Argan pulp as a novel functional ingredient with beneficial effects on multiple metabolism biomarkers

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ABSTRACT

Argan tree is mainly used to obtain the oil from its seeds in a process that generates by-products such as the pulp of the fruit. The aim of this study was to assess the *in vitro* antioxidant activity and profile of bioactive compounds from an argan fruit extract, and to assay the metabolic effects of the argan pulp intake in a mouse experimental model. Our results showed a high *in vitro* antioxidant activity in ethanolic extracts of argan juice and pulp measured as total polyphenol content, reducing capacity, ABTS, DPPH, and lipid peroxidation inhibition capacity. In addition, several bioactive compounds with antioxidant, hypoglycemic, and anti-inflammatory activity were identified (vaccihein A, bergapten, methyl chloragenate, 2-O-caffeoyl glucaric acid, avicularin, quercetin-3-O-sophoroside, prodelphinidin B4 and mirificin). Dietary intake of argan pulp induced a remarkable hypoglycemic activity and decreased the gene expression of inflammatory markers. Gene expression and enzyme activity of liver antioxidant and detoxifying enzymes were differentially affected by argan pulp intake, whereas gut microbiota was modulated decreasing the Firmicutes/Bacteroidetes ratio. In conclusion, argan pulp can be considered a functional food or ingredient with beneficial effects on several metabolism biomarkers.

1. Introduction

In recent decades, the field of nutrition and food technology has directed many efforts towards the search for novel human foods. Specifically, the search for new functional foods, bioactive compounds and new nutraceutical formulations have been the focus of scientific community. In this regard, a wide range of traditional foods are being analyzed not only because of their nutritional value but also their functional potential. One of the most up-to-date examples is the argan tree (*Argania spinosa* (L.) Skeels). Argan tree is a plant found mainly in southwestern Morocco, where its forests were declared a biosphere reserve by UNESCO in 1998 (Gharby & Charrouf, 2022). Due to its relevance, its cultivation is being introduced in other countries such as Spain (Labarca-Rojas et al., 2022). This tree is highly appreciated in its native regions for its usefulness as source of fodder in livestock feed, but, specifically, it is widely known for the oil extracted from its seeds that is used for nutritional and, specially, cosmetic applications. The roasted argan kernels are used for oil extraction with food uses, whereas the unroasted argan kernels are extracted and used as ingredient in cosmetic preparations. Argan oil contains nearly 80% of unsaturated fatty acids, with oleic (45 g/100 g) and linoleic acid (33 g/100 g) among those with higher concentration (Zarrouk et al., 2019). Food uses of argan oil comprise products like salad dressing or sauces to complement toast. In cosmetics, argan oil is used for both hair and skin applications due to its moisturizing properties. In addition, the therapeutic properties of argan oil have shown beneficial effects on metabolic syndrome alterations (El Midaoui et al., 2017), protective effects against oxidative stress caused by bacterial lipopolysaccharides (Essadek et al., 2022), protection against nephrotoxicity (Orabi et al., 2020), in the treatment of inflammations or pain (Kamal et al., 2019), as well as its biological activity on age-related diseases characterized by increased oxidative stress conditions such as neurodegeneration (Badreddine et al., 2017).

The exploitation of this crop, and, specifically, the extraction of oil from its seeds, generates different by-products. These agro-industry by-products have been undervalued for many years. Nowadays, they are also being used in view of their potential applications within the field of biotechnology, nutrition, and biomedicine. The by-product named "seed oil cake", which is the residual mass from the seed oil extraction process, is being studied for use in a variety of industrial applications such as film matrix for pharmaco-cosmetic (Mirpoor et al., 2022), anticrystallization capacity against urolithiasis (El oumari et al., 2022), animal feedstuff (Hilali et al., 2022) and biodiesel production (Zeghlouli et al., 2022).

Other by-products include the pulp or pericarp of the seed that constitutes up to 40 percent of the entire fruit and stands as a good energy source due to its high carbohydrate content and valuable nutrient composition. For this reason, it has been included into animal and human diets (Musial et al., 2020). In addition, several phenolic compounds such as gallic acid, protocatechuic acid, rhamnetin-O-rutinoside, isorhoifolin, hesperidin, hyperoside, isoquercetin, naringenin-7-O-glucoside, quercetin-3-O-arabinoside, naringenin, quercetin, luteolin and procyanidin dimer as well as compounds known as amino phenols argaminolic A-C and arganimide A were identified in methanol/water extracts carried out on argan pulp (Charrouf et al., 2007). This variety of compounds confers this product great potential in the fields of food technology, nutrition, and biomedicine aimed to the formulation of nutraceuticals or the prevention and/or treatment of several pathologies where oxidative stress and inflammation play a major role. Among them, obesity and metabolic syndrome that share the presence of metabolic alterations (Swarup et al., 2022) are highly prevalent world-wide, representing a high cost for national health systems. Phenolic compounds have shown effectiveness against most of the disorders present in both obesity and metabolic syndrome such as insulin resistance and type II diabetes (Islam et al., 2022), dyslipidemia (Feldman et al., 2021), changes in gut microbial community composition (Li et al., 2022) or high blood pressure (Edwards et al., 2007), among others. In fact, some recent studies in experimental animal models has described positive effects of argan pulp (AP) intake on glucose metabolism and other metabolic parameters using a freeze-dried aqueous extract from the fruit pericarp prepared according to the traditional decoction method used in Morocco (Hebi et al., 2018). However, bioavailability of supplemented polyphenols is not well known, and limited research has focused on the functional properties of AP.

Therefore, the aim of this work was to assess the functional activity of argan pulp from an organic farming facility in Andalusia (Spain), using *in vitro* chemical analyses and *in vivo* mouse experimental model. Specifically, the main objectives were to test: i) the profile of bioactive compounds and antioxidant capacity from argan juice and pulp, ii) the *in vitro* availability of bioactive compound present in argan pulp included in an experimental diet, iii) the lack of toxic effects from argan pulp intake and its benefits on glucose and lipid metabolism. It is also the first time that the effect of argan pulp consumption on oxidative metabolism has been studied in an *in vivo* model, as well as its relationship with inflammatory parameters and the intestinal microbiome. For this purpose, we used a standard chow with AP inclusion levels of 5 or 10%.

2. Materials and methods

2.1. Plant material: Argan fruit

The argan fruit was obtained from an organic farming facility in Córdoba (Andalusia, Spain) and provided by the biotechnology company Cellbitec S.L. (Almería, Spain). The juice was obtained from a blending process of the argan fruit (Ariete 1827, Campi Bisenzio, Italy). The residue after the process was also collected and designated as pulp. Both juice, and pulp were freeze-dried to further analysis.

2.2. Ethanolic extracts

To carry out the extraction of bioactive compounds, ethanolic extracts were made from the freeze-dried powder of juice and pulp. Briefly, ten grams of freeze-dried powder of juice or pulp was mixed with 20 mL of hydroalcoholic extraction solution (50:50:0.25; ethanol: type I water: 12 N HCl) at pH 2, 4 °C. The samples were bubbled with nitrogen gas to avoid oxidation processes of the samples and the extraction was carried out for 30 min. Then, the extracts were centrifuged (3500 rpm, 7 min). The supernatants were stored, and the extraction was repeated once again. Both supernatants were mixed and stored at −20 °C. Three aliquots of 1 mL were evaporated using a Savant DNA120 vacuum evaporator (ThermoSci, Waltham, MA, USA) and freeze-dried for 24 h (Cryodos-50 lyophilizer, TELSTAR, Madrid, Spain) to evaluate the ethanolic extract yield.

2.3. Antioxidant activity assays

Total polyphenol content was assessed using a modified Folin–Ciocalteu colorimetric assay (Martínez et al., 2022). A 12.5 μL aliquot of ethanolic extracts or a standard solution of gallic acid (0–600 mg/L) was mixed with 50 μL of double-distilled water and 125 μL of Folin–Ciocalteu reagent. After 6 min of incubation, 1.25 mL of a 10% (w/v) Na_2CO_3/1M NaOH solution was added, and the volume was made up with water to 3 mL and incubated for 90 min. Then, the optical density of the supernatant was measured at 750 nm (Multiskan FC Microplate Photometer, Thermo Fisher Scientific, Waltham, MA, USA). The results were expressed as μg of gallic acid equivalent (GAE) per mg of sample. The 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical uptake assay was performed based on the method of Miller et al. (1993) to measure the total antioxidant capacity of a fluid. For this, 6 μL of evaporated ethanolic extract or a standard solution of gallic acid (0–60 mg/L), was mixed with 294 μL of ABTS and incubated for 3 min. The optical density of the samples was then measured at 620 nm (Multiskan FC, Microplate Photometer, Thermo Fisher Scientific). The blank was made with 6 μL of water and 294 μL of ABTS. The results were expressed as μg of gallic acid equivalent (GAE) per mg of extract. The ability of juice and argan pulp to inhibit lipid peroxidation was assessed using thiobarbituric acid reactive substances (TBARS) measured in rat brain homogenate as described by Ohkawa et al. (1979). Brain tissue was mixed (1:10 w/v) with cold 1.15% KCl/0.1% Triton X-100 with the use of a homogenizer. The mixture was centrifuged at 3000 rpm, 4 °C, for 5 min, and the supernatant was aliquoted and stored at −20 °C. Brain homogenates were treated with a mixture of $FeCl_3$/H_2O_2 to induce lipid peroxidation, and the percentage of inhibition of TBARS formation caused by the juice or AP was determined as previously described by Kapravelou et al., 2015. The capacity of juice and argan pulp to scavenge free radicals was determined by the DPPH radical scavenging assay performed according to Galisteo et al. (2006). The decrease in absorbance at 493 nm of DPPH is proportional to the activity of the antioxidant components of the samples. Briefly, to 1450 μL of a methanol solution containing 0.02 mM DPPH, 50 μL of ethanol extract or a standard solution of Trolox (0–0.5 mM) was added and left to react for 15 min. The optical density of the samples was then measured at 515 nm (Thermo Fisher Scientific). The blank was made with methanol. The results were expressed as μg of Trolox equivalent per mg of sample. Reducing capacity of Fe^{3+} to Fe^{2+} by juice and argan pulp, and Fe^{2+} chelating capacity of dialyzates were determined spectrophotometrically according to the methodologies previously described by Kapravelou et al., 2015.

2.4. Mass spectrophotometry

An Ultra Performance Liquid Chromatography (UPLC) (ACQUITY H CLASS, Waters) coupled by mass spectrometry of QTOF (SYNAP G2, Waters) was employed for all high-resolution mass spectrometry

analysis to identify the main bioactive compounds from the extracts. Prior to mass spectrometry analysis, samples were filtered through 0.22 μm nylon disk filters (Millipore). Ten microliters of the final solution were injected into the chromatograph. Analytical separation of phenolic compounds was performed on an ACQUITY HSS T33 analytical column (100 mm × 2.1 mm internal diameter, 1.8 μm) following the protocol described by Martínez et al., (2016). After chromatographic separation, a high-resolution mass spectrometry analysis was carried out in negative electrospray ionization (ESIve). High-purity nitrogen was used as a gas for desolvation (600 L/h) and cone (30 L/h). Spectra were recorded over the mass/charge (m/z) range 50–1200. All the compounds were identified based on their retention times (RT) and mass (MS) fragments. Based on these data the compounds were tentatively identified using MassLynx software.

2.5. In vitro digestion

Argan pulp was used to prepare two experimental diets to be administered to animals at 5 and 10% inclusion levels. The *in vitro* digestibility of the two experimental and one control diet was assessed by the methodology described by Porres et al. (2006) with minor modifications (Martínez et al., 2022) using a pepsin digestion period of 2 h followed by pH equilibration and pancreatin digestion for another 2 h coupled with equilibrium dialysis (MWCO 12,000–14,000 Da, Medicell International Ltd., London, UK). Briefly, 20 mL of 0.01 N HCl was mixed with 1 g of each sample, and 1 N HCl was further added to the mixture until pH 2 was reached. Then, 1 mL of pepsin solution (0.16 g/mL in 0.1 N HCl) was added for gastric digestion, and the mixture was incubated in a shaking water bath at 37 °C for 2 h. Negative controls were made with the same volume of 0.01 N HCl instead of the sample. Before the intestinal digestion, a pH compensation step of 30 min was performed with 0.1 N NaHCO₃ added into dialysis bags which were placed in the digestion vessels in a shaking water bath at 37 °C. Then, 5 mL of a 0.1 N NaHCO₃ solution with pancreatin (4 mg/mL) and bile salts (25 mg/mL) was added, and the mixture was incubated in a shaking water bath at 37 °C for 2 h. Once digestion was finished, the contents inside (sample dialyzed and potentially absorbable) and outside (sample retained that could potentially reach the colon) the dialysis bags were collected and kept at −20 °C to be used for the analysis of dry matter, antioxidant capacity (total polyphenol contents, ABTS, reducing capacity and Fe chelating capacity).

2.6. In vivo experiments

2.6.1. Animals experimental design

A total of 24 brown male C57BL/6J mice (weight 18–20 g, 6 weeks old) were purchased from Charles River Laboratories Inc, Barcelona). The animals were randomly divided into three experimental groups of eight animals per group and housed in colony cages (n = 4) in a well-ventilated and thermostatically controlled room (21 ± 2 °C) to ensure animal welfare (Unidad de Experimentación Animal, CIC, University of Granada). All the cages in each specific experimental group were labeled accordingly and placed in order to avoid possible identification errors. All the researchers and animal facility personnel were trained to ensure the correct identification of cages in each experimental group. The handling of the animals was refined to the minimum necessary to ensure their comfort during the experiments and to avoid causing unnecessary stress. Mice consumed diet *ad libitum* and had free access to type 2 water. The experiments lasted for 90 days, and in the first three days, the animals were allowed to adapt to the experimental diet and housing conditions. All experiments were performed according to Directional Guides Related to Animal Housing and Care (EU 2010) and all procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada, Spain (16/07/2019/132). To select the number of mice assigned to each experimental group (n = 8), we implemented the 3Rs principle (Hubrecht & Carter, 2019). The three experimental

groups consisted of a control group (CT), fed a standard chow (SAFE® 150, Scientific Diets) and two experimental groups fed the standard chow supplemented by 5 or 10% of freeze-dried argan pulp (AP5 and AP10 respectively). Each mouse was considered an experimental unit. General health monitoring of all animals was performed every day. Criteria for the health monitoring include wound, bleeding, hair brilliance, nasal discharge, eye discharge, convulsions, alterations in heart rate, anal and genital discharge, and general motor activity. An end point criterion was established if the animals suffered any type of physical damage, showed symptoms of anorexia with a decrease in intake of 30% or more, or weight loss of 25% or more. If, based on the manifestations presented by the animal, it is decided that it is in an irreversible state of suffering, the experimental death of the specific animal is declared, and its sacrifice is considered, in case it should be necessary. The dietary intake of the animals was recorded daily and the body weight weekly. No adverse effect derived from the inclusion of functional ingredient in the diet were observed in the experimental animals during the experimental period. In addition, no animal became severely ill or died before the experimental endpoints. At the end of the experimental period, the animals were anesthetized with ketamine (75 mg kg^{-1} body weight) and xylazine (10 mg kg^{-1} body weight), and the blood was collected by abdominal aorta puncture using heparin as an anticoagulant. An aliquot was used to assess blood parameters (KX-21 Automated Hematology Analyzer, Sysmex Corporation), and the rest was centrifuged at $1458 \times g$ for 15 min to separate plasma that was subsequently frozen in liquid nitrogen and stored at − 80 °C until its analysis. Different organs and tissues were collected, weighted and a visual test of their general appearance was carried out to verify the absence of damage associated with the consumption of argan pulp.

2.6.2. Oral glucose tolerance test

An oral glucose tolerance test was carried out the previous week to the end of the experimental period in 8 h-fasted animals. A glucose overload of 6.9 μmol/g body weight was performed as described Prieto et al. (2004). Blood glucose levels were analyzed prior to and 15, 30, 60, 90 and 120 min after glucose overload using a Bayer Breeze 2® glucometer (Bayer Healthcare, Spain). The area under the curve was calculated following the trapezoidal rule.

2.6.3. Body composition analysis

Assessment of body composition was performed in 4 h-fasted animals before they were sacrificed, using a whole-body composition analyzer based on magnetic resonance imaging (EchoMRI™; EchoMedical Systems, Houston TX). This analyzer estimated fat and lean body content in live animals.

2.6.4. Antioxidant activity and lipid peroxidation assays in liver

A fresh liver aliquot was homogenized (1:10 w/v) in 50 mM phosphate buffer (pH 7.8) containing 0.1% Triton X-100 and 1.34 mM of DETAPAC. Liver homogenates were centrifuged at 13,000 × g, 4 °C for 45 min, and the supernatant was used to assess the activity of antioxidant enzymes. Catalase activity was assayed according to Cohen et al. (1996), total cellular Glutathione Peroxidase (GPX) activity by the coupled assay of NADPH oxidation (Lawrence et al., 1974) using cumene hydroperoxide as substrate, and total superoxide dismutase (SOD) activity following the methodology of Ukeda et al. (1997), Mn-SOD activity was determined by the same method after treating the samples with 4 mM KCN for 30 min. Cu/Zn-SOD activity resulted from the subtraction of Mn-SOD activity from the total SOD activity. Protein concentration was assayed by the method of Bradford. Lipid peroxidation was determined in kidney homogenates according to the methodology of Ohkawa et al. (1979). Thiobarbituric acid reactive substances (TBARS) were assayed spectrophotometrically at 532 nm.

2.6.5. Gene expression assays

Total RNA was isolated from a portion of liver by homogenization in

1 mL of Tri-Reagent (Sigma-Aldrich). RNA was solubilized in Rnase-free H$_2$O and treated with DNase (Applied Biosystems) to remove any DNA present in the sample. A total of 100–250 ng of RNA was reverse transcribed according to standard protocols using a Lifepro Thermal Cycler (Bioer Serves Life, China). Quantitative RT-PCR was performed with QuantStudio 12 K Flex Real-Time PCR System (Applied Biosystems) using primer/probes for genes involved in oxidative metabolism as *Nfe2l2* (Mm00477784_m1), *sod1* (Mm01344233_g1), *sod2* (Mm01313000), *cat* (Mm00437992), *gpx2* (Mm01286848), genes coding for detoxification pathways as *Nqo1* (Mm00500822_g1) and *gsta2* (Mm03019257_g1), and markers involved in inflammatory processes as *Tnf* (Mm00443258_m1), *IL-1b* (Mm00434228_m1) and *IL-6* (Mm00446190_m1) (Applied Biosystems). The PCR master mix reaction included the first-strand cDNA template, primers/probes, and 2X TaqMan Fast Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems). Relative quantification was performed using the comparative Ct (2$^{-\Delta Ct}$) method. ß-actin was used as internal control.

2.6.6. DNA extraction and bacterial identification in cecal samples

Cecal content was collected at the end of the experiment and used for genomic DNA (gDNA) isolation. Extraction was carried out using the QIAamp® PowerFecal® DNA kit following the manufacturer's protocol for process automation with the QIAcube robot. Quantification of gDNA was performed by fluorometry (qubit). gDNA samples were analyzed by sequencing the V4 region (233 bp) of 16S ribosomal RNA (rRNA) genes using the MiSeq system (Illumina, San Diego, CA, USA). Library preparation, pooling, and miniSeq sequencing were performed at the Institute of Parasitology and Biomedicine López-Neyra (IPBLN) from the Spanish National Research Council (CSIC, Granada). Reaction steps were as follows: denaturation 3 min at 95 °C, (denaturation 30 s at 95 °C, annealing 30 s at 55 °C, elongation 30 s at 72 °C) × 25 cycles, and extension 5 min at 72 °C indexed with 8 PCR cycles to amplify the V3-V4 regions of the 16S rRNA gene with the following primers: 16S ProV3V4 forward 5'->3' 5' CCTACGGGNBGCASCAG 3' and 16S ProV3V4 reverse 5'->3' 5' GACTACNVGGGTATCTAATCC 3'. Results were obtained from the Illumina analysis software version 2.6.2.3 and presented for the taxonomic levels of phylum, family, and genus according to the top 8 or 9 for each taxonomic level.

2.7. Statistical analysis

A Student's *t* test was performed to detect differences in yield and antioxidant capacity between the extracts from argan juice and pulp. The level of significance was set at $p < 0.05$. The influence of argan pulp levels of inclusion in the diet on food intake, bodyweight, hematological and plasma biochemical parameters, glucose metabolism, body composition, organ weight, antioxidant activity, lipid peroxidation, and gene expression data were analyzed by a one-way ANOVA. The results are given as mean values and standard error of the mean. Tukey's test was used to detect differences between treatment means. The analyses were performed with Statistical Package for Social Sciences (IBM SPSS for Windows®, version 22.0, Armonk, NY), and the level of significance was set at $p < 0.05$.

3. Results

3.1. Ethanolic extract yield and antioxidant capacity

The yield and the antioxidant capacity of ethanolic extracts from argan juice and pulp are represented in Table 1. The extraction yield was significantly higher from the argan juice *vs* pulp, while all the results obtained in the different chemical tests of antioxidant capacity (ABTS, reducing capacity, TBARs inhibition, total polyphenol content, and DPPH) were significantly higher in the argan pulp compared to argan juice.

Table 1
Yield and antioxidant capacity of ethanolic extracts from argan juice (AJ) and pulp (AP).

	AJ	AP
Yield (g/100 g)	53.4 (0.67)	33.2 (0.21) ***
ABTS (µg GAE/mg extract)	26.7 (0.38)	45.3 (0.56) ***
Reducing capacity (µg GAE/mg extract)	62.7 (1.61)	86.9 (2.47) ***
TBARS inhibiting capacity (U/mg extract)	1.26 (0.08)	4.30 (0.09) ***
Total polyphenols (µg GAE/mg extract)	60.0 (1.71)	100.3 (1.58) ***
DPPH (µmol Trolox equivalent/mg extract)	82.4 (4.4)	113.0 (3.00) ***

GAE: gallic acid equivalent, **TBARS:** thiobarbituric acid reactive substances, **U:** units
  ***$p < 0.001$. Data are means of 4 replicates (standard error medium)

3.2. Mass spectrophotometry/ identification of bioactive compounds

The main bioactive compounds tentatively identified in the ethanolic extracts of argan juice and pulp are shown in Table 2. A greater diversity of bioactive compounds was found in argan pulp compared to juice (sixteen *vs* twelve). Among all these compounds, thirteen were classified as flavonoids (hesperidin, rhoifolin, catechin, peltatoside, myricetin 3-O-galactoside, rutin, isoquercetin and quercetin, avicularin, procyanidin b2, quercetin 3-sophoroside, gossypin, prodelphinidin b4). A diterpene (Fischeroside B) and a phenol glycoside esterified with (+)-oleuropeic (cypellocarpin A) were identified in argan juice. Whereas the isoflavonoid (mirificin), the aromatic polyketide vaccihein, the methyl chlorogenate (a phenylpropanoid) and the glucaric acid derivative, 2-O-caffeoylglucaric acid, were labeled in the argan pulp extract (see Table 2).

The main biological activities attributed to these compounds included antioxidant, anti-inflammatory, lipid-lowering, hypoglycemic and anti-obesity activities.

3.3. In vitro digestibility

The different diets administered to the animals were exposed to an *in vitro* digestibility process. This process was carried out to assess whether the antioxidant capacity of argan pulp remained after subjecting the diet to a simulation of gastrointestinal digestion. After the *in vitro* digestion process, both the dialyzate and the retentate were obtained, and their antioxidant capacity assessed by different chemical tests (Table 3). First, dry matter dialyzability was assessed and a higher value was obtained for argan-supplemented *vs* control diet. Regarding the antioxidant capacity, a higher antioxidant capacity in dialyzates and retentates from the argan-supplemented diets was also found compared to the control except for ABTS radical inhibition test in the dialyzates, where no significant differences were found among the three diets.

3.4. In vivo experiments

3.4.1. Body weight and food intake

Food intake and body weight of the animals during the experiment period are represented in Fig. 1A and 1B. During the first week of experiment, animals fed the experimental diet with 10% of argan pulp had a lower diet intake compared to animals fed the diet with a 5% inclusion level and the control group. On weeks 2, 3 of the experimental periods, the animals that consumed the diet with 5% of argan pulp exhibited a significantly lower food intake compared to the other experimental groups. From that point ahead, food intake among all the experimental groups did not show major differences until weeks 9 and 10, in which the amount of food consumed by rats on the control group was slight but significantly higher compared to the groups fed the argan-supplemented diets. Regarding the animals' body weight (Fig. 1B), we observed that the intake of argan pulp was reflected in lower body weight of the animals compared to the control group. Such decrease was significant for the animals fed the diet with the highest percentage of argan pulp inclusion (AP 10 group). It should be emphasized that the

Table 2
Identification of the main bioactive compounds from argan juice and pulp.

RT	MS	COMPOUND	MF	ppm	% FIT	FRAGMENTS		
Argan juice								
1.17	609.1819	Hesperidin	$C_{28}H_{33}O_{15}$	−6.6	76.46	455.121	371.1375	305.0639
3.97	577.1462	Rhoifolin	$C_{27}H_{29}O_{14}$	−6.4	0.14	293.0585	226.9916	159.0112
4.18	703.2701	Fischeroside B	$C_{35}H_{43}O_{15}$	14.1	92.52	669.2769	293.0609	592.2616
4.28	289.0732	Catechin	$C_{15}H_{13}O_6$	6.9	97.96	275.0596	225.0776	207.0648
4.6	595.1332	Quercetin − 3 - arabinoglucoside (Peltatoside)	$C_{26}H_{27}O_{16}$	5.5	99.64	293.0558	225.0745	159.0141
4.6	479.0866	Myricetin-3-galactoside	$C_{21}H_{19}O_{13}$	8.3	98.21	226.9912	159.0141	–
4.81	609.1531	Rutin	$C_{27}H_{29}O_{16}$	12.3	30.11	403.1323	335.0898	294.02253
5.12	463.0963	Isoquercitrin	$C_{21}H_{19}O_{12}$	–	0.10	249.0202	226.9912	225.0778
5.57	433.0811	Quercetin 3-α-L-arabinofuranoside (AVICULARIN)	$C_{20}H_{17}O_{11}$	9.2	98.14	294.016	293.0598	226.9899
6.41	609.1244	Prodelphinidin B4	$C_{30}H_{25}O_{14}$	7.6	91.94	293.0581	239.0845	225.0726
7.05	497.1731	Cypellocarpin A	$C_{23}H_{29}O_{12}$	14.5	96.07	321.0901	275.0496	239.0986
7.22	301.0374	Quercetin	$C_{15}H_9O_7$	7.3	86.21	–	–	–
Argan pulp								
0.85	377.0927	Vaccihein A	$C_{18}H_{17}O_9$	14.3	99.17	306.0615	263.0520	180.0443
0.96	215.0361	Bergapten	$C_{12}H_7O_4$	7.9	66.72	197.0269	165.0546	159.0010
1.10	367.0999	Methyl chlorogenate	$C_{17}H_{19}O_{19}$	−9.0	91.45	293.0556	239.0853	225.0683
1.38	609.1829	Hesperidin	$C_{28}H_{33}O_{15}$	1.6	94.06	556.2178	321.0901	294.0371
2.18	371.0624	2-O-caffeoylglucaric acid	$C_{15}H_{15}O_{11}$	2.7	78.14	253.1168	239.0847	207.0574
3.26	577.1346	Procyanidin b2	$C_{30}H_{26}O_{12}$	9.0	94.94	321.1044	293.0450	275.0511
3.76	289.0748	Catechin	$C_{15}H_{13}O_6$	12.5	97.48	275.0543	239.0788	225.0721
4.00	577.1549	Rhoifolin	$C_{27}H_{29}O_{14}$	−1.4	83.53	321.0618	294.0135	275.0415
4.10	479.0853	Gossypin	$C_{21}H_{19}O_{13}$	5.6	67.63	321.0564	207.0600	179.0671
4.32	609.1522	Rutin	$C_{27}H_{30}O_{16}$	10.8	4.56	307.0088	275.0408	239.0822
4.60	463.0957	Isoquercetin	$C_{21}H_{19}O_{12}$	17.3	2.22	294.0232	179.0413	159.0064
4.87	433.0853	Quercetin 3-α-L-arabinofuranoside (Avicularin)	$C_{20}H_{17}O_{11}$	18.9	0.22	329.0309	226.9883	159.0125
5.39	625.1421	Quercetin 3-O-sophoroside	$C_{27}H_{29}O_{17}$	2.6	98.19	293.033	239.0834	226.9906
6.10	609.1296	Prodelphinidin B4	$C_{30}H_{25}O_{14}$	8.5	22.78	239.0906	225.0728	207.058
6.52	547.1502	Mirificin	$C_{26}H_{27}O_{13}$	9.1	52.18	293.0367	239.0920	225.0791
6.55	301.0388	Quercetin	$C_{15}H_9O_7$	13.3	86.01	–	–	–

RT: retention time, **MF**: molecular formula, **MS**: mass, **ppm**: error

Table 3
In vitro bioaccesibility of dry matter and antioxidant capacity from experimental diets.

	CT	AP 5	AP 10
Dry matter dialyzability (%)	35.4 (0.33) a	38.5 (0.52) b	39.3 (0.35) b
Dialyzates			
Total polyphenols (μg GAE/mg)	4.42 (0.16) a	5.55 (0.18) b	7.08 (0.25) c
ABTS (μg GAE/mg)	2.24 (0.05) ab	2.18 (0.02) a	2.33 (0.02) b
Fe Chelating activity (CAU/mg)	0.30 (0.01) a	0.40 (0.01) b	0.44 (0.01) c
Reducing capacity of Fe (μg GAE/mg)	0.71 (0.01) a	1.41 (0.02) b	5.18 (0.13) c
Retentates			
Total polyphenols (μg GAE/mg)	3.96 (0.32) a	5.09 (0.12) b	5.92 (0.12) c
ABTS (μg GAE/mg)	1.54 (0.01) a	1.73 (0.02) b	1.73 (0.03) b
Fe Chelating activity (CAU/mg)	0.28 (0.01) a	0.55 (0.01) b	0.56 (0.01) b
Reducing capacity of Fe (μg GAE/mg)	0.91 (0.02) a	1.43 (0.02) b	2.08 (0.05) c

CT: control diet, **AP 5**: diet including 5% of argan pulp, **AP 10**: diet including 10% of argan pulp, **GAE**: gallic acid equivalent, **CAU**: chelating activity units. Data are means of 4 replicates (standard error medium). a,b,c means with different letters are significantly different (ANOVA treatment, $P < 0.05$).

animals had no diarrhea or other digestive symptoms at any time during the experimental period due to the argan pulp intake.

3.4.2. Oral glucose tolerance test

The results for the oral glucose tolerance test of the three experimental groups are presented in Fig. 2. No significant differences were found in the values of basal blood glucose among the three animal groups. However, the intake of argan pulp resulted in a significant reduction of blood glucose values after 15 and 30 min of the oral glucose overload administration which was maintained at 60 and 90 min of the test when the animals consumed the diet supplemented with 10% argan pulp. The area under the curve was significantly lower for both groups consuming argan pulp, regardless of the dose, compared to the animals in the control group (Fig. 2).

3.4.3. Body composition and organ weights

The effects of argan pulp consumption on body composition are presented in Fig. 1C. The intake of argan pulp caused a significant decrease in body fat content of the animals that was associated to increased lean and total water contents. In addition, the consumption of argan pulp, regardless of the dose used, significantly decreased the weight of epididymal fat, kidneys and spleen. However, a significant increase in colon weight was observed (Supplementary material Table 1).

3.4.4. Hematological and plasma parameters

The influence of argan pulp intake on hematological and plasma biochemical parameters is shown in the Supplementary material Table 2. Argan pulp consumption did not cause any alterations in hematological parameters except for platelet count that was lower in argan-fed animals. Regarding plasma biochemical parameters, consumption of argan pulp led to hyperuricemia and to a significant reduction in creatinine levels that was more pronounced in the animals that consumed the lowest dose of argan pulp (5%). Argan pulp consumption led to a decrease in total- and HDL-cholesterol levels, although results only reached statistical significance at the 5% dietary inclusion level.

3.4.5. Plasma antioxidant and liver anti-inflammatory markers

Plasma antioxidant capacity evaluated by the ABTS methodology and hepatic lipid peroxidation marker of thiobarbituric acid reactive
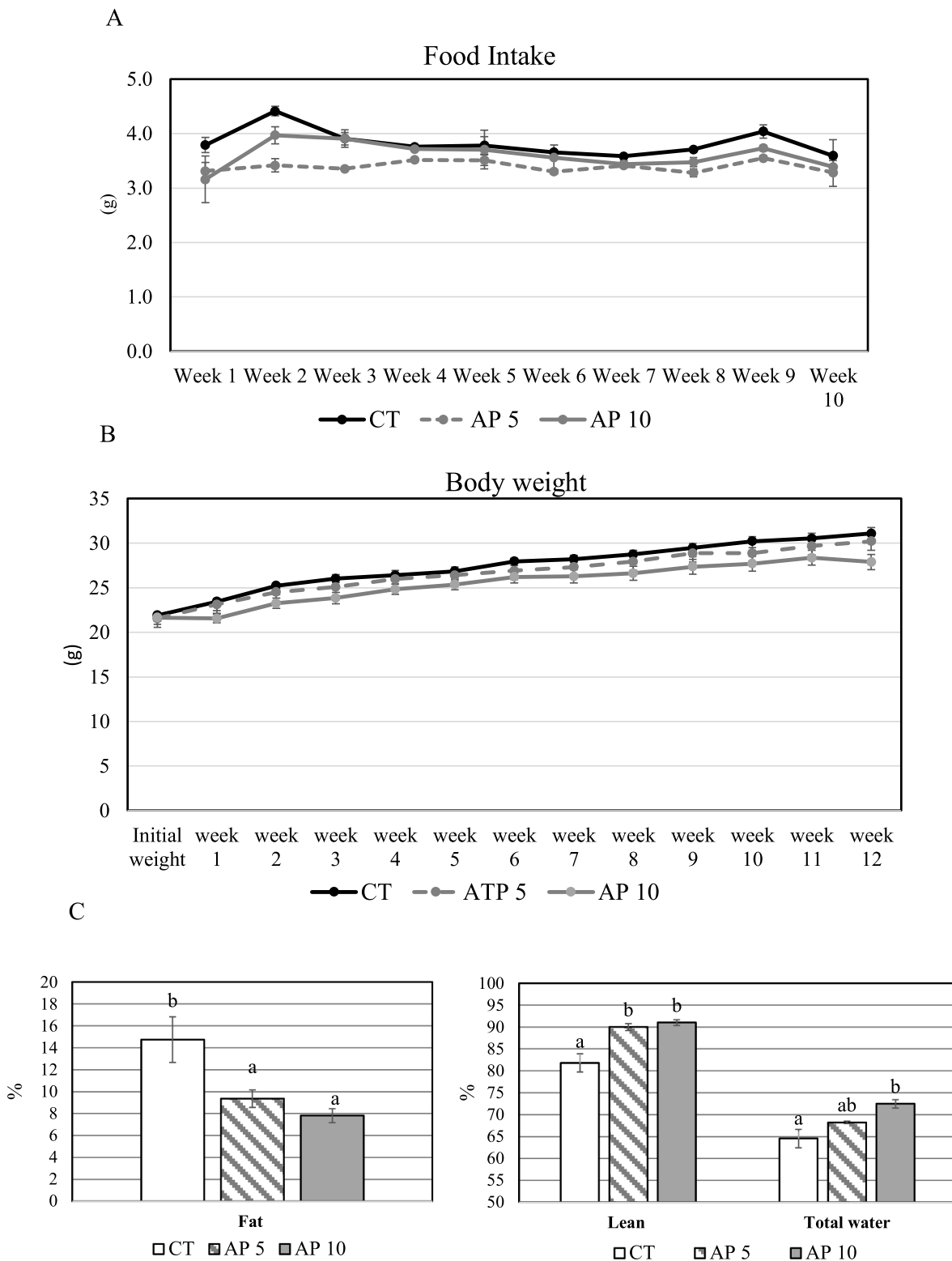
A

Food Intake

CT — · AP 5 — AP 10

B

Body weight

CT — · ATP 5 — AP 10

C

□ CT ▨ AP 5 ▦ AP 10

□ CT ▨ AP 5 ▦ AP 10

Fig. 1. A) Food intake and **B)** body weight of mice fed the different experimental diets. **C)** Effects of argan pulp (AP) intake on body composition (fat, lean and total water) of the animals **CT**: animals fed the control diet, **AP 5**: animals fed the control diet supplemented with 5 % of freeze-dried argan pulp, **AP 10**: animals fed the control diet supplemented with 10 % of freeze-dried argan pulp. Results are means of 8 mice ± standard error of the mean depicted by vertical bars. a, b means with different letters are significantly different (ANOVA treatment, P < 0.05).

substances (TBARs) were measured to assess the influence of argan pulp consumption on the antioxidant status of rats (Fig. 3). Argan pulp intake markedly reduced the concentration of TBARs in liver, whereas a significant increase in total antioxidant capacity measured by the

inhibition of ABTS radical in plasma was observed, although only in mice fed the lowest dose. The effects of argan pulp intake on hepatic gene expression of inflammatory markers such as *Tnf, IL6 and IL-1b* are presented in Fig. 3. Consumption of AP induced a significant reduction
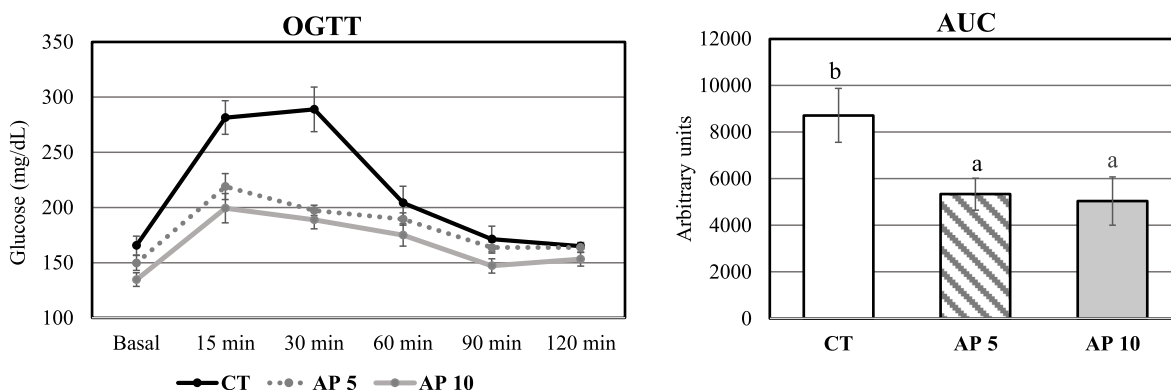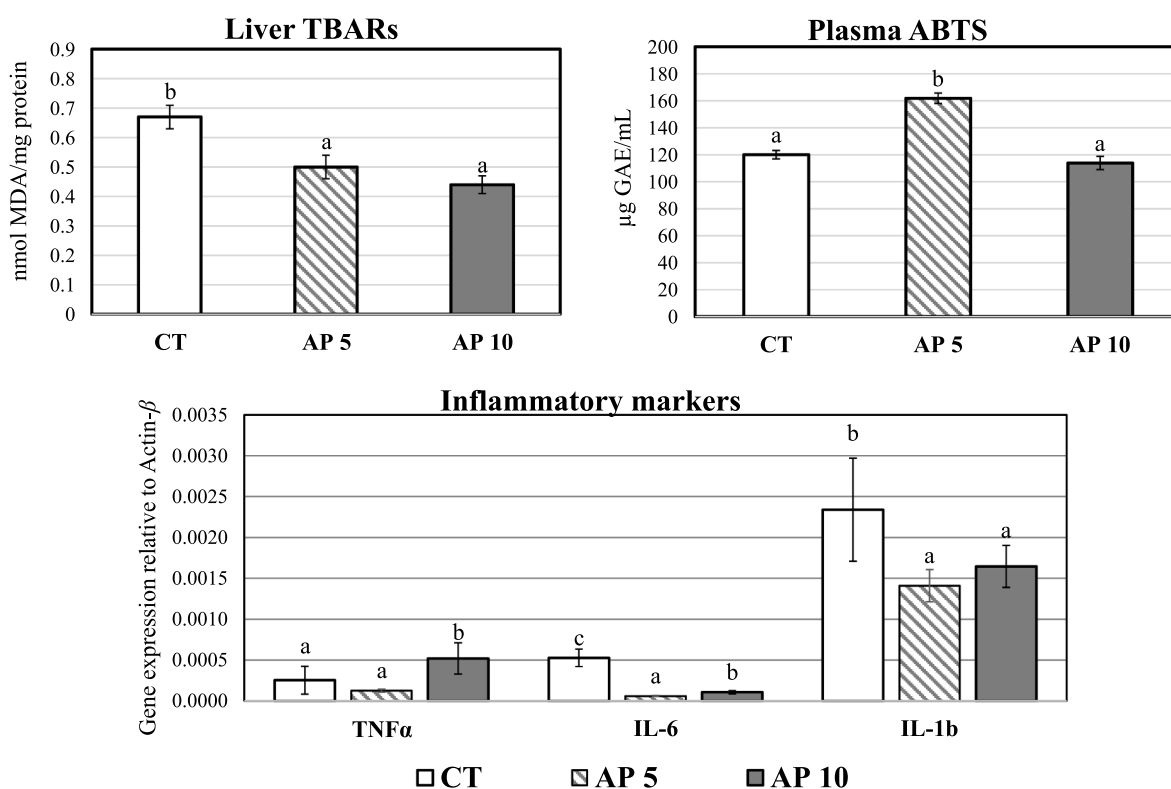
Fig. 2. Effects of argan pulp (AP) intake on glycemic profile and area under the curve (AUC) after an oral glucose tolerance test (OGTT) measured at the end of experimental period. **CT**: animals fed the control diet, **AP 5**: animals fed the control diet supplemented with 5 % of freeze-dried argan pulp, **AP 10**: animals fed the control diet supplemented with 10 % of freeze-dried argan pulp. Results are means of eight mice ± standard error of the mean depicted by vertical bars. a, b means with different letters are significantly different (ANOVA treatment, $P < 0.05$).

Fig. 3. Effects of argan pulp (AP) intake on antioxidant (liver TBARs and plasma ABTS) and anti-inflammatory markers (liver gene expression of TNF-α, IL-6 and IL-1b). **CT**: animals fed the control diet, **AP 5**: animals fed the control diet supplemented with 5 % of freeze-dried argan pulp, **AP 10**: animals fed the control diet supplemented with 10 % of freeze-dried argan pulp. Results are means of eight mice ± standard error of the mean depicted by vertical bars. a,b,c means with different letters are significantly different (ANOVA treatment, $P < 0.05$).

in gene expression of IL-6 and IL-1b, whereas a significant increase in the expression of TNF-α was found upon feeding the animals with the highest dose of AP.

3.4.6. Antioxidant and detoxifying enzyme expression and activity

The hepatic gene expression and activities of antioxidant and detoxifying enzymes superoxide dismutase (*Sod1* and *Sod2*), catalase (*Cat*), Glutathione peroxidase (*GPx*), glutathione transferase (*Gst*), and quinone reductase (*Nqo1*) are presented in Fig. 4. The intake of argan pulp resulted in a significant decrease in the gene expression and enzymatic activity of the mitochondrial enzyme *Sod2* regardless of the administered dose. In contrast, gene expression of *Gpx* was up regulated

by argan pulp consumption. Nevertheless, such increase was accompanied by a significant reduction in enzyme activity. Regarding the detoxifying enzymes GST and QR, a significant increase in gene expression of *Gsta2* was induced by the highest argan inclusion level (10%), while enzyme activity was markedly decreased in animals consuming 5% AP and normalized to values of the control group when the highest argan dose was ingested. In addition, AP intake significantly decreased the gene expression of *Nqo1* at the lowest dose, whereas no-differences were found compared to the rest of experimental groups in enzyme activity.
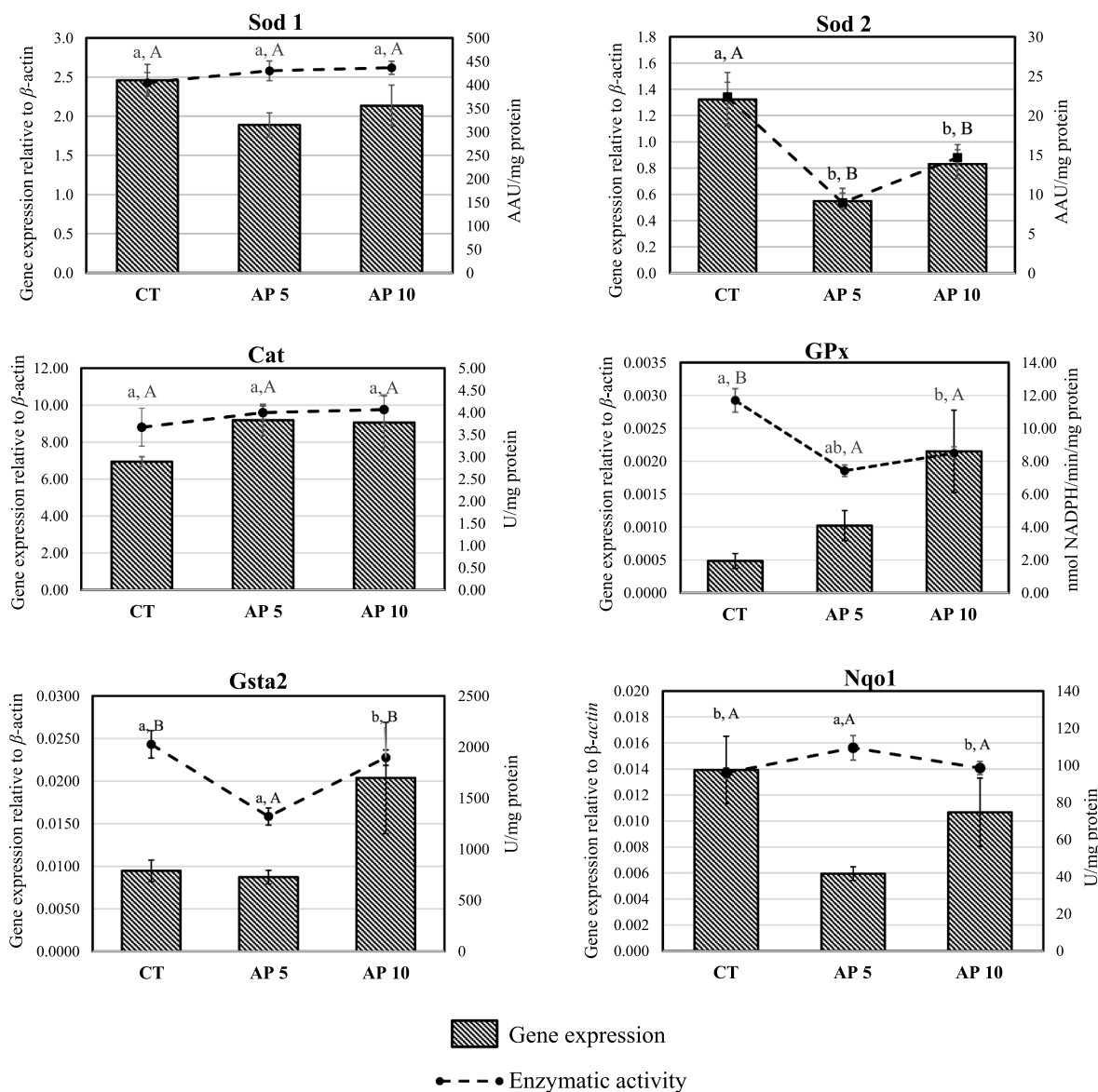
Fig. 4. Gene expression and enzymatic activity assays of different antioxidant and detoxifying enzymes. Gene expression of the enzymes is represented by vertical bars and plotted on the left vertical axis. The dotted line represents the activity of each of the enzymes and is plotted on the right vertical axis. **Sod1:** superoxide dismutase 1 (Cu/Zn dependent), **Sod2:** superoxide dismutase 2 (Mn dependent), **Cat:** catalase, **GPx:** glutathione peroxidase, **Gsta2:** Glutathione S-Transferase Alpha 2, **Nqo1:** NAD(P)H quinone dehydrogenase 1. **CT**: animals fed the control diet, **AP 5**: animals fed the control diet supplemented with 5 % of freeze-dried argan pulp, **AP 10**: animals fed the control diet supplemented with 10 % of freeze-dried argan pulp. Results are means of eight mice $\pm$ standard error of the mean depicted by vertical bars. a,b/A,B means with different letters are significantly different (lower case for enzymatic activity, capitals for gene expression; ANOVA treatment, P < 0.05).

3.4.7. Gut microbiota analysis

Relative abundance analyses at the phylum, family, and genus levels in the three experimental groups studied are presented in Fig. 5. Firmicutes, Bacteroidetes, Verrucomicrobia, Proteobacteria, Actinobacteria, Cyanobacteria and Tenericutes were the most common microorganism phyla found in the cecal content of mice. The proportion of Firmicutes in the control group was roughly 80% of the total population and decreased significantly to 45 and 50% in animals fed the AP5 and AP10 diets, respectively. The Bacteroidetes Phylum represented 10% of the microbiota in the control group, whereas the AP intake raised significantly this percentage to 30%. Further differences among the three experimental groups of animals were found in relation to phylum Proteobacteria that accounted only to 1% of the population in the control group and was significantly raised to 9% and 5%, respectively in the AP5 and AP10 groups. The Actinobacteria phylum was increased

when animals consumed argan pulp, but this increase was only significant between control and AP5 groups. (Fig. 5A).

Regarding the family level, the top 10 were analyzed. Two families were found only in the control group (Streptococcacea and Ruminococcaceae), whereas Bifidobacteriaceae, Coriobacteriaceae and Lactobacillaceae were found only in the argan groups. On the other hand, Lachnospiraceae and Clostridiaceae families were decreased in the AP compared to the control group, although this decrease was only significant in relation to Lachnospiraceae family, while Porphyromonadaceae family was significantly increased (Fig. 5A). The top 10 groups at the genera level were also studied. *Lactococcus* and *Clostridium* were only present in the control group whereas *Parabacteroides* and *Bifidobacterium* were only found in the AP groups. The intake of AP decreased the relative abundance of the genera *Blautia* and *Ruminococcus* compared to the control group whereas the *Akkermansia* genera increased. Finally,
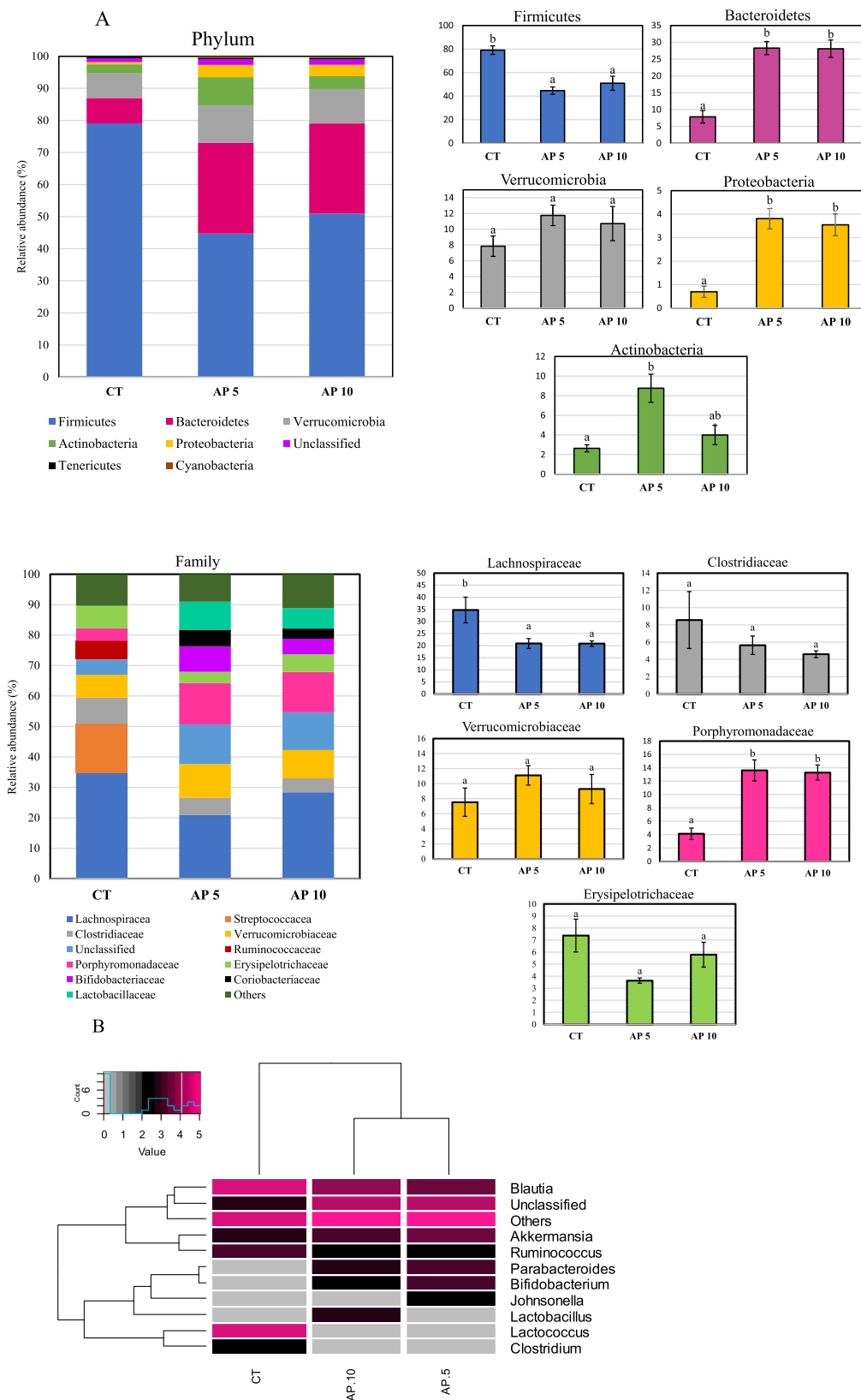
Fig. 5. (A) Relative abundance of the top phyla and families of the three experimental groups. a,b means with different letters are significantly different; ANOVA treatment, $P < 0.05$. (B) Heatmap representing the relative abundance of the top genera.

Johnsonella and *Lactobacillus* were only found in the AP5 or the AP10 groups, respectively (Fig. 5B).

4. Discussion

The search for novel functional foods and the reuse of by-products generated by biotransformation of foodstuffs by the food industry have become an important challenge for researchers in recent years. In this regard, the seed oil extraction from *Argania spinosa* generates useful argan pulp and juice by-products with potential functional activity in the field of food technology and nutrition. Argan pulp (AP) contains a wide variety of bioactive compounds that could be used as novel functional ingredients. This study aimed to assess the profile of bioactive compounds present in an ethanolic extract from AP and its antioxidant capacity as well as its *in vitro* availability when included in the diet. The potential toxicity and effects on general metabolism and gut microbiota were also studied *in vivo*. Our results showed a wide variety of bioactive compounds that provided high antioxidant capacity to the ethanolic extracts assayed. The *in vitro* digestibility of antioxidant capacity from argan pulp-containing diets showed the potential bioavailability of some of the bioactive compounds present in the by-product. The *in vivo* experiment did not show toxicity in AP-fed animals and confirmed its beneficial effects on glucose metabolism and antioxidant status, as well as modulating the gut microbiota.

The ethanolic extract of AP showed a higher antioxidant capacity in all the chemical tests carried out compared to the argan juice that could be related to the greater variety of polyphenolic compounds found in the former extract. Some of these compounds had been identified previously by Charrouf et al., (2007) and Khallouki et al., (2017) in methanolic extracts of the argan fruit. However, to our knowledge this is the first time that other compounds like vaccihein A, bergapten, methyl chlorogenate, 2-O-caffeoylglucaric acid, catechin, gossypin, avicularin, quercetin 3-O-sophoroside, prodelphinidin B4 and mirificin have been reported. For most of the bioactive components found, antioxidant activity has been described. Catechin is widely known and found in many plant-derived foodstuffs and beverages (chocolate, grapes, green tea, and wine) although green tea leaf is the richest source known. The basic function of catechin includes its antioxidant effect. Cypellocarpin A was isolated from the leaves of *Eucalyptus globulus* (Hasegawa et al., 2008) where it exhibited significant DPPH radical scavenging activity. Likewise, Vaccihein A was found in the fruit of rabbiteye blueberry (*Vaccinium ashei*) and described as natural antioxidant (Ono et al., 2002). Methyl chlorogenate was previously isolated from the fruit juice of some plants as *Flacourtia inermis* (Jayasinghe et al., 2012) and the ornamental plant *Ficus macrocarpa,* (Ao et al., 2010) where the antioxidant activity of this compound was demonstrated through the inhibition of superoxide radical formation and the high scavenging activity in DPPH and ABTS tests. Therefore, the antioxidant activity found in the ethanolic extracts of argan juice and pulp could be ascribed to all these bioactive compounds. *In vitro* digestibility showed a higher antioxidant activity in the dialyzates and retentates of the diets including AP compared to unsupplemented control. Therefore, some antioxidant substances of the AP were potentially absorbable and could exert beneficial effects at the systemic level (Di Lorenzo et al., 2021).

After testing the potential bioaccesibility of bioactive compounds into the bloodstream, experimental diets formulated to contain freeze-dried AP at two different levels, 5 and 10%, were tested in an *in vivo* mouse experimental model along 12 weeks of experimental period. Our results showed that during the first three weeks of experiment, the animals underwent an adaptation period in which the intake of AP led to a decrease in food intake that was compensated thereafter. The work of Redondo-Puente et al. (2021) showed that acute polyphenol intake together β-glucan stimulated satiety possibly through the higher levels of plasma ghrelin concentrations after 56 days of polyphenol consumption. In our study, the consumption of AP not only provided polyphenols but also soluble carbohydrates (Zouhair et al., 2020) that

may have contributed to this effect. Besides, the presence of procyanidin b2 which is a dimeric form of grape seed procyanidin, provided astringent characteristic to the fruit which could contribute to this effect. It is worth mentioning that the lower food intake in the AP5 group, compared to the AP10 and CT groups was not reflected in a lower body weight during the first weeks of the experimental period. However, the highest dose of AP did produce a decrease in mice bodyweight from week one of experiment. This effect can be attributed to the presence in the diet of significant amounts of certain polyphenols from AP that have been shown to be effective at body weight reduction. Specifically, the administration of 30 mg per kg body weight of procyanidin b2 per day during 10 weeks in a model of diabetic db/db mice induced a decrease in food intake and body weight (Rauf et al., 2019). Furthermore, hesperidin, a glycosidic flavanone also found in citrus fruits (Meneguzzo et al., 2020) and identified in the AP, reduced body weight in a high-fat diet-induced NAFLD mice model while maintaining feed intake levels unchanged using a dose of 16 mg/kg body weight per day for 16 weeks (Li et al., 2022). The lower body weight gain in animals fed argan pulp may be also related to polyphenol action at intestinal level that can increase the size of lipid droplets and decrease the contact area with digestive enzymes. In addition, hydrogen bonds could be formed between digestive enzymes and the hydrophilic head of bile salts or biliary phosphatidylcholine, leading to diminished fat digestion and absorption, and decreasing plasma levels of HDL-Cholesterol. At the same time, stability of polyphenols would be enhanced during their passage through the small intestine, thus reaching the colon in greater concentrations (Gu et al., 2020; Kardum & Glibetic, 2018). In fact, in our study, changes in body weight appeared to be related to alterations in gut microbiota. A high Firmicutes/Bacteroidetes ratio, has been linked to obesity in several animal and human studies (Houtman et al., 2022; Ley et al., 2006). Here, the lowest body weight was related to a decrease in the Firmicutes/Bacteriodetes ratio. The Phylum Bacteriodetes has been suggested to be less efficient at extracting energy from food than Firmicutes, thus promoting lower calorie absorption and the consequent lower body weight (Krajmalnik-Brown et al., 2012). At genus level, relevant changes were also observed related to argan by-product consumption among which inhibition of pathogenic *Clostridium* spp. and stimulated growth of beneficial bacteria as *Bifidobacterium* and *Lactobacillus* spp., which could indicate a prebiotic effect of AP, are of special relevance. Previous studies have described that the administration of a *Bifidobacterium* strain in high-fat diet-induced obesity promoted lower body weight (Kondo et al., 2010), thus linking its increased presence to this effect. In addition, the lower weight of animals fed AP was complemented by a decrease in epididymal ant total body fat, as well as in plasma total cholesterol. Phytochemicals present in the AP have been described to reduce markers of lipid metabolism. In fact, administration of 50 mg/Kg/day of procyanidin b2 during 10 weeks in a high fat diet–induced obese mouse model decreased the hepatic weight, triglyceride, and cholesterol levels (Su et al., 2018). Hesperidin at a dose of 30 mg/kg/day ameliorated typical alterations of metabolic syndrome such as plasma total cholesterol, triglycerides, and HDL-cholesterol in high-fat/high-fructose diet-fed rats (Apaijit et al., 2022). Its administration in the mouse adipocytic 3 T3-L1 cell line repressed the accumulation of intracellular lipids through suppression of C/EBPα-activated GLUT4-mediated glucose uptake due to decreased transcription level of the *Glut4* gene (Ono & Fujimori, 2011).

Modulation of the gut microbiota has been linked to polyphenols intake (Z. Li et al., 2015; Pérez-Burillo et al., 2020) since most of them are poorly absorbed at digestive level (only 7.2% of ingested polyphenols are found in blood and tissues) and they would reach the large intestine and exert their effect there (Mansoorian et al., 2019). In particular, hesperidin supplementation induced beneficial changes in the gut microbial profile (Li et al., 2022) when administered to C57BL/6J mice at 0.2% (wt/wt) for 16 weeks. Dietary fiber also displays an important impact on gut microbiota, since it provides a wide variety of fermentable substances for the microorganisms. Indeed, we found a

higher colon size in animals fed argan pulp that can be attributed to an increased release of gut fermentation-derived short-chain fatty acids that are able to stimulate the hypertrophy in colon and the differentiation of colon cells to L-cells type where GLP-1 is secreted (Petersen et al., 2014). In turn, this could induce an increase in the insulin released from β–pancreatic cells and lower glycemia levels found after 15, 30 and 60 min of oral glucose overload.

In addition, the polyphenolic profile could had contributed to the hypoglycemic activity of the AP. Procyanidin B2 has shown an inhibitory effect on tyrosine phosphatase-1B (PTP1B) expressed in insulin-sensitive peripheral tissues that can cause the dephosphorylation and subsequent inactivation of insulin receptors and insulin receptor substrates. The administration of 30 mg per kg body weight per day during 10 weeks in a model of diabetic db/db mice reverted serum insulin and HOMA–IR index (Rauf et al., 2019). Isoquercetin (quercetin-3-glucoside), a flavonol found in the leaves of several plants as well as in the mango fruit (Ojeda et al., 2022) showed hypoglycemic effects on *in vivo* experiments (Panda & Kar, 2007), where the administration of 15 mg/kg for 10 days increased serum insulin and decreased glucose concentration and hepatic glucose-6-phosphatase activity. In recent years, the gut microbiota has been identified as a key factor and potential contributor to metabolic disorders like insulin resistance. Furthermore, a higher Firmicutes/Bacteroidetes ratio has been related with decreased phosphorylation of the insulin receptor, insulin receptor substrate (IRS) and Akt, as well as greater inhibitory serine phosphorylation of IRS-1 which would in turn lead to a lower glucose tolerance by target tissues (Caricilli & Saad, 2013). In this study, the lower ratio found could explain an increase in the glucose uptake of target tissues and thus a decrease in plasma glucose levels and insulin resistance.

In general, consumption of AP has been very relevant for antioxidant status of the animals measured as hepatic thiobarbituric acid reactive substances (TBARs), inhibition of ABTS by plasma samples, or hepatic antioxidant enzyme activity. The increase of plasma antioxidant activity exhibited by the AP5 group may be due to the bioavailability of some bioactive compounds that passed into the systemic blood. The reduced TBARs formation in the liver has been related to the administration of polyphenolic compounds. MDA is one of the resulting products from lipid peroxidation and is used as biomarker in lipid peroxidation assays among other products. Hydroxyl radical (•OH) is essential to start the ROS chain reaction, and flavonoids are known to be capable of scavenging it (Rafat Husain et al., 1987). Under our experimental conditions, the lower MDA levels in AP groups was related to three events: i) the presence of bioactive compounds in the diets with radical scavenging properties, ii) the lower SOD2 activity found in these experimental groups that led to lower amounts of H_2O_2 produced as well as, iii) higher activity of GPx enzyme that catalyzes the conversion of H_2O_2 in H_2O. These effects have been assigned to some polyphenolic compound found in AP as Quercetin 3-O-sophoroside (Plumb et al., 1997), Fisheroside B (Oidovsambuu et al., 2013), Avicularin (Samant & Gupta, 2022) and bergapten (Adakudugu et al., 2020). Although these effects can be positive for the treatment of pathologies characterized by high oxidative stress levels, some patients with age-related diseases, specifically neurodegenerative diseases, have been described to exhibit an increased oxidized form of cholesterol, 7-ketocholesterol, which increases ROS production and decreases antioxidant activity of GPx and SOD (Badreddine et al., 2017; Yammine et al., 2020). Therefore, the treatment with argan pulp could be counterproductive in the above-mentioned patients, since it could also lead to a higher decrease in the antioxidant defense systems, and a higher accumulation of ROS. On the other hand, Tung et al. (2014) have reported that the polyphenol resveratrol and physical exercise act as hormetic compounds that activate antioxidant mechanisms especially in aged animals in which oxidative damage was increased and antioxidant activity decreased. Further studies are needed in specific models to select the adequate treatment for each pathology and stage of life.

The redox and inflammatory status are clearly linked. A high level of ROS generates an increase in the inflammatory environment. Here, the improved redox status achieved by AP intake was associated with a remarkably down-regulation in hepatic gene expression of the interleukins IL-6 and IL-1b. Furthermore, the anti-inflammatory properties of flavonoids have been extensively reported. In particular, rhoifolin which is found in edible plants such as grapefruit, bitter orange, lemon, grapes, tomatoes, and bananas, inhibited inflammatory serum factors like TNF-α, IL-6 and IL-1β in an alcoholic liver disease model of mouse at a dose of 40 mg/kg (Mai et al., 2022). Moreover, rhoifolin has been used in a formalin-induced rat paw edema model where it exhibited anti-inflammatory activity inhibiting the edema formation and progression as well as decreasing mRNA expression of IL-1β and TNF-α (Al-Shalabi et al., 2022). In the macrophage RAW 264.7 cell line, avicularin inhibited the release of the pro-inflammatory citokine IL-1β when the cells were treated with LPS from *Escherichia coli.* (Vo et al., 2012) Procyanidin b2 present in the fruit pulp extracts from *Annona crassiflora* (Carvalho et al., 2022) at dose of 150 mg/kg/day caused a significant decrease in pro-inflammatory markers such as TNF-α and IL-6. Bergapten has been described as potent anti-inflammatory in an isoproterenol (ISO)-induced myocardial infarction model in rats at dose of 50 mg/kg/day, leading to a remarkably down-regulation of pro-inflammatory markers such as TNF-α, IL-1β and IL-6 (Yang et al., 2022).

Although inflammation is a response of the immune system used to defend the organism from injury, in some circumstances such as in chronic inflammatory diseases, these effects are detrimental. Indeed increased IL-1β has been described in atherosclerosis, type II diabetes or smoldering myeloma among others (Gabay et al., 2010). High levels of IL-6 have been related to inflammatory bowel diseases, myeloma, rheumatoid arthritis and different forms of arteritis (Choy et al., 2020). Both interleukins are being used as therapeutic targets for the treatment of these pathologies. Argan pulp intake decreased the mRNA expression of both, and it could be considered as new strategy in the prevention or treatment of the above-mentioned pathologies, among others.

Our experimental results also showed a remarkable decrease in the spleen weight of the animals. This effect has been associated with a lower inflammation grade caused by the administration of polyphenols. Finally, we found a higher level of plasma uric acid in animals fed AP diets. This effect could be attributed to the higher intake of fructose contained within in the AP, since it has been described that>50% of fructose is metabolized to uric acid (Zou et al., 2021).

Conclusion

In conclusion, argan pulp is a by-product of the argan oil industry with interesting health benefits that can be considered a new functional food to be consumed in the usual diet. Due to its high content of biologically active components, it holds great potential to reduce the risk of developing certain pathologies and their deleterious effects. In addition, it can also be taken into account as an interesting functional ingredient to be used in the formulation of nutraceuticals for both animal and human nutrition. The multiple functional properties shown by argan pulp such as anti-obesity, hypolipidemic, hypoglycemic, antioxidant, anti-inflammatory and gut microbiota modulator, make it ideal for its use in the treatment and/or prevention of metabolic or inflammatory and some specific diseases characterized by high oxidative stress levels such as insulin resistance, hyperlipidemia, obesity, and metabolic syndrome.

Ethics statement

We follow and agree with all the standards of ethics in authorship, originality of the paper, acknowledgement of funding sources and conflicts of interest. The animal experiment protocol was carried out according to the according to Directional Guides Related to Animal Housing and Care (EU 2010) and all procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada,

Spain (16/07/2019/132).

CRediT authorship contribution statement

Rosario Martínez: Conceptualization, Conceptualization, Formal analysis, Writing – original draft. **Ana Guzmán:** Conceptualization, Formal analysis. **Garyfallia Kapravelou:** Writing – original draft, Validation, Methodology, Investigation, Formal analysis. **Consolación Melguizo:** Resources, Funding acquisition, Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Francisco Bermúdez:** Conceptualization, Resources, Supervision, Writing – review & editing, Funding acquisition. **José Prados:** Resources, Validation, Funding acquisition, Conceptualization, Supervision, Writing – review & editing. **María López-Jurado:** Supervision, Resources, Investigation, Conceptualization, Writing – review & editing, Funding acquisition. **Jesús M. Porres:** Conceptualization, Investigation, Resources, Writing – original draft, Supervision, Writing – review & editing, Funding acquisition.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi. org/10.1016/j.jff.2023.105864.

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