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# Synthesis and characterization of a (+)-catechin and L-(+)-ascorbic acid cocrystal as a new functional ingredient for tea drinks

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ABSTRACT

Tea (*Camellia Sinensis*) is one of the most popular drink, consumed as infusion or bottled ready to drink beverages. Although tea leaves contain many antioxidants compounds, after processing they can drastically decrease, sometimes up to a full degradation, as in the case of catechin, a very healthy flavan-3-ol. In this context, the synthesis of a cocrystal between (+)-catechin and L-(+)-ascorbic acid, was proved to be a useful strategy to make a new ingredient able to ameliorate the antioxidant profile of both infusions and bottled teas. The obtained cocrystal showed a three-fold higher solubility than (+)catechin and its formation was elucidated unambiguously by FT-IR, thermal (DSC) and diffraction (PXRD) analyses. Antioxidant characteristics of the samples were evaluated by colorimetric assays. As expected, infusions showed much better antioxidant features than ready-to-use lemon and peach teas. The same trend was confirmed after the addition of the cocrystal at two concentration levels. In particular, supplementation at concentration of 2 mg $mL^{-1}$ improved the bottled tea antioxidant values to the level showed by the not-added infusion tea.

## 1. Introduction

Tea (*Camellia sinensis*) has become the world's most popular drink after water and its industry involve more than 13 million people around the world. Due to agronomic constraints, tea production is mostly located in Asia and Africa, with about 75% of the global production hold by China, India, Kenya, Sri Lanka and Turkey (FAO, 2015).

Today, tea is available for consumption mostly as green, oolong, black and pu-erh. They can be discriminated by the production process, leading to different oxidization and fermentation levels. Green tea is unfermented, oolong tea is semi-fermented and black tea is fully fermented, while pu-erh is a post-fermented tea involving the action of microorganisms. Black tea drives the market with about 60% of the production, while green tea accounts for about 30%; the remaining part is devoted to oolong and pu-erh teas production (FAO, 2015). In recent years, to support the expansion of the demand, diversification into other segments of the market have been widely encouraged, including canned and bottled tea flavoured beverages.

Tea contains many bioactive compounds. Among them, polyphenols have been proved to be responsible of some beneficial health effects related to tea consumption, including antioxidative, anti-inflammatory, antimicrobial, anticarcinogenic, antihypertensive, neuroprotective, cholesterol-lowering and thermogenic properties (Hayat et al., 2015; Vuong, 2014). Fresh tea leaves contain up to 30% (dry weight) of polyphenols (Stewart et al., 2005), mainly flavan-3-ols and, to a lesser extent, flavonol glycosides, proanthocyanidins, gallic acid derivatives and hydroxycinammatequinic esters. The most abundant catechins are generally (–)-epigallocatechin-3-*O*-gallate and (–)-epicatechin-3-*O*-gallate, followed by (–)-epigallocatechin, (+)-gallocatechin, (+)-catechin and (–)-epicatechin, also present as gallates. During processing of fresh leaves, polyphenols undergo a series of modifications, increasingly drastic passing from the manufacture of commercial green tea, through the processing of semi-fermented, full or post-fermented teas (Kilmartin and Hsu, 2003). In particular, during the black tea production, either by the orthodox or by the cut-tear-curl method, polyphenol oxidases, with the contribution of peroxidases, lead to degradation of flavan-3-ols up to 90% or more. Galloylquinic acids, quercetin and myricetin glycosides as well as proanthocyanidins seem also to be affected by oxidation (Stodt et al., 2014). All these transformations support the formation of typical black tea compounds with higher molecular weights, i.e. theaflavins and thearubigins. In black tea, they are responsible for the astringent taste and the dark colour of the infusion, accounting for about 90% of its phenolic components (Stewart et al., 2005). Irrespective of the kind of tea, other changes occur during the

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domestic brewing process and the production of instant tea beverages in relation to processing phases of manufacture, transport and storage (Wang et al., 2008; Watanabe et al., 1998). To this regard, if black tea is the raw material to produce bottled tea beverages, is reasonable to expect a further decrease in the antioxidant properties of the commercial product (Hocker et al., 2017). In all these cases, it was shown that catechins experience auto-oxidation and epimerization reactions, prompted by heat treatments, pH, presence of other ingredients and/or prolonged storage (Chen et al., 2001; Xu et al., 2003, 2004bib_Xu_et_al_2003bib_Xu_et_al_2004). To overcome the catechin poor stability in water, different strategies have been suggested, including the use of reducing agents and formation of micro- and nanoparticles (Ye; Augustin, 2018; Dube et al., 2010). To this regard, the addition of ascorbic acid is a very common approach (Chen et al., 2001) as it can be oxidized in place of catechins forming dehydroascorbic acid. However, dehydroascorbic acid can further react with cathechins, mostly (–)-epigallocatechin-3-*O*-gallate, forming ascorbyl conjugates whose presence has been reported in bottled green tea by Hung et al. (2018). This effect decreases the catechin content of the product and thus its global quality.

A typical feature of polyphenols, including flavonoids and flavan-3-ols is their poor bioavailability, requiring plasma concentrations prohibitively high to elicit their favourable health effects (Lambert et al., 2006). Over the years various strategies have been explored to overcome these limitations, including chemical modifications (Badolato et al., 2017; Tundis et al., 2019; Carullo et al., 2019a; Carullo and Aiello, 2018), polymerization processes (Restuccia et al., 2019; Carullo et al., 2019b) or cocrystallization. In particular, the latter has been proved to influence the solid state properties of a drug substance, in particular its solubility and hence bioavailability (Schultheiss and Newman, 2009; Aitipamula et al., 2012; Karimi-Jafari et al., 2018). Mostly exploited in pharmaceutical sciences, cocrystals are formed from two or more components; one is generally a drug while the other is a cocrystal former (coformer). Many compounds can be used as a conformer, including polyphenols and many other nutraceuticals (Steed, 2013; Smith et al., 2011, 2013bib_Smith_et_al_2011bib_Smith_et_al_2013).

In this context, the goal of the present research was the synthesis and the characterization of a new cocrystal formed by L-(+)-ascorbic acid and (+)-catechin. This solid form was then applied to bottled black teas in order to improve the antioxidant properties of the product, assessed by colorimetric *in vitro* tests. The same parameters for black tea infusions were also evaluated for comparison.

## 2. Materials and methods

### 2.1. Chemicals

All starting materials used in this study are commercially available from Sigma-Aldrich (Sigma Chemical Co., St Louis, MO, USA). The solvents were used as received.

### 2.2. Preparation of cocrystal by slurry method (ASC)

The (+)-catechin hydrate, commercially available, (99.8% pure 200.0 mg, 0.689 mmol) and L-(+)-ascorbic acid (99% pure, used as received, 121.0 mg, 0.689 mmol) were kneaded with 20 mL of ethanol 96% and stirred at room temperature for 24h in a vial. The vial was left in the hood undisturbed, and after two days, a beige microcrystalline powder (ASC cocrystal) was obtained. The characterization of the powder was carried out by FT-IR spectroscopy, Differential Scanning Calorimetry and Powder X-Ray Diffraction.

### 2.3. FT-IR analysis

Infrared spectra were acquired by a Spectrum Two Fourier transform infrared (FTIR) spectrometer (Perkin Elmer) equipped with an attenuated total reflection (ATR) accessory.

ATR measures the variation of an infrared beam when it comes in contact with the sample. Samples were placed on the ATR surface and spectra were recorded in the wave length range between 4000 and 450 $cm^{-1}$. Scan number and resolution were optimized and 16 scans and 4 $cm^{-1}$ were selected for analysis. The spectrum of the ATR element (after cleaning and drying) versus room air was used as background.

### 2.4. Preparation of cocrystal ASC by liquid assisted grinding (ASC-LAG)

The cocrystal ASC was prepared also by Liquid Assisted Grinding (LAG). (+)-catechin (50 mg, 0.172 mmol) and L-(+)-ascorbic acid (30.3 mg, 0.172 mmol) were grinded in an agate ball miller for 3 h in the presence of a small amount of ethanol (20 μL). The resulting powder was analysed by PXRD.

### 2.5. Powder X-Ray Diffraction (PXRD)

The PXRD patterns were acquired on a Bruker D2-Phaser equipped with a Cu Kα radiation (λ = 1.5418 Å) and a Lynxeye detector, at 30 kV and 10 mA, with step size of 0.01°, over an angular range of 5–50° 2θ. The obtained diffractograms were analysed with DIFFRAC.EVA diffraction software. The interplanar distances (*d*) were calculated according to the Bragg equation.

### 2.6. Differential Scanning Calorimetry (DSC)

DSC analysis was performed using a TA DSC Q2000 instrument with a refrigerated cooling unit (RCS 90). Indium metal standard was used for the temperature calibrations. The pristine materials ((+)-catechin and L-(+)-ascorbic acid) and the cocrystal (ASC) were accurately weighted (1.5–2 mg) and crimped in non-hermetic aluminium pans. The samples were heated from 30 to 220 °C at a heating rate of 10 °C $min^{-1}$, under a dry nitrogen atmosphere (flow rate 50 mL $min^{-1}$), using an empty non-hermetic aluminium pan as reference.

### 2.7. Samples

Tea beverages (peach (PT) and lemon (LT)) have been selected in grocery stores in Cosenza, Italy. PT and LT were chosen because, in Italy, they are the only commercialized beverages based on black tea. ASC cocrystal was added to commercial teas to achieve a final concentration of 1.0 and 2.0 mg $mL^{-1}$. Infusion tea (IT) was prepared according to the International Organization for Standardization (ISO) guidelines titled: "Tea-Preparation of liquor for use in sensory test" (ISO 3103) and employing a procedure already described (Spizzirri et al., 2016). Briefly, a volume of 70 mL of double boiling distilled water was added to 2.00 g of tea leaves. After 20 min the infusion was filtered and the cooled filtrate was placed in a volumetric flask. A final volume of 100 mL was reached with water. Fortified infusions were prepared by the same protocol just reported, but tea leaves (about 2.00 g) were supplemented with 100 and 200 mg of (+)-catechin L-(+)-ascorbic acid cocrystal before the addition of the boiling water. ASC was dissolved in water to obtain a 2 mg $mL^{-1}$ solution employed to evaluate antioxidant performances.

### 2.8. Antioxidant properties of ASC cocrystal, commercial and reinforced beverages

#### 2.8.1. Total phenolic groups by Folin–Ciocalteu procedure

The total phenolic content of the samples (ASC in water, black tea infusion, PT, LT and fortified counterparts) was determined using the Folin-Ciocalteu reagent procedure (Iemma et al., 2010). Results were expressed as Gallic Acid Equivalents (GAE) per grams of cocrystal or as GAE per litre of beverage, by using the equation obtained from the calibration curve of the reference antioxidant compound (gallic acid). In a standard procedure, a volume of 6.0 mL of aqueous solution of the sample was placed in a volumetric flask (10 mL) and then 1.0 mL of the

Folin-Ciocalteu reagent was added. The solution was mixed thoroughly and, after 3 min, 3.0 mL of $Na_2CO_3$ (2%) were added. The mixture was then allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm against a control prepared under the same reaction conditions, but without sample. Calibration curve was recorded by employing five different standard solutions of gallic acid. 0.5 mL of each solution was added to the Folin-Ciocalteu system leading to the final concentration of 8.0, 16.0, 24.0, 32.0, and 40.0 μM, respectively. After 2 h, the absorbance of the solutions was measured to obtain the calibration curve equation and the correlation coefficient ($R^2$). The slope and the intercept of the obtained equation were calculated by the least square method. Each measurement was performed in triplicate and data expressed as means (±SD). UV-Vis absorption spectra were recorded with a Jasco V-530 UV/Vis spectrometer (Jasco, Tokyo, Japan).

#### 2.8.2. Determination of total antioxidant activity

The total antioxidant activity of samples (ASC in water, black tea infusion, PT, LT and fortified counterparts) was evaluated following a literature protocol, with some changes (Cirillo et al., 2012). Briefly, 0.3 mL of aqueous solution of each matrix were mixed with 1.2 mL of reagent solution (0.6 M $H_2SO_4$, 28.0 M $Na_3PO_4$, and 4.0 M $(NH_4)_2MoO_4$). The reaction mixture was incubated at 95 °C for 150 min and, after cooling at room temperature, the absorbance of the solution was measured at 695 nm in comparison to the control prepared in the same conditions. Measurement was carried out in triplicate and data expressed as means (±SD). For ASC cocrystal, the total antioxidant activity was expressed as GAE per grams of cocrystal, while for beverages as GAE per litre of beverage, respectively. By using five different antioxidant standard solutions, a gallic acid calibration curve was recorded. A volume of 0.3 mL of each solution was mixed with 1.2 mL of phosphomolybdate reagent solution to obtain the final concentrations of 8.0, 16.0, 24.0, 32.0, and 40.0 μM, respectively. After 150 min of incubation at 95 °C, the solutions were analysed by using a UV-Vis spectrophotometer. Once the regression equation was obtained, its correlation coefficient ($R^2$), slope, and intercept were all calculated by the least square method.

#### 2.8.3. Determination of scavenging activity on DPPH radicals

The free radical scavenging properties of samples (ASC in water, black tea infusion, PT, LT and fortified counterparts) were estimated towards DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, following a literature protocol, with some changes (Spizzirri et al., 2011). In a standard procedure 1.0 mL of sample solution was placed in a volumetric flask (10 mL) and then 4.0 ml of ethanol and 5.0 mL of ethanol solution of DPPH (200 μM) were added, obtaining a solution of DPPH with a final concentration of 100 μM. The sample was incubated in a water bath at 25 °C and, after 24 h, the absorbance of the remaining DPPH was colorimetrically determined at 517 nm. The scavenging activity of the sample was measured considering the DPPH deceasing absorbance. Results were therefore expressed as percent of inhibition of the DPPH radical and calculated according the following Eq. (1):

$$inhibition\% = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where $A_0$ is the absorbance of a blank solution prepared in the above-mentioned conditions but without the sample, and $A_1$ is the absorbance of the sample solution. Each measurement was carried out in triplicate and data expressed as means (±SD).

#### 2.8.4. Determination of scavenging effect on the ABTS radical cation

The free radical scavenging properties of ASC solution, black tea infusion, PT, LT and fortified counterparts were estimated towards ~~(~~2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid~~))~~ (ABTS) radical (Restuccia et al., 2017). ABTS was dissolved in water (concentration 7.0 mM). ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Because ABTS and potassium persulfate react at a ratio of 1:0.5, this leads to an incomplete oxidation of the ABTS. Oxidation of the ABTS started immediately, but the absorbance was not maximal and stable until 6 h, at least. Finally, the concentration of the resulting $ABTS^{\cdot+}$ solution was adjusted to an absorbance of 0.970 ± 0.020 at 734 nm. The radical was stable in this form for more than two days when stored in the dark at room temperature. In order to evaluate the scavenging effect of all samples, 500 μL of each solution were mixed with 2.0 mL of the ABTS radical solution. The obtained mixture was incubated in a water bath at 37 °C and protected from light for 5 min. The decrease of absorbance at 734 nm was measured at the endpoint of 5 min. The antioxidant activity was expressed as a percentage of scavenging activity on the ABTS radical according to Eq. (1). All samples were assayed in triplicate and data expressed as means (±SD).

## 3. Results and discussion

### 3.1. Synthesis and characterization

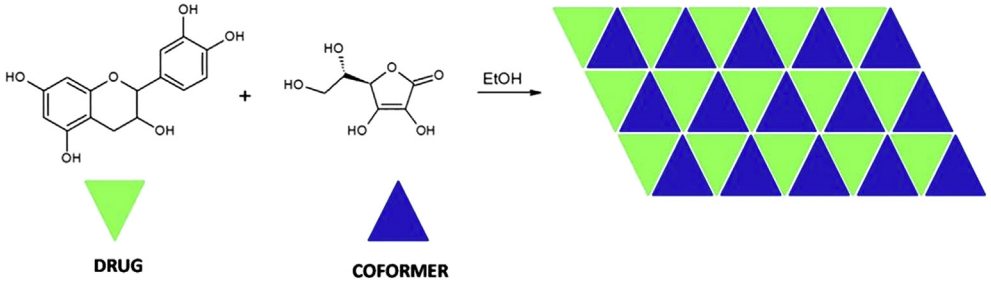
To improve the water solubility of (+)-catechin the basic concepts of crystal engineering were employed (Scheme 1). In particular, ASC cocrystal was prepared in bulk using the slurry method.

The growing interest towards the cocrystals preparation and employment is due to their high water solubility, in turn positively affecting bioavailability. It is well known that the solubility of cocrystals is proportional to those of its precursors, rising as the co-former solubility increases. Usually the solubility of the co-former should be about 10 folds higher than that of the other component. Taking into consideration that the water solubility of (+)-catechin at room temperature is 0.45 mg $mL^{-1}$ (Cuevas-Valenzuela et al., 2014) and that L-(+)-ascorbic acid shows a water solubility of 330 mg $mL^{-1}$, the latter can be a suitable coformer to prepare the cocrystal (Wouters and Quèrè, 2012). The ASC cocrystal finally exhibited a solubility of 3.4 mg $mL^{-1}$, a value three fold bigger than that of (+) catechin.

The formation of the ASC cocrystal was confirmed by comparing the FT-IR spectrum of the cocrystal (Fig. 1) with the spectra of the individual components (Semalty et al., 2012; Wang et al., 2015). In the cocrystal spectrum (green line) it was evident a large stretching band of phenolic groups OH around 3300 $cm^{-1}$, indicating hydrogen bond formation not present in the pure compounds (blue and red lines). Furthermore, the bending of O–H and stretching of C–O are clearly visible at 1075 and 1066 $cm^{-1}$ respectively, confirming the new interaction like H-bonds formed between (+)-catechin and L-(+)-ascorbic acid.

DSC is a useful cocrystal screening technique, allowing the construction of binary phase diagrams (Yamashita et al., 2013; Zhou et al., 2016) and providing information about the possible cocrystal formation through thermal methods, e.g. Hot Melt Extrusion, Co-Melting and Kofler's technique (Berry et al., 2008; Rodrigues et al., 2018). Besides, thermal analysis (DSC and DTA) have been used to prove the effective formation of a cocrystal by comparing the pristine materials and the cocrystal thermal responses (Sevukarajan et al., 2011; Yuliandra et al., 2018). In this study, the cocrystal DSC curve was compared to those of (+)-catechin and L-(+)-ascorbic acid (Fig. 2). The L-(+)-ascorbic acid DSC plot (Fig. 2a) is characterized by an endothermic peak at 191 °C associated to its melting with decomposition. On the contrary, as already reported by Junior et al. (2017) and Ferreira-Nunes et al. (2018), (+)-catechin displays a more complex thermal profile (Fig. 2c), presenting many peaks in the temperature range of 75–150 °C related to water loss, an endothermic peak at 176 °C associated to anhydrous catechin melting, followed by its decomposition above 200 °C. The first part of the cocrystal DSC profile resembles that of (+)-catechin, however, at 186 °C a new peak, related to the cocrystal melting, arises (Fig. 2b). This observation clearly indicates the formation of a new solid phase.

However, the hard evidence of the cocrystal formation has been provided by Powder X-Ray Diffraction (PXRD) analysis. Indeed, PXRD




**Scheme 1.** Schematic description of the cocrystal formation.

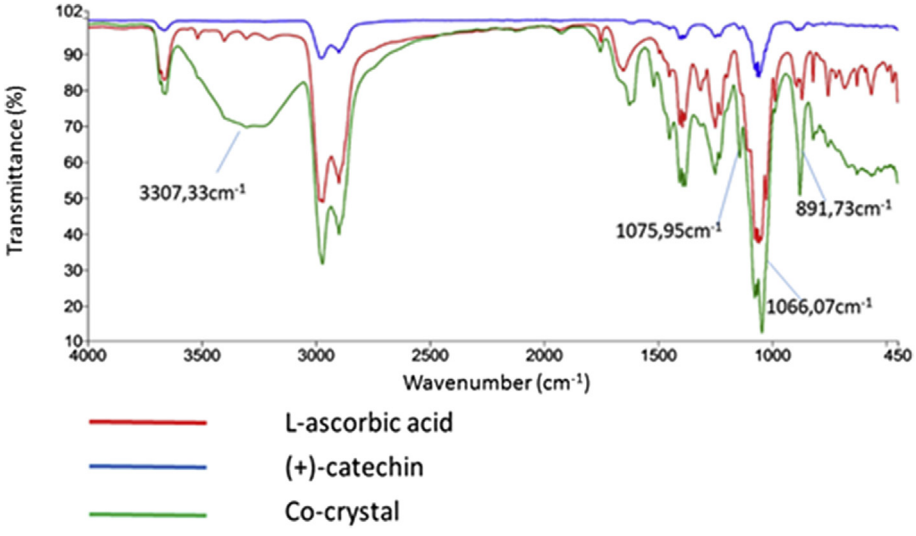



**Fig. 1.** FT-IR spectra and reference peaks of ascorbic acid, catechin and cocrystal ASC.

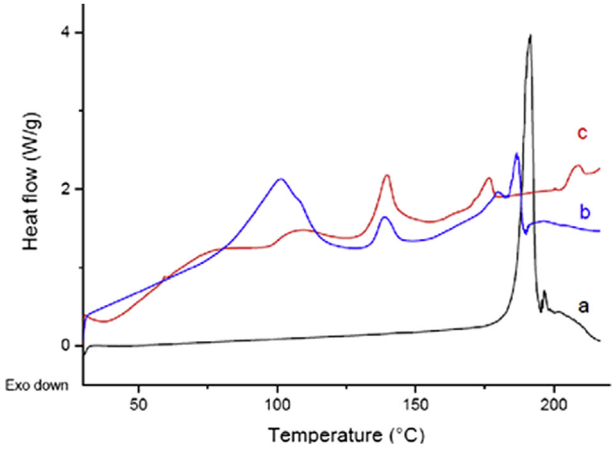



**Fig. 2.** DSC curves of: a) ascorbic acid (black line), b) cocrystal ASC (blue line) and c)catechin (red line).

pattern of the cocrystal ASC (Fig. 3, blue line) shows significant differences with respect to the PXRD profiles of the pristine materials (Fig. 3, black and red lines).

In particular, new reflection peaks (marked with blue stars and whose *d* values are reported in Fig. 3), absent in the (+)-catechin and L-(+)-ascorbic acid PXRD profiles, are evident in the cocrystal diffractogram, while the distinctive reflections of the individual constituents are missing, all pointing out the generation of a new solid form.

As further confirmation, the PXRD profile of the ASC cocrystal (Fig. 4d) was compared with the diffraction pattern obtained by merging the PXRD profiles of the two individual components (Fig. 4c). Juxtaposition of the PXRD profile of a mixture obtained by gentle grinding equal amounts of (+)-catechin and L-(+)-ascorbic acid powders (Fig. 4b) was accomplished as well. This superposition highlights again that the XRD profile of the cocrystal ASC is a unique diffraction pattern, and not just the overlap of the diffractograms of each individual components (Fig. 4a and e). However, unexpectedly, the PXRD pattern of the mixed powders (Fig. 4b) appears not exactly superimposable when compared with the pristine materials merged diffraction profile. The slight differences were more emphasized by the comparison of the physical mixture pattern with those of the pristine materials (Fig. 5). Indeed, the physical mixture XRD profile presents some reflection peaks, marked with green stars in Fig. 5, absent in the merged pattern and into that of the cocrystal.

This finding suggested that the solid grinding of the starting materials, even though briefly and lightly performed, may induce the formation of a new solid form. On this basis and in order to use a green method which avoids the employment of large amount of solvent, the cocrystal ASC was also prepared through Liquid Assisted Grinding (LAG). Here, LAG was performed by adding a small amount of ethanol during the grinding of the starting materials in an agate ball miller for 3 h. The PXRD pattern of the powder derived (ASC-LAG) is reported in Fig. 6, coupled with the pristine materials profiles. The patterns overlay shows again the formation of a new material, with a unique diffraction profile and with unique diffraction peaks (marked with pink stars and whose *d* values are reported in Fig. 6).

Moreover, the PXRD pattern of the cocrystal obtained (ASC-LAG) was compared to that originated from the ASC cocrystal produced by slurry method (Fig. 7). This comparison highlighted relevant differences between the cocrystals generated by the two methods, which were characterized by different diffraction profiles. This is not surprising as different polymorphs of a cocrystal can be obtained using different cocrystallization techniques (Eddleston et al., 2013; Aitipamula et al., 2009; Trask et al., 2004). Considering that mechanochemical methods, such as

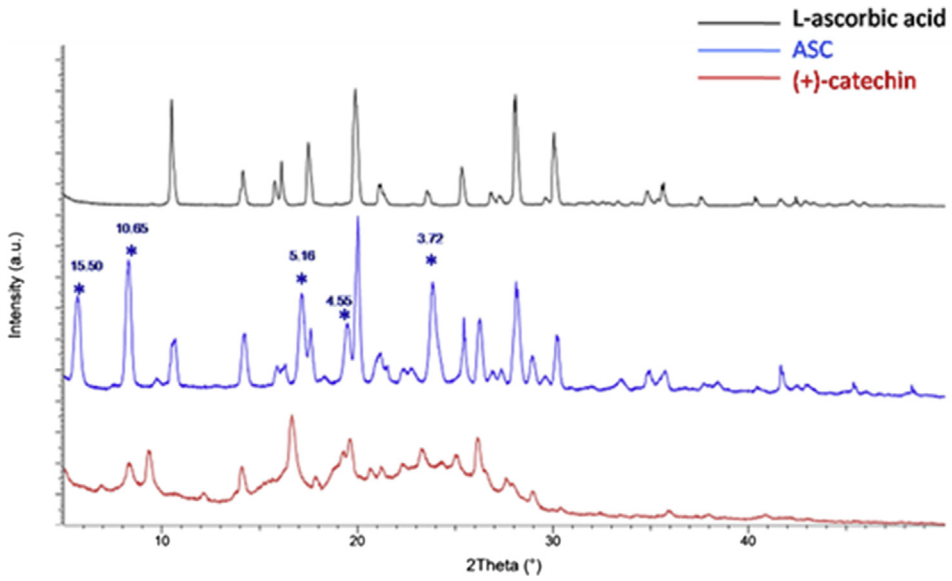



**Fig. 3.** PXRD patterns of catechin (red line), ascorbic acid (black line) and cocrystal ASC (blue line). Position of peaks reported in Å.

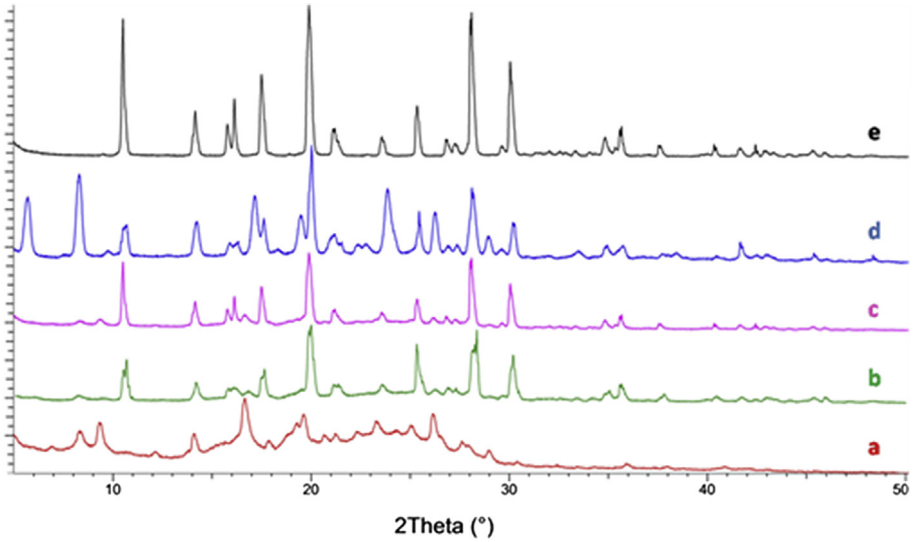



**Fig. 4.** PXRD patterns of: a) catechin (red line); b)physical mixture 1:1 M ratio of catechin and ascorbic acid (green line); c) the pristine materials merged patterns (pink line); d) cocrystal ASC (blue line); e) ascorbic acid (black line).

neat or liquid assisted grinding, allow to isolate metastable forms of cocrystals hardly attainable through solution-based techniques (Douroumis et al., 2017), the cocrystal ASC-LAG can be reasonably considered a new polymorphic form of the cocrystal ASC.

The existence of different polymorphs of the cocrystal composed by (+)-catechin and L-(+)-ascorbic acid in 1:1 M ratio can be plausibly ascribed to the different interaction modes between the precursors. Indeed, taking into account the large number functional groups present within the starting compounds, possibly contributing to different type of hydrogen bonds, several polymorphs, varying in their hydrogen bonds motifs (Aitipamula et al., 2014), can be generated. Some of the possible type of hydrogen bonds between the starting reagents and producing the different cocrystal ASC polymorphs are reported in Fig. 8. In particular, Fig. 8a and b show the hydrogen bonds formation between two proximal functional groups of ascorbic acid and a –OH groups as well as activate hydrogen atoms of (+)-catechin; this interaction mode has been already found in the crystal structure of (-)-epicatechin (Fronczek et al., 1984). Another possible interaction, presented in Fig. 8c, can be established between the lactonic group of ascorbic acid and two proximal –OH groups of catechin; a similar interaction has been described by Zhou et al. (2005). Finally, catechin and ascorbic acid can be linked together by means of hydrogen bonds between two proximal –OH group of catechin and two –OH functions of ascorbic acid (Fig. 8d), as in the case of the crystal structure of catechol (Brown, 1966).

However, the here obtained two polymorphic forms of the cocrystal composed by catechin and ascorbic acid in 1:1 M ratio, present the same water-solubility value, therefore only the ASC polymorph, prepared through slurry method, was used in the antioxidant studies.

### 3.2. Antioxidant performances

#### 3.2.1. Evaluation of antioxidant properties of tea infusion and commercial beverages

In the last years phenolic compounds received much attention due to their antioxidant properties, their abundance in the human diet, and their ability to prevent many diseases usually associated with the oxidative stress (Manach et al., 2004). Additionally, the interest of consumers, food science researchers and nutritional experts toward the knowledge of the

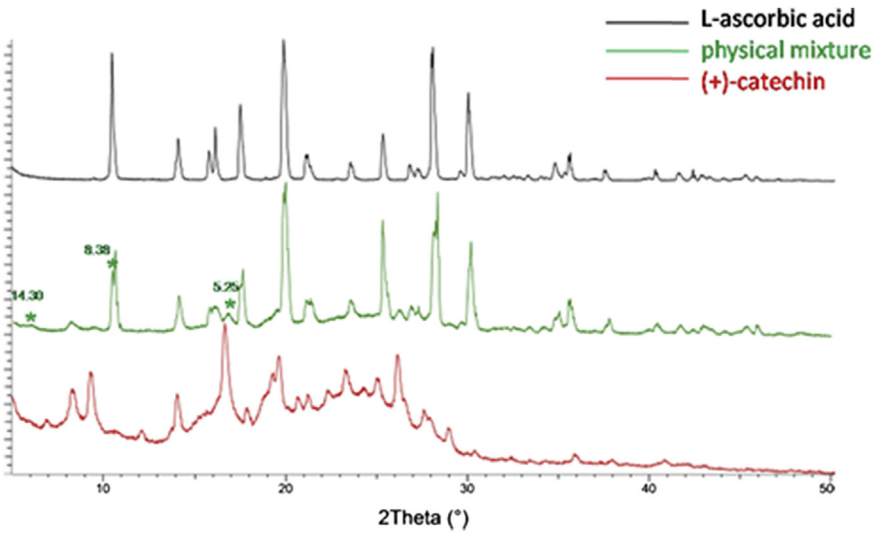



**Fig. 5.** PXRD patterns of catechin (red line), ascorbic acid (black line) and their physical mixture 1:1 M ratio (green line). Position of peaks reported in Å.

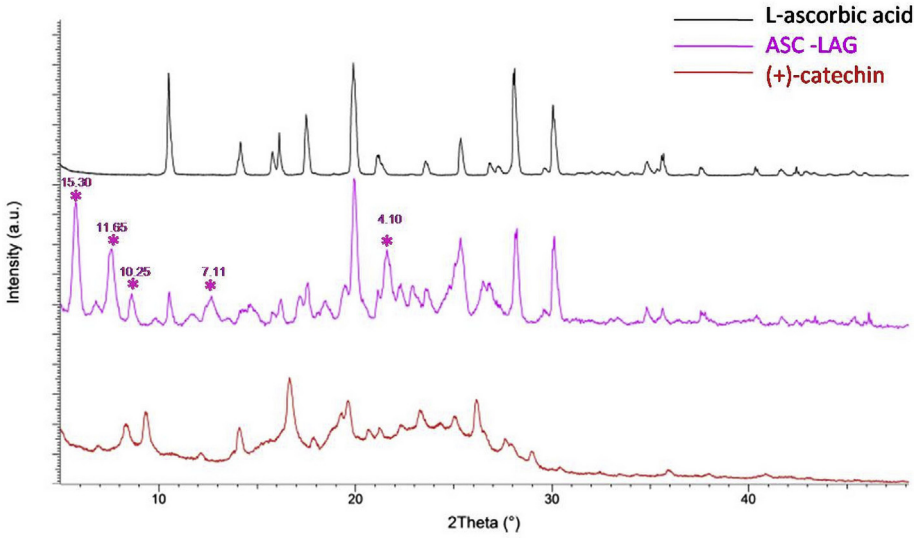



**Fig. 6.** PXRD patterns of ascorbic acid (black line), cocrystal ASC-LAG (pink line) and catechin (red line). Position of peaks reported in Å.

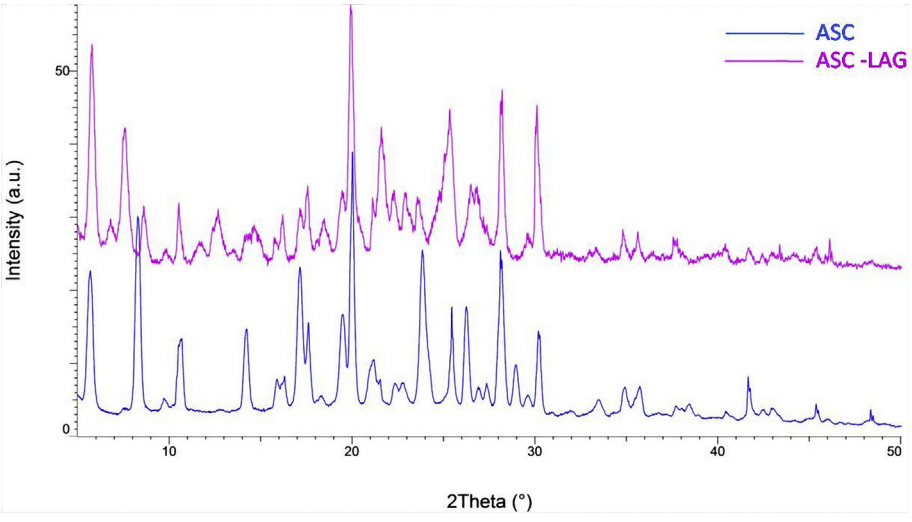



**Fig. 7.** PXRD patterns of ASC-LAG cocrystal (pink line) and ASC cocrystal obtained through slurry method (blue line).

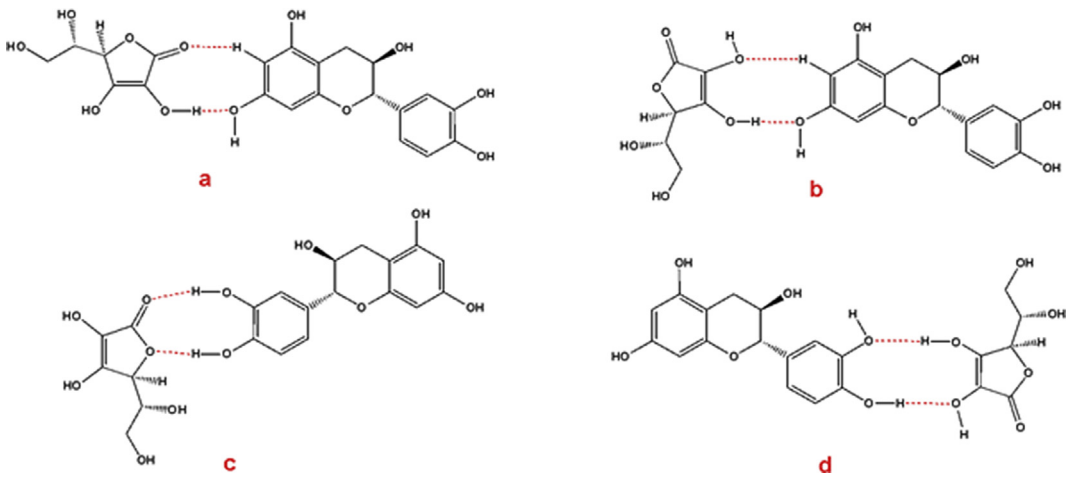



**Fig. 8.** Possible cocrystal ASC polymorphs due to the formation of different hydrogen bonded motifs: a) the ascorbic acid lactonic group forming dimer with the hydrogen bond donor and acceptor –OH and –H of catechin; b) two proximal –OH groups of ascorbic acid forming dimer with the hydrogen bond donor and acceptor –OH and –H of catechin; c) the ascorbic acid lactonic group forming dimer with two proximal –OH groups of catechin; d) two proximal –OH groups of ascorbic acid and two proximal –OH groups of catechin.

antioxidant capacity of the consumed foods rapidly increased in the last time (Huang et al., 2005). In this context, improving the antioxidant properties of IT and mostly ready-to-use PT and LT represented an exciting challenge. These features were investigated by the evaluation of total phenolic groups (DPG), total antioxidant activity (TAA) and scavenging performances against hydrosoluble (ABTS) and liposoluble (DPPH) radical species. Additionally, the ASC cocrystal was added in different concentration to all beverages to obtain reinforced-drinks deeply analysed concerning their antioxidant properties. All the results are reported on Table 1.

Total phenolic groups, performed by Folin-Ciocalteu procedure, were expressed in term of Gallic Acid Equivalent (GAE) per grams of cocrystal and GAE per litre of beverage, respectively. DPG of drinks were in the range 0.00191–0.01170 GAE $L^{-1}$, with significant differences between infusion tea and commercial beverages. Specifically, IT showed DPG almost one order of magnitude higher in comparison to the ready-to-use commercial beverages (PT and LT), highlighting that tea leaves represent an excellent source of antioxidant molecules (Das and Eun, 2018; Pérez-Burillo et al., 2018). This observation was confirmed by the study by Flores-Martinez et al., (2018) certifying that the flavonoid content decreases from infused tea to ready-use commercial beverages (hot infused tea > instantaneous preparations > iced tea > ready-to-use tea drinks). In addition, concerning ready-to-use teas, the major DPG amount was recorded in lemon-flavored tea, and this could be attributed to the antioxidant vitamins content of the concentrated lemon juice employed.

Additionally, the analysis of the TAA of drinks displayed values enclosed in a broad range (0.3516–1.0397 GAE $L^{-1}$), and also in this case infusion tea displayed the best performances.

Finally, the scavenger activity in organic and aqueous environments, expressed in term of $IC_{50}$ (the concentration of ASC that gives half-maximal response), showed remarkable differences. According to the literature data, the bottled PT and LT teas displayed highest scavenging activity in the aqueous medium ($IC_{50}$ values in the ABTS tests were one order of magnitude lower than DDPH assays) (Gramza-Michałowska et al., 2018). Additionally, the scavenger activity of IT appeared almost two times higher respect to PT and LT, in concordance with the data recorded in the DFG and TAA experiments.

### 3.2.2. Evaluation of antioxidant properties after ASC adding

In order to evaluate the cocrystal performances, the same experiments were performed by adding ASC to the tea beverages, allowing the preparation of reinforced teas employing different ASC concentrations (1.0 and 2.0 mg $mL^{-1}$, respectively) (Table 1). The results clearly indicated that the supplemented drinks exhibited improved antioxidant performances respect to their no added counterparts.

**Table 1**
Antioxidant properties of ASC, commercial and enriched beverages. Data represent mean ± RSD (n = 3), $p < 0.05$.

| Sample | DPG* | TAA* | $IC_{50}$** | |
|---|---|---|---|---|
| | | | DPPH radical | ABTS radical |
| ASC | 0.00670 ± 0.0008 | 0.0847 ± 0.0002 | 0.157 ± 0.005 | 0.01900 ± 0.0020 |
| LT | 0.00339 ± 0.0005 | 0.3867 ± 0.0012 | 0.068 ± 0.005 | 0.00195 ± 0.0018 |
| LT# | 0.00851 ± 0.0004 | 0.4009 ± 0.0008 | 0.051 ± 0.004 | 0.00180 ± 0.0015 |
| LT ## | 0.01785 ± 0.0005 | 0.8861 ± 0.0011 | 0.043 ± 0.004 | 0.00170 ± 0.0015 |
| PT | 0.00191 ± 0.0003 | 0.3516 ± 0.0005 | 0.063 ± 0.005 | 0.00375 ± 0.0021 |
| PT# | 0.00845 ± 0.0005 | 0.4577 ± 0.0012 | 0.043 ± 0.002 | 0.00310 ± 0.0011 |
| PT## | 0.01776 ± 0.0005 | 1.0321 ± 0.0015 | 0.037 ± 0.003 | 0.00270 ± 0.0009 |
| IT | 0.01170 ± 0.0004 | 1.0397 ± 0.0014 | 0.033 ± 0.001 | 0.00295 ± 0.0018 |
| IT # | 0.01546 ± 0.0008 | 2.0238 ± 0.0016 | 0.018 ± 0.002 | 0.00215 ± 0.0018 |
| IT## | 0.03931 ± 0.0008 | 5.1128 ± 0.0021 | 0.017 ± 0.002 | 0.00150 ± 0.0008 |

ASC = Ascorbic Acid Catechin cocrystal; LT = Lemon Tea; PT = Peach Tea; DPG = Total Phenolic Groups; TAA = Total Antioxidant Activity; $IC_{50}$ = ASC concentration giving half-maximal response.

* Express as Gallic Acid Equivalent (GAE) per g for cocrystal and GAE per L for the beverages.

** Express as mg $mL^{-1}$ for cocrystal and mL $mL^{-1}$ for the beverages.

\# ASC was added to a final concentration of 1.0 mg $mL^{-1}$.

\## ASC was added to a final concentration of 2.0 mg $mL^{-1}$.

In particular, a positive correlation between DPG and TAA against ASC concentrations was recorded and the increase in DPG and TAA followed the same order: IT > PT > LT. However, no linear correlation appeared between added ASC and scavenging performances. In this case, it is reasonable to suppose that antioxidant-radical reaction rate influenced the observed results. It is known that such processes occur more slowly in the presence of several hydroxyl groups, ring groups, bulky adducts and if the active molecule concentration is higher (Schaich et al., 2015). Mishra et al. (2012) showed that, when the amount of the

antioxidant species increased the reaction did not reach completeness in the 30 min commonly foreseen by the protocol and, therefore, the possible reduction of the $IC_{50}$ could not be properly appreciated.

The trend observed increasing the ASC concentration, appeared to be in accordance with scavenging properties against both DPPH and ABTS radicals. However, considering the antioxidant performances in relation to the kind of sample a certain discrepancy in the ABTS experiments was recorded. In the latter case, in fact, IT seemed to be less performing than LT ready-to-use drink. This dissimilar behaviour against the DPPH and ABTS radicals, can be due to several factors such as, type of solvent, nature of the radicals, different kinetics of reaction and interference of other substances usually present in commercial beverages, such as sweeteners and dyes. In particular, the different solvent (water against ethanol) in the ABTS test, seems to play a crucial role. In fact, the aqueous environment supports the formation of hydrogen bonds between polyphenols and water molecules, hindering the proton transfer to the radical species and thus reducing the scavenging capacity of the food matrix (Pérez-Jiménez and Saura-Calixto, 2006). Furthermore, Shalaby et al. (2016) showed that the presence of sweeteners can interfere with the absorbance of the solution. Ready-to-use teas contain considerable amounts of sugar and this may have influenced the $IC_{50}$ values towards the ABTS cationic radical.

Finally, in comparison to their unenriched counterparts, the addition of ASC at concentration level of 2.0 mg $mL^{-1}$ significantly improved the antioxidant values of LT and PT beverages. Total phenolic content increased more than three times and TAA of almost five times. These values appeared to be in the same order of magnitude of unenriched IT. Specifically, TAA tends to the values of the no added IT, DPG exceeds that of the reference, while the scavenging properties become comparable to those of the IT, both in aqueous and in organic environments.

## 4. Conclusions

The synthesis of ASC cocrystal, involving (+)-catechin and L-(+)-ascorbic acid, was exploited to obtain a new ingredient to be used for enriching tea infusions and tea-based ready-to-use beverages. The ASC cocrystal, obtained through slurry method, was exhaustively characterized through FT-IR, DSC and PXRD analysis, confirming the successful of the formation of a new adduct. Moreover, another polymorphic form of ASC cocrystal, was obtained through the LAG preparation method (ASC-LAG), clearly proving that polymorphism in cocrystal is depending on the synthetic routes followed for their preparation.

*In vitro* colorimetric assays, confirmed that the addition of ASC cocrystal improved the antioxidant features of teas (ready-to-use and infusion), although a linear correlation was not recognized. In comparison with infusion tea, ready-to-use teas showed poor antioxidant performances as consequence of the manufacturing phases characterized by a considerable loss of the antioxidant molecules. As the addition of ASC at a concentration of 2 mg $mL^{-1}$, led ready-to-use beverages to antioxidant properties similar to those of the not reinforced infusion tea, it can be concluded that the new additive can be suitable to restore the antioxidant features that the product lost during manufacturing. On the other hand, also ASC addition to IT improved the antioxidant performances of the drink, suggesting a possible limitation of the degradation processes induced by the brewing procedure.

Further studies seem to be necessary to underline the biological features of the functional products, as well as which polymorph accounts for better antioxidant properties and to what extent.

## Declarations

### *Author contribution statement*

U Gianfranco Spizzirri, Gabriele Carullo, Lucia De Cicco, Alessandra Crispini, Francesca Scarpelli, Donatella Restuccia, Francesca Aiello: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### *Competing interest statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

## References

Aitipamula, S., Chow, P.S., Tan, R.B.H., 2009. Dimorphs of a 1 : 1 cocrystal of ethenzamide and saccharin: solid-state grinding methods result in metastable polymorph. Cryst. Eng. Comm. 11, 889–895.

Aitipamula, S., Banerjee, R., Bansal, A.K., Biradha, K., Cheney, M.L., Choudhury, A.R., Desiraju, G.R., Dikundwar, A.G., Dubey, R., Duggirala, N., Ghogale, P.P., Ghosh, S., Goswami, P.K., Goud, N.R., Jetti, R.R.K.R., Karpinski, P., Kaushik, P., Kumar, D., Kumar, V., Moulton, B., Mukherjee, A., Mukherjee, G., Myerson, A.S., Puri, V., Ramanan, A., Rajamannar, T., Reddy, C.M., Rodriguez-Hornedo, N., Rogers, R.D., Row, T.N.G., Sanphui, P., Shan, N., Shete, G., Singh, A., Sun, C.C., Swift, J.A., Thaimattam, R., Thakur, T.S., Thaper, R.K., Thomas, S.P., Tothadi, S., Vangala, V.R., Variankaval, N., Vishweshwar, P., Weyna, D.R., Zaworotko, M.J., 2012. Polymorphs, salts, and cocrystals: what's in a name? Cryst. Growth Des. 12, 2147–2152.

Aitipamula, S., Chow, P.S., Tan, R.B.H., 2014. Polymorphism in cocrystals: a review and assessment of its significance. Cryst. Eng. Comm. 16, 3451–3465.

Badolato, M., Carullo, G., Perri, M., Cione, E., Manetti, F., Di Gioia, M.L., Brizzi, A., Caroleo, M.C., Aiello, F., 2017. Quercetin/oleic acid-based G-protein-coupled receptor 40 ligands as new insulin secretion modulators. Future Med. Chem. 9, 1873–1885.

Berry, D.J., Seaton, C.C., Clegg, W., Harrington, R.W., Coles, S.J., Horton, P.N., Hursthouse, M.B., Storey, R., Jones, W., Friščić, T., Blagden, N., 2008. Applying hot-stage microscopy to cocrystal screening: a study of nicotinamide with seven active pharmaceutical ingredients. Cryst. Growth Des. 8, 1697–1712.

Brown, C.J., 1966. The crystal structure of catechol. Acta Crystallogr. 21, 170–174.

Carullo, G., Aiello, F., 2018. Quercetin-3-oleate. Molbank 3, M1006.

Carullo, G., Perri, M., Manetti, F., Aiello, F., Caroleo, M.C., Cione, E., 2019a. Quercetin-3-oleoyl derivatives as new GPR40 agonists: molecular docking studies and functional evaluation. Bioorg. Med. Chem. Lett 29, 1761–1764.

Carullo, G., Durante, M., Sciubba, F., Restuccia, D., Spizzirri, U.G., Ahmed, A., Di Cocco, M.E., Saponara, S., Aiello, F., Fusi, F., 2019b. Vasoactivity of Mantonico and Pecorello grape pomaces on rat aorta rings: an insight into nutraceutical development. J. Funct. Foods 57, 328–334.

Chen, Z.Y., Zhu, Q.Y., Tsang, D., Huang, Y., 2001. Degradation of green tea catechins in tea drinks. J. Agric. Food Chem. 49, 477–482.

Cirillo, G., Puoci, F., Iemma, F., Curcio, M., Parisi, O.I., Spizzirri, U.G., Altimari, I., Picci, N., 2012. Starch-quercetin conjugate by radical grafting: synthesis and biological characterization. Pharm. Dev. Technol. 17, 466–476.

Cuevas-Valenzuela, J., González-Rojas, A., Wisniak, J., Apelblat, A., Pérez-Correa, J.R., 2014. Solubility of (+)-catechin in water and water-ethanol mixtures within the temperature range 277.6–331.2K: fundamental data to design polyphenol extraction processes. Fluid Phase Equilib. 382, 279–285.

Das, P.R., Eun, J.B., 2018. A comparative study of ultra-sonication and agitation extraction techniques on bioactive metabolites of green tea extract. Food Chem. 253, 22–29.

Douroumis, D., Ross, S.A., Nokhodchi, A., 2017. Advanced methodologies for cocrystal synthesis. Adv. Drug Deliv. Rev. 117, 178–195.

Dube, A., Ng, K., Nicolazzo, J.A., Larson, J., 2010. Effective use of reducing agents and nanoparticle encapsulation in stabilizing catechins in alkaline solution. Food Chem. 122, 662–667.

Eddleston, M.D., Sivachelvam, S., Jones, W., 2013. Screening for polymorphs of cocrystals: a case study. CrystEngComm 15, 175–181.

Ferreira-Nunes, R., Gratieri, T., Gelfuso, G.M., Cunha-Filho, M., 2018. Mixture design applied in compatibility studies of catechin and lipid compounds. J. Pharm. Biomed. Anal. 149, 612–617.

Flores-Martınez, D., Urıas-Orona, V., Hernandez-Garcıa, L., Rubio-Carrasco, W., Silva-Gutierrez, K., Guevara-Zambrano, M., Prieto-Cadena, J., Serna-Mendez, T., Muy-Rangel, D., Niño-Medina, G., 2018. Physicochemical parameters, mineral composition, and nutraceutical properties of ready-to-drink flavored-colored commercial teas. J. Chem. Article ID 2861541.

Food and Agriculture Organization of the United Nations (FAO), 2015. World tea Production and Trade. Current and Future Development. Retrieved from. http://faostat.fao.org.

Fronczek, F.R., Gannuch, G., Mattice, W.L., Tobiason, F.L., Broeker, J.L., Hemingway, R.W., 1984. Dipole moment, solution, and solid state structure of (-)-Epicatechin, a monomer unit of procyanidin polymers. J. Chem. Soc., Perkin Trans 2, 1611–1616.

Gramza-Michałowska, A., Kobus-Cisowska, J., Kmiecik, D., Korczak, J., Helak, B., Dziedzic, K., Górecka, D., 2016. Antioxidative potential, nutritional value and sensory profiles of confectionery fortified with green and yellow tea leaves (*Camellia sinensis*). Food Chem. 211, 448–454.

Hayat, K., Iqbal, H., Malik, U., Bilal, U., Mushtaq, S., 2015. Tea and its consumption: benefits and risks. Crit. Rev. Food Sci. Nutr. 55, 939–954.

Hocker, N., Wang, C., Prochotsky, J., Eppurath, A., Rudd, L., Perera, M., 2017. Quantification of antioxidant properties in popular leaf and bottled tea by high-performance liquid chromatography (HPLC), spectrophotometry, and voltammetry. Anal. Lett. 50, 1640–1656.

Huang, D., Ou, B., Prior, R.L., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53, 1841–1856.

Hung, W.-L., Wang, S., Sang, S., Wand, X., Wanga, Y., Hob, C.-T., 2018. Quantification of ascorbyl adducts of epigallocatechin gallate and GallocatechinGallate in bottled tea beverages. Food Chem. 261, 246–252.

Iemma, F., Puoci, F., Curcio, M., Parisi, O.I., Cirillo, G., Spizzirri, U.G., Picci, N., 2010. Ferulic acid as a comonomer in the synthesis of a novel polymeric chain with biological properties. J. Appl. Polym. Sci. 115, 784–789.

Junior, O.V., Dantas, J.H., Barão, C.E., Zanoelo, E.F., Cardozo-Filho, L., de Moraes, F.F., 2017. Formation of inclusion compounds of (+)catechin with β-cyclodextrinin different complexation media: spectral, thermal and antioxidant properties. J. Supercrit. Fluids 121, 10–18.

Karimi-Jafari, M., Padrela, L., Walker, G.M., Croker, D.M., 2018. Creating cocrystals: a review of pharmaceutical cocrystal preparation routes and applications. Cryst. Growth Des. 18, 6370–6387.

Kilmartin, P.A., Hsu, C.F., 2003. Characterisation of polyphenols in green, oolong, and black teas, and in coffee, using cyclic voltammetry. Food Chem. 82, 501–512.

Lambert, J.D., Lee, M.J., Diamond, L., Ju, J., Hong, J., Bose, M., Newmark, H.L., Yang, C.S., 2006. Dose-dependent levels of epigallocatechin-3-gallate in human colon cancer cells and mouse plasma and tissues. Drug Metab. Dispos. 34, 8–11.

Manach, C., Scalbert, A. Morand C., Remesy, C., Jimenez, L., 2004. Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr. 79, 727–747.

Mishra, K., Ojha, H., Chaudhury, N.K., 2012. Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. Food Chem. 130, 1036–1043.

Pérez-Burillo, S., Giménez, R., Rufián-Henares, J.A., Pastoriza, S., 2018. Effect of brewing time and temperature on antioxidant capacity and phenols of white tea: relationship with sensory properties. Food Chem. 248, 111–118.

Pérez-Jiménez, J., Saura-Calixto, F., 2006. Effect of solvent and certain food constituents on different antioxidant capacity assays. Food Res. Int. 39, 791–800.

Restuccia, D., Sicari, V., Pellicanò, T.M., Spizzirri, U.G., Loizzo, M.R., 2017. The impact of cultivar on polyphenol and biogenic amine profiles in Calabrian red grapes during winemaking. Food Res. Int. 102, 303–312.

Restuccia, D., Giorgi, G., Spizzirri, U.G., Sciubba, F., Capuani, G., Rago, V., Carullo, G., Aiello, F., 2019. Autochthonous white grape pomaces as bioactive source for functional jams. Int. J. Food Sci. Technol. 54, 1313–1320.

Rodrigues, M., Baptista, B., Almeida Lopes, J., Cruz Sarraguça, M., 2018. Pharmaceutical cocrystallization techniques. Advances and challenges. Int. J. Pharm. 547, 404–420.

Schaich, K.M., Tian, X., Xie, J., 2015. Hurdles and pitfalls in measuring antioxidant efficacy: a critical evaluation of ABTS, DPPH, and ORAC assays. J. Funct. Foods 14, 11–125.

Schultheiss, N., Newman, A., 2009. Pharmaceutical cocrystals and their physicochemical properties. Cryst. Growth Des. 9, 2950–2967.

Semalty, A., Semalty, M., Singh, D., Rawat, M.S.M., 2012. Phyto-phospholipid complex of catechin in value added herbal drug delivery. J. Inclusion Phenom. Macrocycl. Chem. 73, 377–386.

Sevukarajan, M., Thanuja, B., Sodanapalli, R., Nair, R., 2011. Synthesis and characterization of a pharmaceutical cocrystal: (aceclofenac: nicotinamide). J. Pharm. Sci. Res. 3, 1288–1293.

Shalaby, E.A., Mahmoud, G.I., Shanab, S.M.M., 2016. Suggested mechanism for the effect of sweeteners on radical scavenging activity of phenolic compounds in black and green tea. Front. Life Sci. 4, 241–251.

Smith, A.J., Kavuru, P., Arora, K.K., Kesani, S., Tan, J., Zaworotko, M.J., Shytle, R.D., 2013. Crystal engineering of green tea epigallocatechin-3-gallate (EGCg) cocrystals and pharmacokinetic modulation in rats. Mol. Pharm. 10, 2948–2961.

Smith, A.J., Kavuru, P., Wojtas, L., Zaworotko, M.J., Shytle, R.D., 2011. Cocrystals of quercetin with improved solubility and oral bioavailability. Mol. Pharm. 8, 1867–1876.

Spizzirri, U.G., Picci, N., Restuccia, D., 2016. Extraction efficiency of different solvents and LC-UV determination of biogenic amines in tea leaves and infusions. J. Anal. Methods Chem. Article ID 8715287.

Spizzirri, U.G., Restuccia, D., Chiricosta, S., Parisi, O.I., Cirillo, G., Curcio, M., Iemma, F., Puoci, F., Picci, N., 2011. Olive stones as source of antioxidants for food industry. J. Food Nutr. Res. 50, 57–67.

Steed, J.W., 2013. The role of cocrystals in pharmaceutical design. Trends Pharmacol. Sci. 34, 185–193.

Stewart, A.J., Mullen, W., Crozier, A., 2005. On-line high-performance liquid chromatography analysis of the antioxidant activity of phenolic compounds in green and black tea. Mol. Nutr. Food Res. 49, 52–60.

Stodt, U.W., Blauth, N., Niemann, S., Stark, J., Pawar, V., Jayaraman, S., Koek, J., Engelhardt, U.H., 2014. Investigation of processes in black tea manufacture through model fermentation (oxidation) experiments. J. Agric. Food Chem. 62, 7854–7861.

Trask, A.V., Motherwell, W.D.S., Jones, W., 2004. Solvent-drop grinding: green polymorph control of cocrystallisation. Chem. Commun. 890–891.

Tundis, R., Frattaruolo, L., Carullo, G., Armentano, B., Badolato, M., Loizzo, M.R., Aiello, F., Cappello, A.R., 2019. An ancient remedial repurposing: synthesis of new pinocembrin fatty acid acyl derivatives as potential antimicrobial/anti-inflammatory agents. Nat. Prod. Res. 33 (2), 162–168.

Vuong, Q.V., 2014. Epidemiological evidence linking tea consumption to human health: a review. Crit. Rev. Food Sci. Nutr. 54, 523–536.

Wang, Q., Wu, R., Wu, J., Dai, N., Han, N., 2015. A new alkaloid from Helianthemumordosicum. Magn. Reson. Chem. 53, 314–316.

Wang, R., Zhou, W., Jiang, X., 2008. Reaction kinetics of degradation and epimerization of EGCG in aqueous system over a wide temperature range. J. Agric. Food Chem. 56, 2694–2701.

Watanabe, T., Nishiyama, R., Yamamoto, A., Nagai, S., Terabe, S., 1998. Simultaneous analysis of individual catechins, caffeine, and ascorbic acid in commercial canned green and black teas by micellar electrokinetic chromatography. Anal. Sci. 14, 435–438.

Wouters, J., Quèrè, L., 2012. Pharmaceutical Salts and Cocrystals. RSC Publishing, USA, pp. 230–260.

Xu, J.Z., Leung, L.K., Huang, Y., Chen, Z.Y., 2003. Epimerization of tea polyphenols in tea drinks. J. Sci. Food Agric. 83, 1617–1621.

Xu, J.Z., Yeung, S.Y.V., Chang, Q., Huang, Y., Chen, Z.Y., 2004. Comparison of antioxidant activity and bioavailability of tea epicatechin with their epimers. Br. J. Nutr. 91, 873–881.

Yamashita, H., Hirakura, Y., Yuda, M., Teramura, T., Terada, K., 2013. Detection of cocrystal formation based on binary phase diagrams using thermal analysis. Pharm. Res. 30, 70–80.

Ye, J.-H., Augustin, M.A., 2018. Nano- and micro-particles for delivery of catechins: physical and biological performance. Crit. Rev. Food Sci. Nutr. 18, 1–17.

Yuliandra, Y., Zaini, E., Syofyan, S., Pratiwi, W., Novita Putri, L., SahraPratiwi, Y., Arifin, H., 2018. Cocrystal of ibuprofen–nicotinamide: solid-state characterization and in vivo analgesic activity evaluation. Sci. Pharm. 86, 23.

Zhou, F.-Y., Jian, S.-Z., He, X.-Y., Wang, Y.-G., 2005. (5R,5aR,8aR,9S)-5-(3,4-dihydroxy-5-methoxyphenyl)-9-fluoro-5,8,8a,9-tetrahydrofuro-[30,40:6,7]naphtho[2,3-d]-1,3-dioxol-6(5aH)-one acetone solvate. Acta Crystallogr. E61, 1374–1376.

Zhou, Z., Chan, H.M., Sung, H.H.Y., Tong, H.H.Y., Zheng, Y., 2016. Identification of new cocrystal systems with stoichiometric diversity of salicylic acid using thermal methods. Pharm. Res. 33, 10301039.