phenolics and cynamonic acids derivates, these substances being also responsible for the high H-ORACFL of smoked paprika.

Smoked paprika, purple garlic and red wine showed statistically significant differences between them ($P < 0.001$). As well as smoked paprika, purple garlic and red wine presented good antioxidant capacity, with AC values of 6780 ± 671 μmol TE per 100 g and 3135 ± 312 μmol TE per 100 g, respectively.

Biological activities, including antioxidant properties, are attributed to fresh and processed garlic. *In vitro* assays have shown that derivates from 1, 2, 3, 4-tetrahidro-β- carbolina (THβCS) present in aged garlic extract (AGE) and possess a high propensity to scavenge hydrogen peroxides. These mentioned derivates, organosulphur and alkaloids, are responsible for the high AC presented by purple garlic (Ide *et al*., 2003).

On the other hand, wine is a frequently consumed food in the Mediterranean diet, and it is widely recognised in the scientific community as one the most important sources of poliphenolic antioxidants, including a wide variety of flavonoids and no flavonoid compounds. Seeram *et al*. (2008) observed that red wine possesses an AC of 2570 μmol TE per 100 g. This value is very similar to that provided in the present study. Comparing the data of AC present in meat products (Fig. S2) to the data on AC in red wine (Table S1), it can be observed that the AC of Iberian cured ham was considerably higher than that of red wine, showing statistically significant differences ($P < 0.001$).

In addition to these vegetable products, another analysed product from animal origin was semi-skimmed milk, providing an H-ORACFL of 818 ± 65 μmol TE per 100 g, this data being very similar to that provided by orange juice ($P > 0.05$).

The AC of semi-skimmed milk can be attributed to the presence of protein-rich cysteine in milk serum; this form of protein promotes the synthesis of glutathione (intracellular antioxidant). The components of milk, like milk serum and casein, have demonstrated activity-inhibiting lipid peroxidation and generation of peroxyl/superoxide radicals (Korpela *et al*., 1995).

Total Antioxidant Capacity of a reference Spanish standard diet

The estimation of the total antioxidant capacity (TAC) of a reference Spanish standard diet is indicated in Table S2. TAC of the diet was calculated combining the consumption of each food (g) with the H-ORACFL and L-ORACFL values of selected foods published by the USDA (2010) and along with H-ORACFL data obtained in the present study.

The AC of a wide variety of vegetables and fruits has been extensively reported; however, only a very limited number of studies show an estimation of the TAC of food groups or complete diets, to compare with our results. The TAC of the diet designed in this study was 29 006 μmol TE per whole diet per day (Table S2). The contribution of each specific food to the TAC is related to both food intake and food AC.

The largest contributors to the TAC were fruits and fruit beverages (red wine and orange juice), with 18 496 μmol TE per intake day (about 64% per intake day of the TAC). On the contrary, although olive oil is frequently consumed food in the Mediterranean diet, its contribution was only 0.58% of the TAC. These findings highlight that meat and fish intake provided an AC of 3049 μmol TE per intake day, a significant value, corresponding to 10.51% per intake day of the TAC. On the other hand, Pulido *et al.* (2003) estimated the AC intake from beverages (coffee, wine, tea, bear, orange juice, milk and cola) in the Spanish diet by FRAP and ABTS methods, which was 3894 and 2575 μmol TE per intake day. Wu *et al.* (2004) calculated the total hydrophilic and lipophilic antioxidant capacity intakes of a diet rich in fruit and vegetables by an ORACFL assay; this TAC intake provided 5724 μmol TE per day. Among all fruits and vegetables of this diet, we can note that fruits such as apple, orange and grape and vegetables such as beans, peas, tomato and lettuce are the major contributors to this TAC. The previous estimated TAC was much lower than that provided by us, but we have to consider that this diet does not represent the real daily intake of foods, because fruit and vegetables are consumed in the diet with other kinds of foods like cereals, legumes, fats, meat, fish and sugars. Brighenti *et al.* (2005) established a correlation between the TAC of the diet (a controlled diet received by workers of an Italian factory) and markers of systemic inflammation; the two diets evaluated in this study provided a TAC of 5300 and 4900 μmol TE per intake day, respectively, using the ABTS assay. The estimation of this TAC was based on the use of foods consumed in the diet and data of the AC of plant foods, beverages and oils consumed in Italy and was published by Pellegrini *et al.* (2003). Saura-Calixto & Goñi (2006) estimated the TAC in the Spanish diet, which provided 6014 and 3549 μmol TE per intake day by FRAP and ABTS assays, respectively. This diet underestimates the TAC, because it does not contain the foods from animal origin (meat and fish) present in the Mediterranean diet, and a natural source of proteins. In addition to this, Martínez-Álvarez & Izquierdo-Pulido (2006) also estimated the TAC of a meal, which was 19 401 μmol TE; this meal was based solely on fruit and vegetables, and did not contain either meat or fish. For this reason, it is not very representative of the real intake of foods and does not represent a balanced diet. More recent research has shown that the contribution of fruits and vegetables to the AC of a Mexican rural diet is around 1000–2000 μmol TE per day, evaluated by FRAP and ABTS assays. This low AC is attributed to the poor intake of fruits and vegetables (400 g day^{-1}) and is also related to the absence of all food groups (Hervert-Hernández *et al.*, 2011).

The different TAC found between authors can be attributed to several factors: food type, portions, and quantity of each food ingested, as well as the differences between assays employed in the quantification of the TAC. Between the different methods used, FRAP and ABTS assays are colorimetric assays, and ORAC is a fluorimetric assay. The measured antioxidant capacity of a sample depends on which technology and which free radical generator or oxidant is used in the measurement. Consequently, the comparison of different analytical methods for determining TAC can generate different results and controversy. In addition, the AC of each food depends on many factors, including the colloidal properties of the substrates, the conditions and stages of oxidation, and the localisation of antioxidants in different phases (Frankel & Meyer, 2000).

Another factor that could explain the differences found between the TAC of countries like Spain, México or Italy could be the different consumption patterns and food habits. The TAC estimated by us provides a global measurement of the AC of all food groups consumed daily in the Mediterranean diet, and not merely two food groups (such as fruits and vegetables) and the subsequent data, as was the case with the abovementioned studies.

Conclusions

Using a modified H-ORACFL extraction procedure, the results of this study show that meat has a high antioxidant capacity, little known to date. This AC is superior to that from other foods of animal origin such as sardine and hake, and it is also superior to the AC provided by food rich in antioxidants, like orange juice. The AC of meat and fish were negatively affected by thermal treatment, showing a decrease by the heating process in the oxygen radical-scavenging assay, an effect that was more pronounced in fish with AC losses of around 40%. Meat products like Iberian cured ham have higher AC than that provided by raw meat and red wine. The balanced and healthy diet proposed in the present study provided a TAC of 29 006 μmol TE per intake per day. The amount of meat ingested in this diet provided 3049 μmol TE per intake day, this value being 10.51% per intake day of the TAC of the whole diet. These findings highlight that the consumption of meat may significantly contribute to the total antioxidant capacity of a reference Spanish standard diet.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Influence of heat treatment on the antioxidant capacity of meat. Bars with different letters (a, b) or (*) are significantly different ($*P < 0.05$, $***P < 0.001$).

Figure S2. Antioxidant capacity of meat products (μmol Trolox Equivalents per 100 g of fresh weight. Bars with different letters are significantly different.

Figure S3. Effects of cooking on the hydrophilic H-ORACFL of sardine and hake fish (μmol Trolox Equivalents per 100 g of fresh weight).Bars with different letters are significantly different.

Table S1. Total antioxidant capacity vegetable products and milk.

Table S2. Total antioxidant capacity of a balanced diet (hydrophilic: H-ORAC; and lipophilic: L-ORAC).