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# Interaction of coffee compounds with serum albumins. Part II: Diterpenes

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ABSTRACT

Cafestol and 16-*O*-methylcafestol are diterpenes present in coffee, but whilst cafestol is found in both *Coffea canephora* and *Coffea arabica*, 16-*O*-methylcafestol (16-OMC) was reported to be specific of only *C. canephora*. The interactions of such compounds, with serum albumins, have been studied. Three albumins have been considered, namely human serum albumin (HSA), fatty acid free HSA (ffHSA) and bovine serum albumin (BSA). The proteins interact with the diterpenes at the interface between Sudlow site I and the fatty acid binding site 6 in a very peculiar way, leading to a significant change in the secondary structure. The diterpenes do not displace reference binding drugs of site 2, but rather they enhance the affinity of the site for the drugs. They, therefore, may alter the pharmacokinetic profile of albumin – bound drugs.



## 1. Introduction

Coffee is undoubtedly one of the most consumed and appreciated beverage in the world. The two commercially exploited species of coffee, *Coffea arabica* (arabica) and *Coffea canephora* (var. Robusta), have been extensively studied, so far as chemical composition is concerned, particularly for sensory, traceability and authenticity purposes. However, there is a great focus on understanding the chemical properties of coffee major constituents and the biological effects as witnessed by the body of articles appearing in the literature every year. The genome of *C. canephora* has just been sequenced, giving origin to new frontiers in the comprehension of the biosynthesis of coffee secondary metabolites (Denoeud et al., 2014). The chemical composition of coffee beans depends on the coffee species (*arabica* or *robusta*), on the geographical region of the cultivars (Kitzberger et al., 2013), on the roasting process (Eloy Dias, Ferreira, Zerlotti Mercadante, Bragagnolo & de Toledo Benassi, 2014) and on the method used to prepare the coffee beverage. All these variables influence the perceived sensory properties of coffee brands and for this reason industry pays great attention to monitor all these factors.

Among the vast array of compounds present in coffee brew, the biological active classes are usually considered to be phenolic compounds (chlorogenic acids), alkaloids (caffeine and trigonelline), diterpenes (cafestol and kahweol) and melanoidins. These compounds have been shown, at least *in vitro*, to possess various properties, including antioxidant, chemopreventive, antihypertensive and hypoglycemic activity. A recent review, by Croizer et al., considered the overall literature regarding the potential impact on health of the phytochemicals present both in green and roasted coffee beans (Ludwig, Clifford, Lean, Ashihara & Croizer, 2014). The diterpene alcohols of the kaurene family, cafestol, kahweol and 16-*O*-methylcafestol (Fig. 1) are produced only by plants of the *Coffea* genus, but while cafestol was found in both *C. arabica* and *C. canephora*, kahweol is present in *C. arabica* in large amounts, while only traces are found in *C. canephora*. On the contrary, 16-*O*-methylcafestol was found to be specific to *C. canephora*. The total diterpene content ranges from 1.3% to 1.9% (w/w) in green coffee beans of *C. arabica* and from 0.2% to 1.5% in beans of *C. canephora*. In particular, diterpenes have been extensively studied and show beneficial effects to human health as anti-inflammatory properties, a prevention on DNA damage from oxidative stress, although a hypercholesterolemic effect attributed to cafestol was also observed (Bonita, Mandarano, Shuta, & Vinson, 2007).

To better understand the biological impact on human health of these compounds, a study of their interaction with human serum

*Abbreviations:* HSA, human serum albumin; ffHSA, fatty acid – free HSA, BSA, bovine serum albumin; PBS, phosphate buffer solution; 16-OMC, 16-*O*-methylcafestol; DMSO, dimethyl sulfoxide; Trp, tryptophan.

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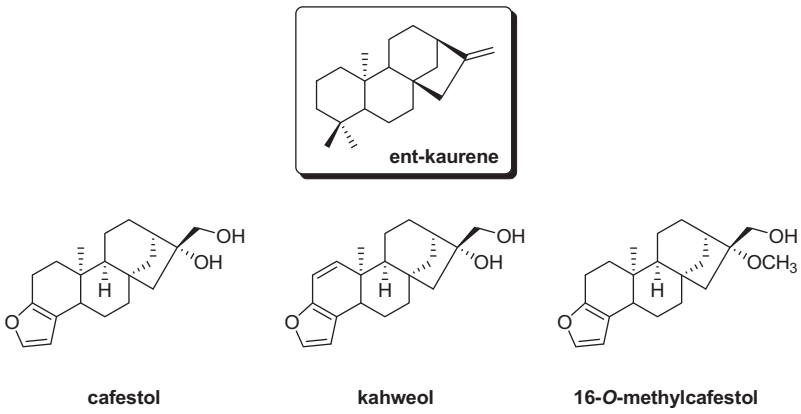


**Fig. 1.** Structures of the ent-kaurene systems, of cafestol and 16-*O*-methylcafestol.

albumin (HSA) is recommended since albumin is the most abundant protein in human plasma. It is a monomeric 585-residue protein, containing three homologous helical domains (I–III), each divided into two subdomains (A and B) (He & Carter, 1992). Two main binding sites for small organic molecules are found, one located in subdomain IIA and one in IIIA, that are known as Sudlow I and Sudlow II sites, respectively (Sudlow, Birkett, & Wade, 1975a). Further sites are present, to bind fatty acids. In the present study we have also considered fatty free HSA (ffHSA). Commercial source albumins are in fact fatty acid-bound and almost all the fatty acid binding sites are occupied. ffHSA is used as a reference as it is known that the occupancy of the fatty acid binding sites may change the affinity of the protein for the drug binding sites, and mostly for the Sudlow site I, which is contiguous to the myristic acid site FA6: in this case tyrosine 210 is turned towards the fatty acid carboxylic head when the FA6 site is occupied, to establish a hydrogen bond with the carboxylate, while it is turned towards the drug site in the absence of fatty acids (Fig. 2A).

Bovine serum albumin (BSA) has been extensively studied in kinetic and affinity drug tests, as a replacement for human serum albumins (HSA), because of its easy accessibility, high stability, ability to bind various ligands and structural similarity to HSA (Shinga Roy, Tripathy Chatterjee & Dasgupta, 2010; Zhang et al., 2013). The structure of BSA is homologous to HSA and consists of three linearly arranged domains (I–III) that are composed of two subdomains (A and B).

In our previous study we have determined by fluorescence spectroscopy the dissociation constants for the complexes of chlorogenic acids and quinides with HSA, which were in the micromolar range (Sinisi, Forzato, Cefarin, Navarini, & Berti, 2015).

In the present work we have considered the diterpenes alcohols cafestol and 16-*O*-methylcafestol, isolated from commercial *C. canephora* blends and we have studied their interactions with albumins by fluorescence and circular dichroism spectroscopies.

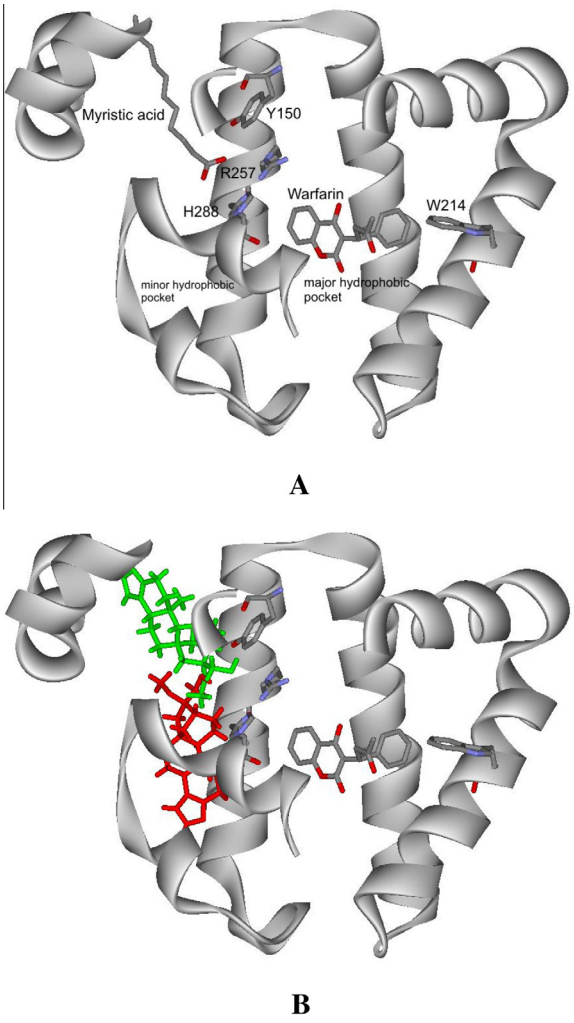


**Fig. 2.** (A) Outline of Sudlow site I and of fatty acid binding site 6 in human albumin. The reference ligand of site I, warfarin, is located inside the main hydrophobic pocket and shows also a phenyl ring pointing towards the fluorescent side chain of tryptophan 214 in the front hydrophobic pocket. The three polar aminoacids Y150, R257 and H288 at the borderline between site I and site 6 are shown. Tyrosine 150 is turned towards the carboxylic head of a molecule of myristic acid. From pdb id 1H9Z. (B) AutoDock Vina calculated poses for 16-OMC in the binding areas of site I and 6, in the presence of warfarin. In the green solution the terpene is fully placed inside the fatty acid binding site, while in the red one the ligand is placed in the minor hydrophobic pocket of site I, but the hydroxyl group and its neighbours lie in site 6 close to the polar aminoacids. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 2. Materials and methods

### 2.1. Materials

HSA (A1653, 96–99%), HSA essentially fatty acid free (A3782, 99%), BSA (A3912, ⩾96%) were purchased from Sigma–Aldrich Co. (St Louis, MO, USA) and used without further purification. Their molecular weights were assumed to be 66.478 Da, 66.478 Da and 66.463 Da respectively. Stock solutions of albumins were prepared by dissolving in PBS (pH 7.4). All stock solutions were kept at 4 °C and then diluted to the required experimental sample concentrations ($1.0 \times 10^{-6}$ M). Cafestol and 16-OMC were provided by