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Effects of consuming diets containing *Agave tequilana* dietary fibre and jamaica calyces on body weight gain and redox status in hypercholesterolemic rats

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ABSTRACT

Dietary fibre (DF) obtained from *Agave tequilana*, which is rich in fructans and insoluble DF, and jamaica calyces (*Hibiscus sabdariffa*), which is rich in DF and phenolic compounds, were assessed as new potential functional ingredients using the hypercholesterolemic animal model. Wistar rats (200–250 g) were divided into 3 groups ($n = 8$) and fed with cholesterol-rich diets supplemented with cellulose (CC, control), agave DF (ADF) or ADF with jamaica calyces (ADF–JC). After consuming the test diets for 5 weeks, weight gain in the ADF–JC group was significantly lower than in the other groups. The ADF and ADF–JC groups had a reduced concentration of cholesterol transporters in the caecum tissue, although no changes were observed in the plasma lipid profile. Both treatments improved the redox status by reducing the malondialdehyde serum levels and protein oxidative damage, compared to the CC group. DF from *A. tequilana* alone, or in combination with jamaica calyces, shows promising potential as a bioactive ingredient.

1. Introduction

Dietary fibre and its associated antioxidant compounds are dietary factors that are being increasingly studied due to their potential health-related properties (Saura-Calixto, 2011). Taking this into account, national and international institutions recommend a daily DF intake of 25–35 grams per day (Block, 1991), and thus DF supplementation can be used to increase the DF intake. Consequently, with the beneficial health effects, new sources of DF are being developed for industrial applications.

Agave tequilana Weber var. *Azul*, also known as blue agave, is a plant grown in arid and semi-arid areas in Mexico, and is often used to make Tequila, one of the most consumed alcoholic beverages worldwide. Moreover, agave is also used to obtain agave fructans and fructose syrup. The agave stem has an average carbohydrate content of 250 g/kg, mainly consisting of highly branched fructans, that have a polymerization degree between 3 and 29 units (Mellado-Mojica & Lopez, 2012). These compounds are resistant to hydrolysis by human digestive enzymes and can be fermented by the colonic microbiota to produce short chain fatty acids (SCFA), and thus they are considered as prebiotic soluble dietary fibre (SDF) (Urias-Silvas & Lopez, 2007). Moreover, agave fructans have shown physiological effects on lipid metabolism (Iniguez-Covarrubias, Lange, & Rowell, 2001, Urias-Silvas et al., 2008).

During the extraction process of agave fructans, an insoluble dietary fibre (IDF)-rich by-product (about 30%) is generated, which is usually discarded (Iniguez-Covarrubias et al., 2001). It is well known that IDF derived from plant food has beneficial effects on health (Schieber, Stintzing, & Carle, 2001). Among other benefits, the combination of slowly fermented IDF with SDF reduces the potential side effects related to the highly fermentable substrate, decreases the fermentation speed and modulates gas formation (Goetze et al., 2008). Moreover, a functional application of agave IDF by-product would have an economical and environmental added value due to the large production of agave heads used to obtain Tequila, which in Mexico may reach one million tonnes annually (CRT., 2011). Taking all this into account, a new source of dietary fibre may be produced by combining the discarded IDF fraction with SDF rich in fructans.

DF associated antioxidant compounds are dietary factors increasingly studied due to their potential health-related proper-

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ties (Saura-Calixto, 2011). Jamaica or Roselle sorrel (*Hibiscus sabdariffa* L.), which is widely cultivated and consumed as an infusion in many tropical regions of Southeast Asia and Central America, produces calyces rich in DF and polyphenolic compounds (Sáyago-Ayerdi, Arranz, Serrano, & Goñi, 2007). Jamaica calyces have recently been described as a promising bioactive ingredient in animal studies (Fernández-Arroyo et al., 2012; Wang et al., 2000). The mixture of agave fibre (rich in IDF), agave fructans (containing SDF) and jamaica calyces (rich in polyphenols) may yield an interesting source of DF and antioxidant compounds. Thus, the aim of this study was to investigate the potential health effects of consuming the DF fraction isolated from *Agave tequilana* (ADF), and its combination with jamaica calyces (JC) on the regulation of body weight gain, plasmatic lipid profile and redox status using an hypercholesterolemic rat model.

2. Materials and methods

2.1. ADF and JC ingredients preparation

Agave tequilana Weber var *Azul* was harvested in Nayarit, Mexico. Agave fructans and ground agave fibre were provided by a local company (Bioagaves de la Costa SA de CV, Tepic, Nayarit, Mexico) and were manufactured following the standard industrial process used. Agave stems were grounded and diluted in water. The juice (18–22 °Brix) was filtered, clarified, and spray-dried (LPG5 Model, Sinotek, S. A. México) (outlet temperature: 80 °C, atomizer speed: 30,000 rpm, air flow: 24 m/s), yielding 145 ± 10 g/kg of a white crystalline powder. As a by-product, the remaining stem was used to obtain the agave IDF by washing, drying and grounding to a fine powder, which was sieved through a 0.05 mm mesh. Jamaica calyces from *Hibiscus sabdariffa* L. '*Criolla*' were purchased from local producers in Huajicori, Nayarit, Mexico. They were washed, dried at 50 °C overnight and grounded (IKA ®Works, Inc, Model M20 S3, Wilmington, NC). For the purpose of the present study, ADF was prepared as a 1:3 (w/w) mixture of agave fructans and agave IDF, providing a IDF/SDF ratio of 70:30, which is similar to that recommended by dietary guidelines (Block, 1991). Protein, fat, ash and moisture contents were analysed following AOAC methods (AOAC, 1990). DF was analysed by the AOAC enzymatic–gravimetric method modified by Mañas, Bravo, and Saura-Calixto (1994). The total carbohydrates in the soluble and insoluble DF fractions were measured using the method described by Englyst and Cummings (1988). The extractable, hydrolysed and condensed polyphenols were also determined (Hartzfeld, Forkner, Hunter, & Hagerman, 2002; Montreau, 1972; Reed, McDowell, van Soest, & Horvath, 1982).

2.2. Animals and diets

The experimental protocol was approved by the Institutional Animal Ethics Committee of La Paz University Hospital (Madrid, Spain) and all procedures were performed in accordance with Spanish law for the protection of experimental animals (RD 1201/2005). Male Wistar rats (200–225 g body weight) were obtained from an accredited supplier (Charles River Laboratories España, S.A. Barcelona, Spain). After an adaptation period of 1 week, animals were distributed into 3 experimental groups ($n = 8$). The hypercholesterolemic diet was prepared as previously described (Tabernero et al., 2013). Test diets corresponding to groups CC, ADF and ADF–JC were supplemented with an equivalent amount of DF, consisting in 10% of cellulose, 10% of ADF or 10% of a 50:50 (w/w) mixture of ADF and JC, respectively. The energy content (255–260 kcal/100 g) and macronutrient composition of the diets (56% carbohydrates, 15% protein, 5% lipids and 12% DF) was similar among the groups. Throughout the intervention, the health of the animals was monitored by a veterinary and the animal weight gain and daily food intake was observed once a week. After 5 weeks, the animals were fasted for 10–12 h. Before sacrifice, they were anaesthetised using isoflurane 20 ml/kg and subjected to complete exsanguination. Plasma and serum fractions were separated by centrifugation (10 min at 2500*g*) using pre-treated EDTA and Silica Act Cot Activator blood collection tubes (BD Vacutainer), respectively. Livers and caecum were collected, weighed and washed with ice-cold PBS. All samples were immediately frozen in liquid nitrogen and stored at −20 °C until analysis.

2.3. Plasmatic biochemical analyses

An automated analyser (Beckman Coulter-Former Olympus Diagnostics AU 5420, Nyon, Switzerland) was used to determine the lipid profile (triglycerides, total-cholesterol, LDL-cholesterol, and HDL-cholesterol) and glucose in the plasma.

2.4. Antioxidant capacity in serum

The oxygen radical scavenging capacity was measured using the hydrophilic ORAC assay, according to the method developed by Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002), with modifications. This assay is based on the fluorescence decay of a reference substance (fluorescein) after the addition of a peroxyl radical AAPH, which acts as an initiator for the oxidative reaction. The analysis was carried out using a 96 well microplate reader (Bio-Tek, Winooski, VT, USA). 25 µl of either trolox, test sample or the solvent as a blank were added to a 96-well microplate. Subsequently, 150 µl of fluorescein stock solution (8.5×10^{-5} mM), prepared in 75 mM phosphate buffer (pH 7.4), was added to the wells. The fluorescence absorbance was recorded every 90 s for 90 min at 485 nm and 528 nm excitation and emission wavelengths, respectively, after the addition of 30 µl of the peroxyl radical generator (AAPH, 153 mM), which was also prepared in 75 mM phosphate buffer (pH 7.4). ORAC results were expressed as nmol Trolox/ml in serum.

2.5. Determination of malondialdehyde (MDA) in serum and liver

MDA, was used as a biomarker for lipid peroxidation. The MDA level was determined in its hydrazone by high-performance liquid chromatography (HPLC) using dinitrophenylhydrazine for derivatization (Mateos, Goya, & Bravo, 2004). This method was a sensible and reproducible alternative to evaluate differences in the oxidative status *in vivo*. Concentrations were expressed as µmoL MDA/mg of protein in liver tissue and µmoL MDA/ml in serum. Protein content in liver homogenates was estimated using the Bradford method (Bradford, 1976).

2.6. Western Blot

Caecum samples were homogenised in a buffer containing 180 mM KCl, 5 mM MOPS, 2 mM EDTA, 1 mM diethylenetriaminepentaacetic acid and 1 µM butylated hidroxytoluene, 10 µg/ml aprotinin and 1 mM phenyl methylsufonyl fluoride at pH 7.3 (Potter–Elvehjem device, at 4 °C). After brief centrifugation (5 min at 500*g*) of pellet cellular debris, protein concentrations were measured in the supernatants using the Bradford method (Bradford, 1976). The total protein (15–40 µg) was resolved by SDS–PAGE and electroblotted onto polyvinylidenedifluoride membranes (Immobilon-P Millipore, Bedford, MA), which had been incubated with the corresponding primary antibody: ABGC5 (H-300): sc-25796, ABGC8 (H-300): sc-30111 and NCP1(H-115): sc-20152, diluted at 1:1000, (Santa Cruz Biotech, Santa Cruz, CA, USA).