JOURNAL OF FUNCTIONAL FOODS 10 (2014) 485–498

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jff

Isolation, comprehensive characterization and antioxidant activities of *Theobroma cacao* extract

M.L. Cádiz-Gurrea [a,b], J. Lozano-Sanchez [a,b], M. Contreras-Gámez [a,b], L. Legeai-Mallet [c], S. Fernández-Arroyo [d,*], A. Segura-Carretero [a,b]

[a] Department of Analytical Chemistry, University of Granada, c/Fuentenueva s/n, 18071 Granada, Spain
[b] Research and Development of Functional Food Centre (CIDAF), PTS Granada, Avda. Del Conocimiento s/n., Edificio BioRegion, 18016 Granada, Spain
[c] Institut National de la Santé et de la Recherche Médicale Unité 781, Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, Hôpital Necker-Enfants Malades, 75015 Paris, France
[d] Centre de Recerca Biomèdica, Institut d'Investigació Sanitària Pere Virgili, Hospital Universitari de Sant Joan, Universitat Rovira i Virgili, C/Sant Joan s/n, 43201 Reus (Tarragona), Spain

ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form 9 July 2014
Accepted 10 July 2014
Available online

Keywords:
Theobroma cacao
HPLC-MSESI-QTOF
Semi-preparative HPLC
Phenolic compounds
Procyanidins
Antioxidant activity

ABSTRACT

Cocoa (*Theobroma cacao*) is a major, economically important, international crop and has been associated with several nutritional benefits including high antioxidant capacity. The aim of the present study was to isolate, characterize and quantify phenolic compounds of *T. cacao* extract using HPLC-MSESI-QTOF. A total of 61 compounds were identified and quantified in the *T. cacao* extract and fractions belonging to various structural classes such as flavan-3-ol and derivatives (including procyanidins), flavonols, *N*-phenylpropenoyl-L-amino acids, and other compounds. These compounds were isolated by semi-preparative HPLC. Afterwards, the composition of each fraction was established by the detailed HPLC-DAD and HPLC-MSESI-QTOF method. The relationship between the chemical structure of flavonoids and their radical-scavenging activities was analyzed. In addition, *T. cacao* extract and fractions show antioxidant activity (by TEAC, FRAP and ORAC methods) decreasing the generation of reactive oxygen species.

1. Introduction

Cocoa, a product derived from the beans of the *Theobroma cacao* tree, consumed by pre-Columbian American civilizations, was introduced to Europe by the Spaniards in the 16th century (Lanaud, Motamayor, & Sounigo, 2003). In the last two decades, the food industry has developed new cocoa-based products, e.g. cocoa liquor, cocoa powder, and chocolate, which are consumed worldwide and used as common ingredients of many food products. The cocoa market has remained stable over the last few years (Ellam & Williamson, 2013), and scientific interest in this potential source of bioactive compounds is growing. Indeed, a large number of studies support the health benefits of cocoa consumption (Ellam & Williamson, 2013; Ramiro-Puig & Castell, 2009; Smith, 2013), being attributed mainly to the flavanol content (Payne, Hurst, Miller, Rank, & Stuart, 2010; Quiñones, Sánchez, Muguerza, Miguel, & Aleixandre, 2011; Ramiro-Puig & Castell, 2009; Smith, 2013). In this respect, the European Food Safety Authority (EFSA) recently issued a positive scientific statement on cocoa flavanols, ascribing beneficial effects primarily to the maintenance of

* *Corresponding author.* Tel.: +34 977 310 300 ext. 55409; fax: +34 977 312 569.
E-mail address: salvador.fernandez@iispv.cat (S. Fernández-Arroyo).
http://dx.doi.org/10.1016/j.jff.2014.07.016
1756-4646/

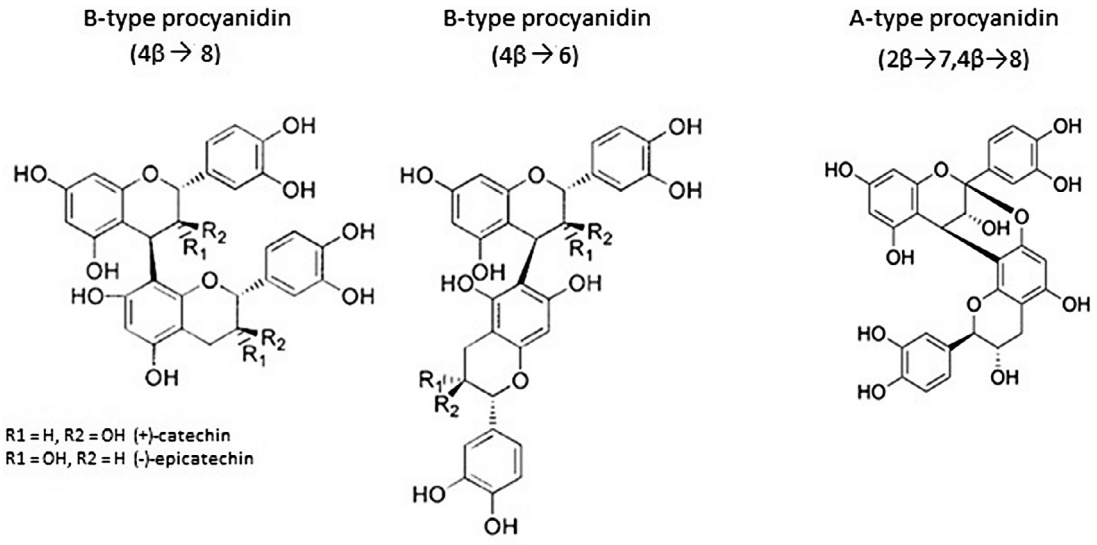

Fig. 1 – Structures of procyanidin (4β→8) and (4β→6)-dimers (B-type) and (2β→7, 4β→8)-dimer (A-type).

normal endothelium-dependent vasodilation (European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies, 2012). Since chocolate contains additional calories, sugar, and fats (Ellam & Williamson, 2013), raw and high-flavanol cocoa powder could receive rapid approval by consumers as well as by legal food authorities defining it as a functional food ingredient.

Cocoa flavanols consist of monomeric (+)-catechin and (–)-epicatechin, and oligomeric flavanols (procyanidins) ranging from dimers to decamers. Concerning the interflavanoid linkage (IFL) nature, B-type procyanidins [C-4 (upper unit)→C-6 or C-8 (lower unit)] are more abundant than A-type procyanidins, which present an additional ether-type bond [C-2 (upper unit)→O→C-5 or C-7 (lower unit)], as well as IFL can be either α or β type (Fig. 1). This list of combinations together with the occurrence of glycosylated and methylated derivatives explains the high diversity of this family and the wide range of biological and biochemical activities in plants (He, Pan, Shi, & Duan, 2008). In addition, processing could lead to the formation of the diasteroisomers (+)-epicatechin and (–)-catechin (Payne et al., 2010). On the other hand, theobromine, a methylxanthine alkaloid, minor amounts of quercetin derivatives and the less known *N*-phenylpropenoyl-L-amino acids have also been reported (Andres-Lacueva et al., 2008; Ortega et al., 2008; Sanbongi et al., 1998; Stark & Hofmann, 2005; Tomas-Barberán et al., 2007), and thus their contribution to the beneficial effects of cocoa should not be ruled out (Ellam & Williamson, 2013; Zeng et al., 2011).

Although the molecular mechanism of these compounds in relation to many diseases could have different cellular targets, the bioactivity of the cocoa polyphenols could be related to different properties, mainly antioxidant activities linked to their chemical structure (Ramiro-Puig & Castell, 2009; Smith, 2013). *In vitro* antioxidant activity of foods and plants is generally studied by $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ based methods (Wojdylo, Oszmianski, & Czemerys, 2007), but these radicals are not as biologically relevant as peroxyl radicals. In this case, the oxygen radical absorbance capacity (ORAC) assay could be used. Regarding the key bioactives, the capacity of polyphenols to inhibit free radicals is governed by their chemical structure. For example, the presence of a catechol group in the B-ring may trap radicals as well as chelating metals (Heim, Tagliaferro, & Bobilya, 2002), and the degree of polymerization of flavanols could also be important (Counet & Collin, 2003; De Gaulejac, Provost, & Vivas, 1999).

Because it is difficult to isolate large amounts of cocoa polyphenols and because there is a lack of commercial standards of procyanidins, almost all *in vitro* and *in vivo* studies make use of whole cocoa matrices. The analytical methodologies applied to purify cocoa bioactives involve laborious pretreatment together with isolation and purification procedures (Hatano et al., 2002; Ortega et al., 2008; Stark & Hofmann, 2005). Among these, isolation by semi-preparative and preparative liquid chromatography (LC) with C18 reversed phase (RP) offers high versatility to separate a wide range of nitrogenous and non-nitrogenous bioactive compounds (Contreras, Carrón, Montero, Ramos, & Recio, 2009; Rzeppa, Von Bargen, Bittner, & Humpf, 2011; Stark & Hofmann, 2005).

The usual technique to analyze polyphenols from cocoa multicomponent extracts or a specific isolated fraction is reversed-phase high-pressure liquid chromatography (RP-HPLC) with C8 (Srdjenovic, Djordjevic-Milic, Grujic, Injac, & Lepojevic, 2008), C12 (Pereira-Caro et al., 2013) and C18 (Andres-Lacueva et al., 2008; Calderón, Wright, Hurst, & Van Breemen, 2009; Quiñones et al., 2011; Tomas-Barberán et al., 2007) stationary phase. As solvent system, all of these authors used linear gradients with acidified water (using formic or acetic acid) and acetonitrile or methanol as organic solvent. This separation technique has been coupled to different detectors for the qualitative and quantitative characterization of these compounds, such as ultraviolet and diode-array detection (DAD) (Quiñones et al., 2011; Srdjenovic et al., 2008), fluorescence (Payne et al., 2010; Pereira-Caro et al., 2013) and/or mass spectrometry (MS) (Andres-Lacueva et al., 2008; Ortega et al., 2010; Pereira-Caro