Development of polymeric formulations using (-)-Epicatechin as an Active Ingredient by Hot Melt Extrusion technique

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Abstract

Polymer formulations rich in flavonoids or other sort of antioxidants may be developed by using hot melt extrusion (HME). The corresponding compound consists of about 50 % wt. of an active nutraceutical ingredient, in this case, (-)-epicatechin, polymers GRAS type (Generally Regarded as Safe) and taste masking agents, if needed. The formulations have to be extruded at a temperature substantially below the melting point of the interest molecules which guaranties that a significant degradation of these molecules is avoided. In order to detect any possible chemical and thermal change on the formulations, the samples ought to be characterized by using laboratory techniques, such as Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA); polarized optical microscopy (POM), likewise, chemical evaluations may be carried out by using chromatographic and spectrophotometric techniques. The main contribution of this research is to shed light on the development of polymeric formulations based on (-)-epicatechin by using Hot Melt Extrusion which may be scaled-up and commercially launched either as nutraceutical products.

Keywords

Catechins; Epicatechin; Hot Melt Extrusion; Encapsulation, Nutraceuticals

Introduction

The human body relies among others on antioxidants. Exogenous antioxidants include phenolic compounds, alkaloids and steroids. An antioxidant may be defined as any substance that at low concentrations, and compared with oxidized substrate significantly delays or prevents its oxidation [1]. Oxidation refers to the loss of electrons, prooxidants and their compounds are those which can oxidize other molecules [2].

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Prooxidants may be in the form of free radicals or non-radical species. Free radicals are molecules that contain one or more unpaired electrons. The presence of an unpaired electron sometimes makes free radicals highly reactive due to the need to ‘‘pair up’’ the unpaired electron [3]. Tea, Coffee and Cacao are high-demand sources of natural antioxidants. Tea (*Camellia sinensis*), is rich in polyphenolic, for example black tea, which is an oxidized product of green tea, has approximately 15–25% polyphenols [4]. Green tea antioxidants have been well-recognised for a wide range of health benefits including protection against cancer [5] and other diseases [6]. Other the hand, Coffee (*Coffea Arabica) approximately* contributes to 64% of total antioxidant intake in the human diet, but its antioxidant activity has been shown to be considerably lower than that of tea. The other antioxidant-source mentioned above is cacao (*Theobroma cacao*), which has been rising interest in Colombia having a pivotal role in crop substitution. In fact, according to the Colombian national federation and the ministry of agriculture, there are *circa* 52,000 families work and rely on cacao growing. Apart from the conventional ways to intake antioxidants, there is a new trend of natural antioxidant consumption, the use and development of nutraceuticals.

In recent years have emerged new strategies for the preparation of either nutraceutical formulations. The challenge is to develop more efficient and technologically more profitable products. In this regard, the main concerns are the solubility and the stability of the acting active-ingredient compound in the final product. The use of solid dispersions is a rather cost-effective method to enhance the dissolution rate of poorly soluble ingredients. By definition an excipient is an inactive substance formulated along with the active ingredient (AI) to protect, support and improve its stability.

Despite the large amount of studies related to antioxidant presented in natural products and their use nutraceuticals, there is few information about the development and preparation of nutraceuticals by using twin screw extruders for Hot-Melt Extrusion. Therefore, the aim of this contribution was to make a comprehensive overview of antioxidants in Cacao, flavonoids, nutraceuticals, hot melt extrusion and analytical techniques associated with the topics herein.

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Antioxidants in Cacao (Theobroma cacao)

Cacao (*Theobroma cacao*) is a small and evergreen tree that belongs to the family of *Malyvaceae* which is originally from in central and South America, but it has been spread all over the world, being also cultivated in Asia and Africa. Its seed and beans are used to making any kind of food products including cocoa mass, cocoa powder, chocolate, butter and other sort of desserts and beverages [7-8].

It is worth pointing out that there is a difference between cacao and cocoa, Cacao is the bean that comes from the cacao tree and are fermented and dried. When they are already cleaned and roasted, the coming products are often referred to as cocoa. According to the International cocoa organization (ICCO), there was a world production of approximately 4645 thousand tonnes in 2014, being Ivory Coast, Ghana, Indonesia, Nigeria and Brazil as the main producers; whereas The Netherlands and Germany are the main grinders. In the Americas the main producers are Brazil, Ecuador and Peru, and United States and Brazil as grinders. With regard to the Colombian production, the country produced about 60.5 tonnes in 2017, its market is low and is based basically internal consumption. Pursuant to Colombian official figures, majority of Colombian cacao is cultivated in small farms and few companies have implemented high technology means [9-10].

Criollo, Forastero, Trinitario and Nacional are deemed as the most cultivated varieties around the world. The Criollo tree originates in Mexico and Central America and is also sown in these areas. Forastero largely cultivated in Africa and represents roughly 80% of the global production. The trinitario is a crossbreed between the Forastero and Criollo is harvested in Central and South America and Asia, and nacional is cultivated in the west of the Andes, it is difficult to grow but it has excellent aroma. The products so-called specialty chocolates are the ones that are made by solely a variety of cacao, while, ordinary chocolate are produced with cheap cacao bean from several places and more than one cacao variety. Bitterness and astringency are important components of cocoa flavor. The former is related in part to purines primarily theobromine caffeine and also 2,5-diketopiperazines. The latter is attributed to phenolic constituents, where flavan-3-ols play

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a pivotal role especially catechins and epicatechins which are the main phenolic substance found in different Cacao varieties (Figure 1) and of their physical properties are summarized in Table 1 [11-14].

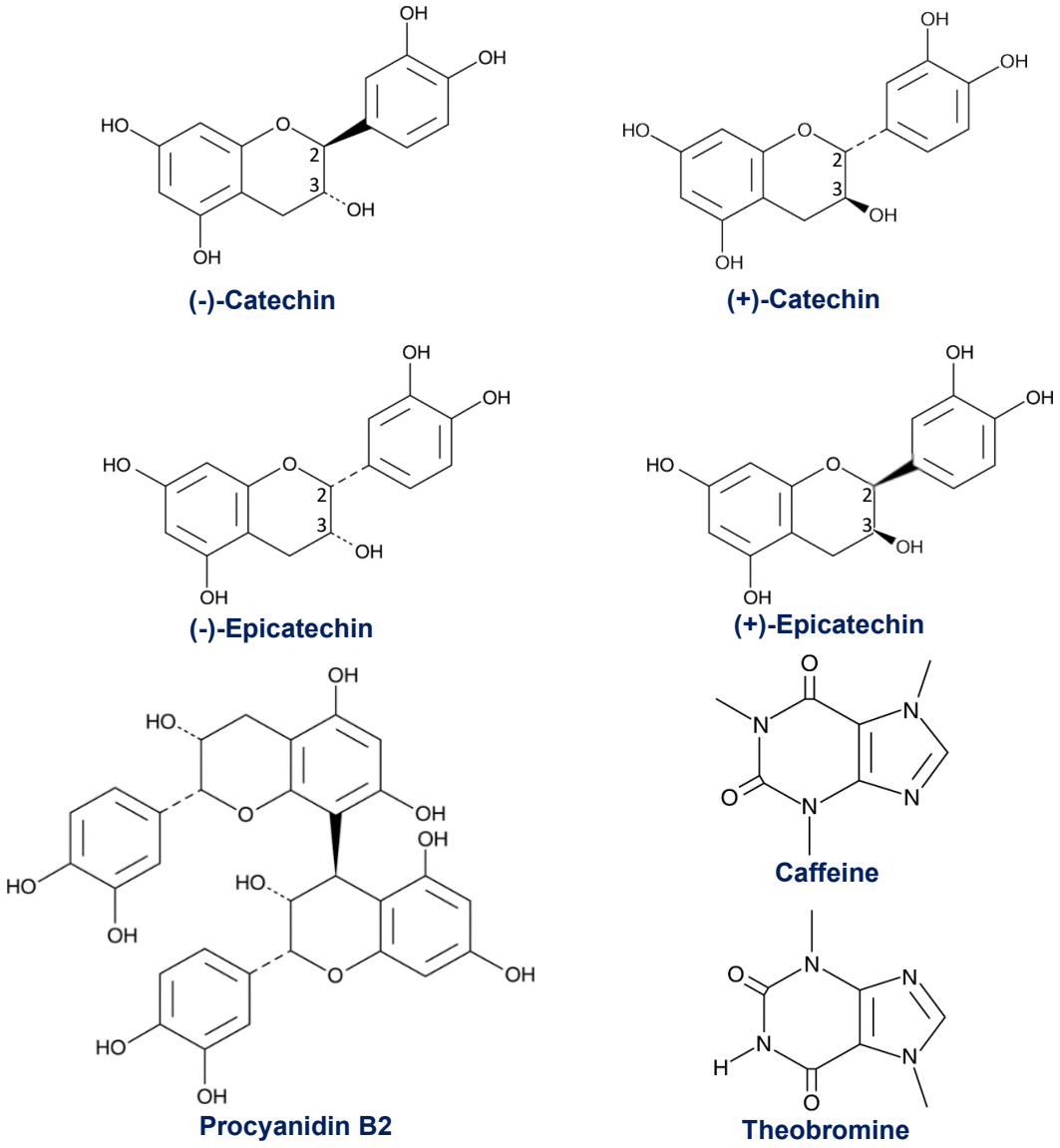

Figure 1: Structures of the catechin /epicatechin enantiomers, procyanidin and purines

It has been comprehensively studied that phenolic cacao ingredients possess strong anti-oxidative properties among then, effects against artery vascular diseases, cancer and inflammatory processes in the human body. The polyphenols content depends on each cacao variety, time of the year, sunshine, rainfall, age of the plant and other kind of factors.

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Molecule	CAS #	Tg (°C)	Tm (°C)	Tdeg (°C)	T boiling (°C)
Theobromine	83-67-0	---	Sublimes 290-295	---	357
Caffeine	58-08-2	---	Sublimes 178	---	236
(+)-Catechin	154-23-4	47	175-177	290	630.4
(-)-Epicatechin	490-46-0	---	240-250	---	630.4
Procyanidin B1	20315-25-7	---	---	---	955.3
Procyanidin B2	29106-49-8	---	197-198	---	955.32

Table 1: Physical properties of some of the main compounds found in Cacao

It has been comprehensively studied that phenolic cacao ingredients possess strong anti-oxidative properties among then, effects against artery vascular diseases, cancer and inflammatory processes in the human body. The polyphenols content depends on each cacao variety, time of the year, sunshine, rainfall, age of the plant and other kind of factors.

All of these phenols come from the shikimate and phenylpropanoids metabolic pathways. Indeed, phenylpropanoids are the largest group of secondary metabolites produced by plants mainly to counteract biotic or abiotic stresses, where the protective action is related to their antioxidant and free radical scavenging properties [15-17].

For instance Epicatechin is a plant secondary metabolite which belong to the group of flavan-3-ols or flavanols, a subclass of flavonoids. This molecule has two chiral centers at the carbon 2 and 3 of the flavonoid backbone; hence and as it is illustrated in Figure 1, there are four diastereomers with different spatial configurations which hold distinct physiological effects. (+)-catechin and (-)-epicatechin (EC) are predominantly biosynthesized, conversely (-)-catechin and (+)-epicatechin, often referred to as ent-catechin and ent-epicatechin are rarely found in nature. High temperature and/or an increasing pH value are able to induce an epimerization reaction (change of one chiral carbon to another). The abundance of either (+)-catechin or (-)-epicatechin (EC) hinges on the cacao variety, geographical location, age of the plant among others. Procyanidins

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are oligomeric compounds formed by catechin and epicatechin molecules which may be found in cacao, apples, wine, and flavonoid-rich natural products [18-19].

Albeit there are different values pertaining to the recommended amount of flavonoids that should be consumed per day, the range of 20-200mg day is the most found in literature [20-22]. There is no a unify value, since there are thousands of different flavonoids, like isoflavones, flavonols, flavones, chalcones amongst other, few of them possess the same set of biological activities. For instance, some flavonoids are cytotoxic, others are not. Hence, there may not be an optimal flavonoid consumption level without defining exactly which specific compounds are referring to. The best way of having a proper flavonoid intake diet is by eating fruits and vegetables. With regard to nutraceuticals, the best is do not exceed the range mentioned above because taking flavonoids as concentrates, extracts or in purified form seems may lead to harmful effects [23-26].

Nutraceuticals Their Market and Its Benefits

Nutraceuticals are a variety of foods that provides positive health benefits and also offers a nutritional value. Other terms related are: dietary supplements, functional food or fortified food [27-28]. According to National Institutes of Health, Office of Dietary Supplements (ODS): Dietary supplements that may be taken in form of tablets, powders or liquids, are products intended to supplement the diet, by increasing the total daily intake and contains among others: vitamin and mineral products, botanical extracts and/or amino acids products [29].

The main advantage of nutraceutical over traditional medicine relies on either the mitigation or the elimination of side effects. Nutraceuticals may be classified as nutrients, herbals, dietary supplements and dietary fibers. There are three types of nutraceuticals, the first ones mainly contain substances of primary metabolism, like carbohydrates, vitamins, and proteins, the second type is connected to secondary metabolites, such as flavonoids, terpenes, polyphenols, organic acids, pigments, etc. The third type, which is emerging is the so-called functional food or pharmafood that are based on the addition of

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special ingredients with certain physiological properties to ordinary foods. A broad classification of Nutraceutical may be seen in Figure 2.

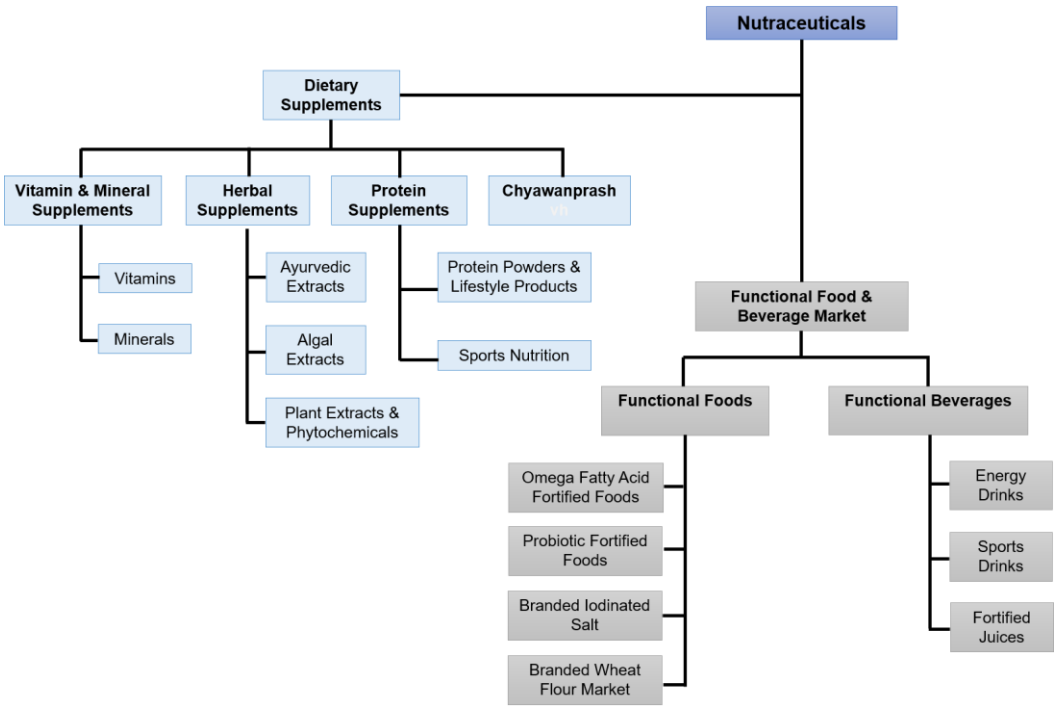

Figure 2: Classification of Nutraceuticals

Currently nutraceuticals represent a fast growing segment in food industry. The global nutraceutical market is approximated 117 billion USD, the US is the leader nutraceutical market in the world, followed by the European Union and India. A result is a considerable increase in research and development. "The nutraceutical industry represents a dynamic, evolving entity that offers novel opportunities to merge scientific discovery with growing consumer interest in health-enhancing foods" [30].

Hot Melt Extrusion (HME)

HME technique offers many advantages including the operation without organic solvents, the speed and continuity of the process, which means, it is more economical compared to other techniques, reducing the number of processing steps. During HME process, a material is fed and transported through a barrel that contains one or two rotating screws [31]. The material is melted and mixed under high temperature and pressure, finally is forced through a die to produce polymeric products of particular shape and density. A

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number of applications has been developed by HME processing, which includes, solid dispersions, microencapsulation, taste masking, drug delivery systems, medical devices incorporating drugs, among others. There are different types of solid dispersions but only three can be achieved by HME, crystalline solid dispersion, amorphous solid dispersion, and solid solutions. Crystalline solid dispersions are systems wherein the crystalline drug substance is dispersed into an amorphous carrier matrix. The Differential Scanning Calorimetry profile for such a system is characterized by the presence of a melting endotherm corresponding to the crystalline Active Ingredient and a characteristic glass transition temperature corresponding to the amorphous polymer matrix. They are generally designed to achieve controlled AI release profiles for highly soluble drugs. [32].

Literature was found regarding the development of dietary supplements based on catechins [33-35], but few works have been conducted in (-)-epicatechin encapsulation by HME, where there is a current potential of R&D and innovation.

The main objective of this work is to use the Hot Melt Extrusion technique to develop a polymer formulation with EC as an active ingredient. This technology is selected, since it has proven to improve the bioavailability of the AI by its effective and efficient encapsulation in polymer melts. Therefore it is possible to mask the characteristic bitter taste of EC, to protect the shelf life of the supplement, and to enhance the dissolution profile to obtain an efficient delivery in the gastrointestinal tract.

Different techniques can be used for characterization of solid dispersions, such as, film casting (FC), polarized optical microscopy (POM), batch melt mixer (BMM), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), infrared spectroscopy (FTIR), dissolution test (DT), High performance liquid chromatography (HPLC); among others.

In a previous research stage, two nutraceutical formulations based on (-)-Epicatechin, as an AI, were developed and published in USPTO and PCT patent applications [36-37]. The main objective of the present research work is to develop polymeric formulations achieved and effective encapsulation (*In Vitro* studies) of the active ingredient, (-) - Epicatechin, in a polymer matrix, that could be used in food applications.

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Experimental

Materials

(-)-Epicatechin 90% (EC) was purchased from Changsha Sunfull Bio-tech Co.,Ltd. (China). Aqualon™ N7 ethylcellulose (AN7), was generously donated by Ashland (Covington, Kentucky, USA), Ethocel ™ Standard 10, ethylcellulose (ES10), was generously donated by DOW (Midland, Michigan, USA), Kollidon® SR polyvinyl acetate and povidone (KSR) (8:2) was generously donated by BASF Company (SE, Ludwigshafen, Germany) and Eudragit L100- methacrylic acid- ethyl acrylate (L100) was generously donated by EVONIK Industries (Darmstadt Germany) . These materials were thermally characterized by TGA and DSC. Methyl Alcohol Anhydrous (MeOH) with a purity of 99.9% and 37% hydrochloric acid were purchased from Mallinckrodt Chemicals. N,N-Dimethylformamide (DMF) 99.8%PS, pure tri-Sodium Phosphate 12-hydrate and pure Sodium Hydroxide were supplied by Panreac, ITW Companies. HPLC grade Acetonitrile and Glacial Acetic Acid were from J.T Baker and Merck respectively. Ultrapure water was used throughout the work.

Methods

Film casting (FC)

Films of about 110 µm thickness were prepared using a bar applicator PA-5567 BYK-Gardner GmbH (Geretsried, Germany) as casting device. Different polymer formulations of EC and polymers AN7, KSR and L100 were dissolved using DMF and MeOH. The ratio of concentration for the polymer formulations were 1:15 and controls (pure EC) were 1:1 respectively. Solvent was evaporated in a vacuum drying cabinet at 40°C and 100 mbar overnight. This technique was suitable to predict the compatibility of EC in the polymeric matrix and combinations of polymers (Screening). A film of 100% EC is prepared as control.

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Polarized light optical microscopy (POM)

For examining the surface morphology of the polymer formulations and check for the presence of crystalline or amorphous phases, a polarized optical microscope (Leitz Laborlux 12 Pool S. Germany) equipped with a Nikon D60 digital camera, 10.2 MB-pixel resolution was used.

Preparation of EC formulation by Batch Melt Mixer (BMM)

A Haake (PolyLab QC, Thermo Scientific) equipped with two counter-rotating Roller rotors (R600) was used to prepare the polymer formulation that showed the best compatibility and the most appropriated % of EC load and is possible to estimate the processing window that will be used in the HME stage.
Near to 57 g of polymer formulation were mixed in the chamber, a design of experiments (DoE) is made to define the processing conditions, having two rotational speeds (70 and 100 rpm), two processing temperatures (130 and 150°C) and two polymers (AN7 and ES10) as the factors and the EC release at 4 hours in gastrointestinal conditions simulated pH medium for release profile as the response.

The formulations were processed at a temperature substantially below the melting point of EC (247.17 °C) to avoid significant degradation of EC.

After 150 seconds of mixing, the samples were taken out of the Haake mixer and cooled down to room temperature.

Thermal Gravimetric Analysis (TGA)

A thermogravimetric analyzer (TA Instruments Q500) as used to perform the thermal characterization of materials. Measurements were made according to the standard ASTM E 1131: In a ramp heating test, 10-15 mg of sample was placed in an aluminum pan and heated from room temperature to 350°C at a heating rate of 10 °C/min. This technique was employed to assess the thermal stability (degradation temperature) of pure EC, polymers, and polymer formulations based on EC.

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Preparation of EC polymer *formulation by HME using twin screw extruder (TSE)*

The Leistritz co-rotating twin screw extruder Nano 16 was used to processing of formulations that showed the best results in the characterization techniques described before. The Nano 16 has a screw diameter of 16 mm and a L/D of 26.25. The temperature profile was set 160°C near the feed zone to 145°C at the die.

The screw rotation speed was set to 250 rpm. The die diameter used was 4 mm.

The polymer formulation was mixed, dried, and fed into the extruder via a powder volumetric Feeder (K-Tron Soder, NJ, USA).

After extrusion, the sample was collected, cooled down to room temperature, and subsequently milled using a PQ-N2 Planetary Ball Mill (Across International) and sieve to obtain a final particle size of 425µm.

Differential Scanning Calorimetry (DSC)

Measurements of the samples were conducted following the standard ASTM D 3418 in a TA Instruments DSC Q200 equipped with a cooling system. The chamber was flushed with nitrogen at a flow rate of 50 ml/min. The samples (about 5-7 mg) were heated from -30°C to 270°C at a flow rate of 20°C/min and quenched during the cooling down cycle to analyze the thermal transitions. The thermal transition temperatures were measured in the first heating cycle due to the EC immediate degradation after melting. DSC was used to evaluate the physical state of the polymer formulations after BMM processing and stability of the sample prepared by HME right after processing and 6 months later.

FTIR Infrared Spectroscopy

A Thermo Scientific Nicolet 6700 FT-IR was used to collect the FTIR spectra of the samples over the range 400 – 4000 cm-1 and each spectrum was an average of 16 scans. KBr was used to form disks with samples. FTIR spectrum of pure EC has a fingerprint region (1800-700 cm-1) that is suitable for spectral analysis of samples, because the targeted functional groups appear in this region [38]. This technique is used to collect the

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FTIR spectra of the samples and evaluate possible changes in chemical structure of EC after processing.

EC content in polymer formulation by HPLC

EC quantification in polymer formulation was performed using an Agilent Technologies HPLC 1200 with Agilent Zorbax Eclipse XDB-C18 column, 150 mm, 4.6 mm x 5 µm. The mobile phase, composed of 90% Acetic acid solution pH 2.55 and 10% Acetonitrile, was delivered at 1.5 ml/min. The column temperature was set to be 30°C, 10 µl of sample was injected into the column. The EC was monitored using a DAD detector at 280nm. Data was acquired and processed using Agilent Chemstation Data Analysis software. HPLC is used for control of formulations and for EC quantification in the In Vitro studies

In vitro EC release

A certain amount of the polymer formulation containing 200 mg of EC with a particle size 425µm (Mesh 40) was added to 750 mL of HCl 0.1 M pH 1.2 medium prepared to simulate enzyme-free gastric dissolution at 37 ± 0.5°C with a stirring speed of 100 rpm. After 2 hours, an approximate volume of 100 mL of a Phosphate Buffer Solution 0.6 M was added, then the volume was adjusted to 900 mL with ultrapure water and the solution was adjusted to pH 6.8 with 0.2 M NaOH or 0.2 M HCl, simulating enzyme-free intestinal dissolution for another 2 hours. Aliquots of 4 mL solution were withdrawn every hour and filtered through 0.45 µm PTFE filters for HPLC analysis. The retired volume was replaced by the same medium. The experiments were conducted in triplicate.

Despite specific guidelines have not been proposed for the dissolution testing of nutraceutical products, these studies have been considered as an important quality evaluation parameter [39], thus the criterion for a minimum release of EC was set to > 80% over a 4 hours period, to ensure that the EC could be assimilated properly by the human body (simulated conditions). This threshold estimation was discussed with experts of the food industry. [36-37].

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Results and Analysis

Materials analysis by Differential Scanning Calorimetry (DSC) and Thermogravimetry (TGA)

Melting point (Tm) of EC was around 247.2°C, its degradation temperature was around 248 °C, and its heat of fusion (ΔHm) was 175.9 J/g. AN7 has three glass transition temperatures (Tg) around 123,8 ; 164,3 and 196,7°C, ES10 has a Tg around 174.9 and a Tm at 127.6°C, for KSR the Tg was around 42.6°C and for L100 a Tg around 81.2°C.

Film casting and polarized optical microscopy

FC is a screening technique suitable to predict the compatibility of EC in the polymeric matrix and is carried out before BMM, so this technique is used to reject AI-polymer systems that are not compatible [40]. Also, it is used to find the highest content or loading of EC in the formulations.

According to these criteria, the polymer formulations that showed the best results were the following:

Polymer Formulation 1(PF1): 50%wt.EC-30%wt.AN7-10%wt.KSR-10%wt.L100

Polymer Formulation 2(PF2): 50%wt.EC-30%wt.ES10-10%wt.KSR-10%wt.L100

The control sample containing pure Epicatechin at 90% formed an opaque film, due to the large crystals of EC and the polymer formulations (PF) films were morphologically stable within a period of 4 weeks. These findings suggested that AN7, ES10, L-100 and KSR were suitable polymeric materials for EC, since EC showed good compatibility with these polymers.

Figure 3 shows the morphology of the films after 24 hours and 4 weeks, there are no observable changes by the POM technique among the films evaluated 4 weeks later, however when realizing the DSC (Figure 4) it is observed that, when formulations are heated, a recrystallization of the EC takes place, which subsequently melts while heating continues, and it was observed that the amount of crystalline EC in the formulation PF2

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(ΔH: 24.19J/g) is higher than in PF1 (ΔH: 15.36J/g), this results could explain the granulations observed in PF2 formulations. However, XRD studies will be carried out to analyze morphology of films more in detail.

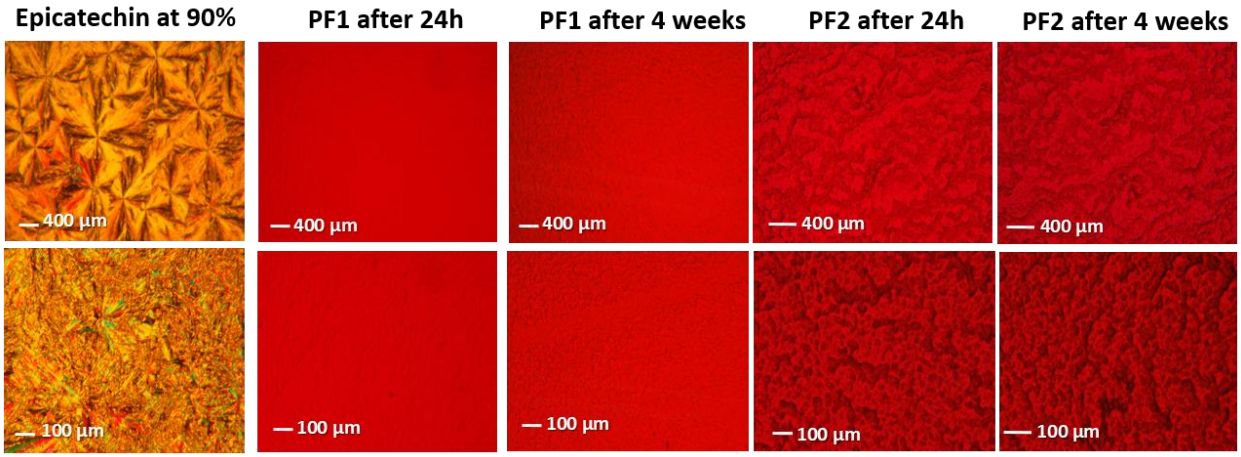

Figure 3: Micrographs of PF1 and PF2

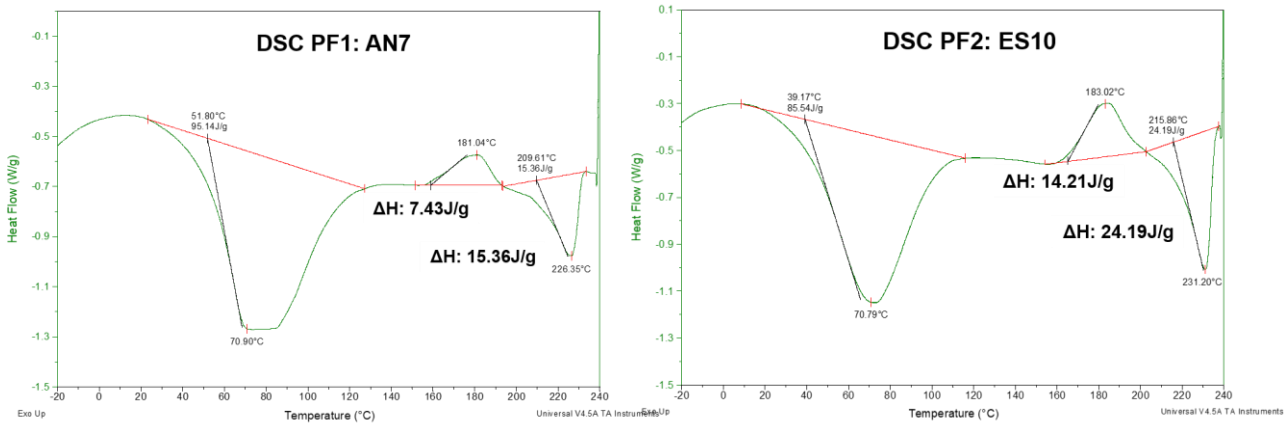

Figure 4: DSC of polymer formulations prepared by Film Casting

Preparation of EC polymer formulations by Batch Melt Mixer (BMM)

Figure 5 shows the behavior of the torque and melt temperature in the batch melt mixer (BMM) of the prepared samples. It can be seen that the final melt temperature was higher than the set temperature, due to the viscous dissipation.

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As expected, the torque decreases as the processing temperature increases from 130°C to 150°C for PF1 and PF2, suggesting that dispersion process is better under 150°C condition due to a faster stabilization. This is the first approach to HME conditions.

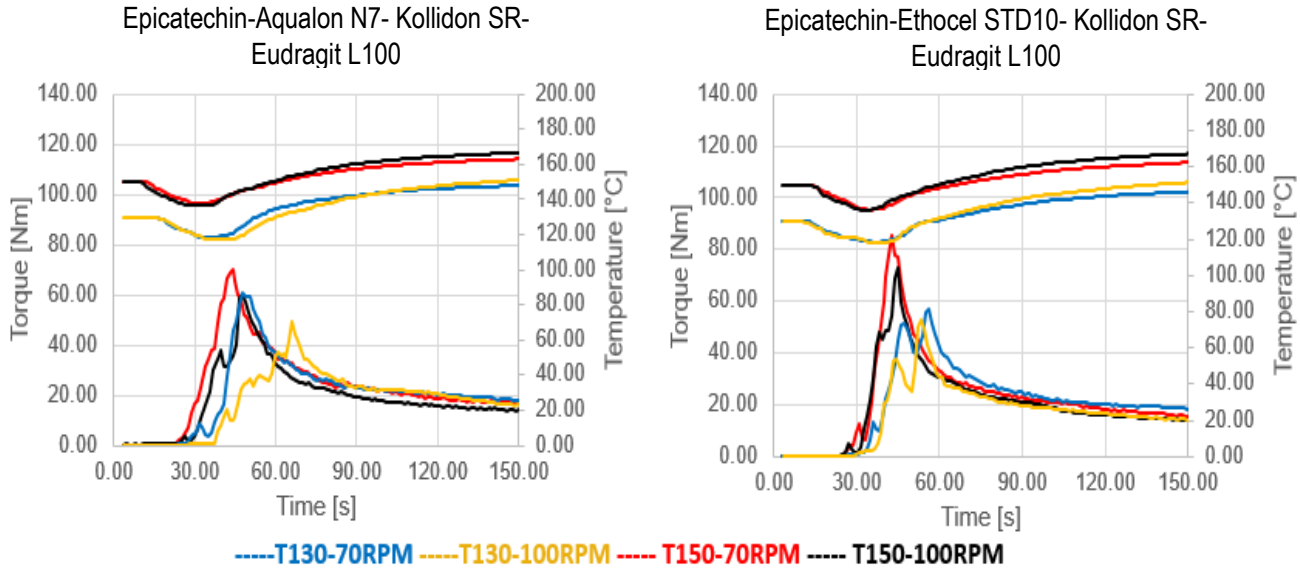

Figure 5: Torque and melt temperature of PF1 and PF2 during BMM process

In vitro EC release of polymer formulations prepared by BMM

Dissolution profile of pure EC and polymer formulations PF1 and PF2 can be seen in figure 6.

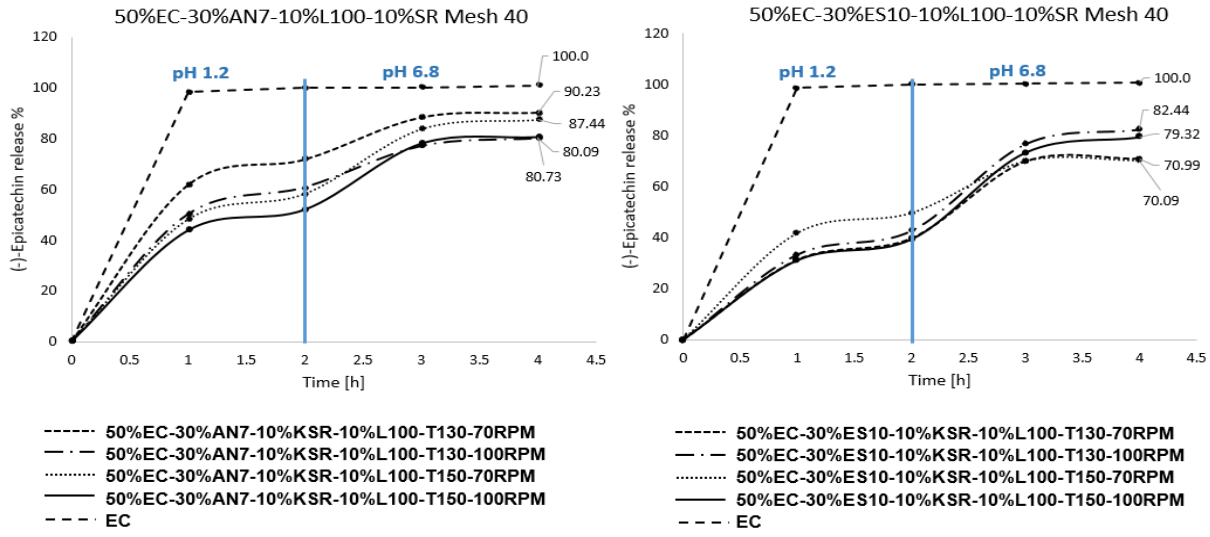

Figure 6: Release Profile in pH 1.2 and pH 6.8 at 37°C for PF1 and PF2

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Polymer formulation in PF1 promoted solubility and allowed EC release above 80% in 4h as required, but PF2 formulation did not meet the release requirement.

At this stage, PF1 samples: 50%EC-30%AN7-10%KSR-10%L100, continue being analyzed because they meet release criterion.

Figure 7 shows DSC analysis for physical mixture of polymers (PM), pure EC and PF1 processed by BMM. Two thermal transitions appeared in polymer formulations: A Tg corresponding to polymeric materials and a Tm of EC, suggesting the formation of crystalline solid dispersions (Table 2).

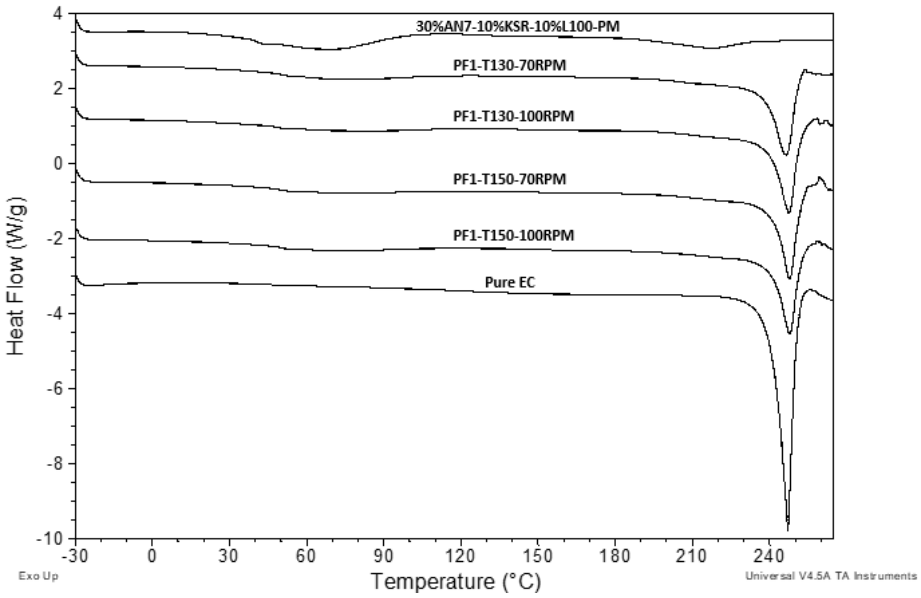

Figure 7: DSC thermograms of polymers PM, Pure EC and PF1 prepared by BMM at DoE conditions

Sample	Tg [°C]	Tm [°C]	ΔHm [J/g]
30%AN7-10%KSR-10%L100- PM	41 / 125 /200	---	---
PF1-T130-70RPM	49.34	246.52	121.8
PF1-T130-100RPM	49.47	247.58	121.0
PF1-T150-70RPM	49.94	247.83	120.6
PF1-T150-100RPM	50.55	247.84	119.6
Pure EC	---	247.17	175.7

Table 2: Transition temperatures and enthalpies of formulations prepared by BMM

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Preparation of EC formulation by HME using twin screw extruder (TSE)

Figure 8 shows the DSC thermograms of the 50%EC-30%AN7-10%KSR-10%L100 polymer formulations prepared by Physical mixture (PM), BMM, and HME. It can be seen that EC continues still in crystalline form after BMM and HME processing, and every sample showed one Tg and one Tm, suggesting the formation of crystalline solid dispersion, whereas the PM showed several thermal transitions due to unprocessed polymers and EC. The heat of fusion (ΔHm) of the PF1 processed by HME (114.9 J/g) was lower than ΔHm of the same formulation processed by BMM (119.6 J/g), this results can be explained because of the better dispersive and elongational mixing in HME process.

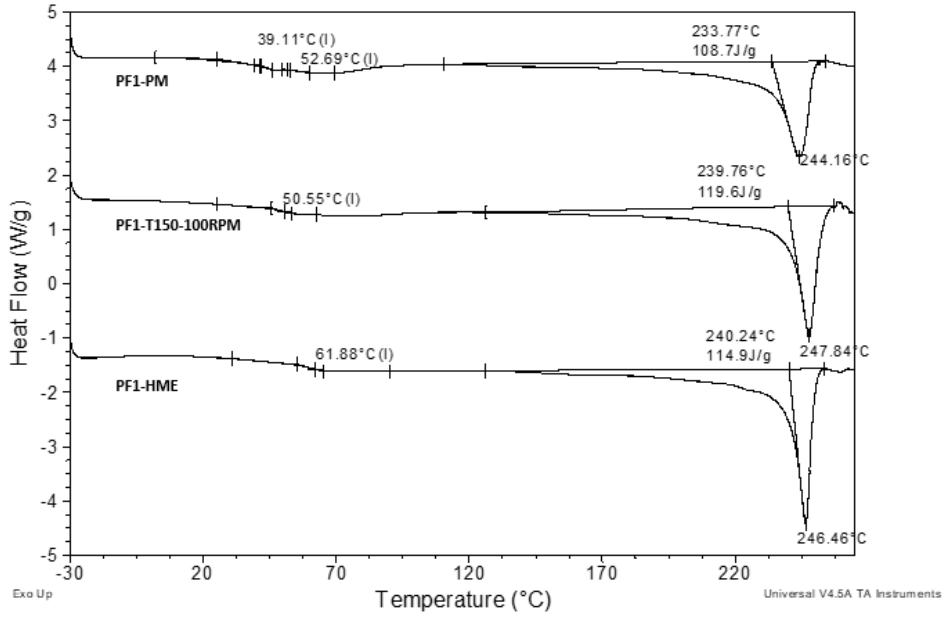

Figure 8: DSC thermograms of PF1 prepared by PM, BMM and HME

FTIR infrared spectroscopy

FTIR analysis of PF1 prepared by BMM and HME, pure EC and PM of polymers were conducted to determine the interactions between polymers and EC. EC spectrum has a fingerprint region (1800-700cm^{-1}) that is suitable to spectral analysis of samples, because the targeted functional groups appear in this region, and are shown in Figure 9.

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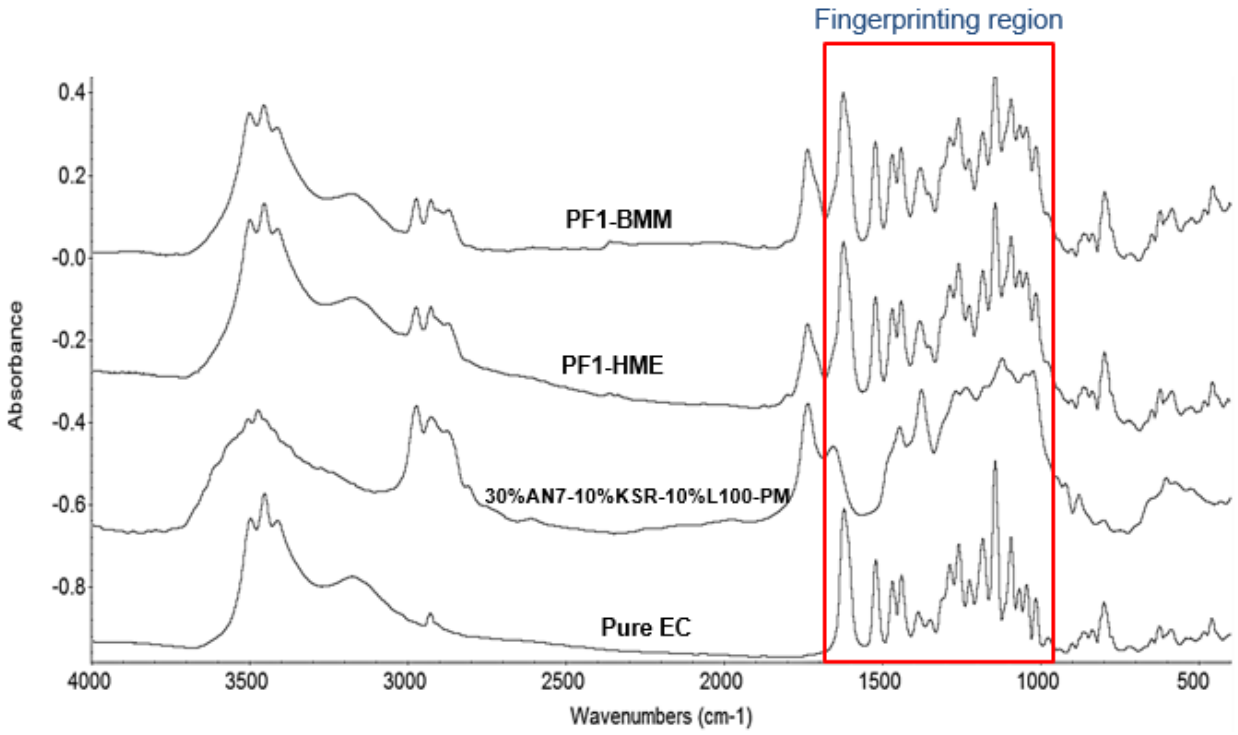

Figure 9: FTIR spectrum of PF1– BMM, PF1 -HME, polymers-PM and Pure EC

Bands are assigned as follows [38]: 1522 cm^{-1} in-plane C–H bending of phenyl, 1288 cm^{-1}, 1389 cm^{-1}, 1469 cm^{-1} and 1441 cm^{-1} are associated with O–C–H, C–C–H, and C–O–H bending modes, 963 cm^{-1}, 1016 cm^{-1}, 1069 cm^{-1}, and 1112 cm^{-1} were assigned to C–O and C–C stretching modes, Bands reflecting the ring structures occur at 822 cm^{-1} ring C-H deformation, 1144 cm^{-1} C–OH of phenyl, and 1626 cm^{-1} C–C stretching of phenyl.

The characteristic bands of EC were observed in the spectrums of PF1, suggesting a consistent chemical structure of EC before and after processing by BMM and HME. Additional characteristic bands were observed in the samples due to the polymers.

Stability assessment by Differential Scanning Calorimetry (DSC)

Figure 10 shows DSC thermograms, where transition temperatures and ΔHm of the samples are displayed.

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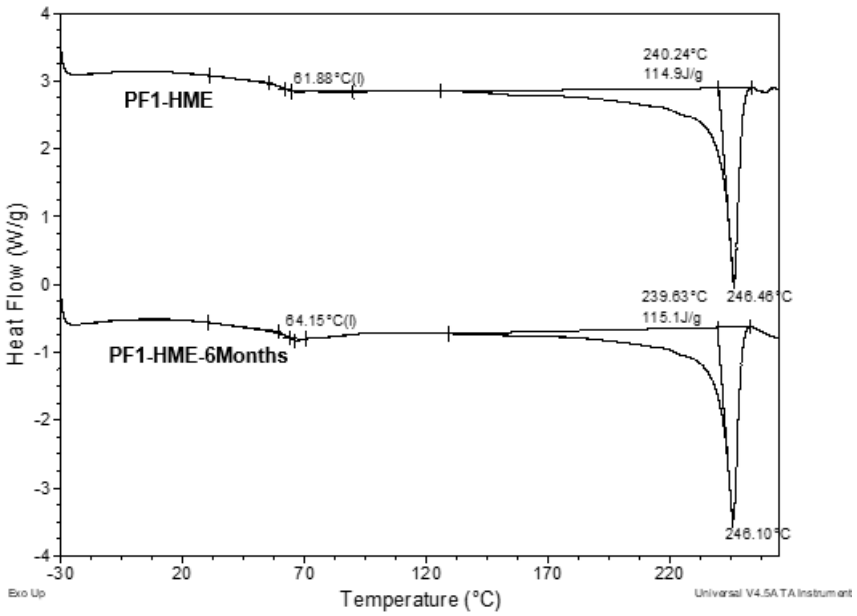

Figure 10: DSC thermograms of PF1 formulation just after HME processing and after 6 months of storage

It can be seen, there was no significant differences between ΔHm values, for PF1 just after HME processing (ΔHm =114.9 J/g) and after 6 months of storage (ΔHm =115.1 J/g) for PF1 after 6 months of storage. Even though this results suggest the stability of the formulation over time, DSC technique is considered as a primary screening technique to evaluate stability and other stability testing methods should be considered, i.e. stability test chambers.

In vitro EC release

The dissolution profiles of pure EC and PF1-HME formulation can be seen in Figure 11. PF1 exhibited a slower dissolution rate in the first two hours, releasing near 45% of EC compared to pure EC that showed a release of 99.96%. After pH adjustment from 1.2 to 6.8, the dissolution increased to 88.64% for the PF1 formulation, releasing more than 80% in 4 hours. Based on the dissolution profiles, It could be deduce that EC would be released under gastrointestinal conditions GIT (*in vitro* studies).

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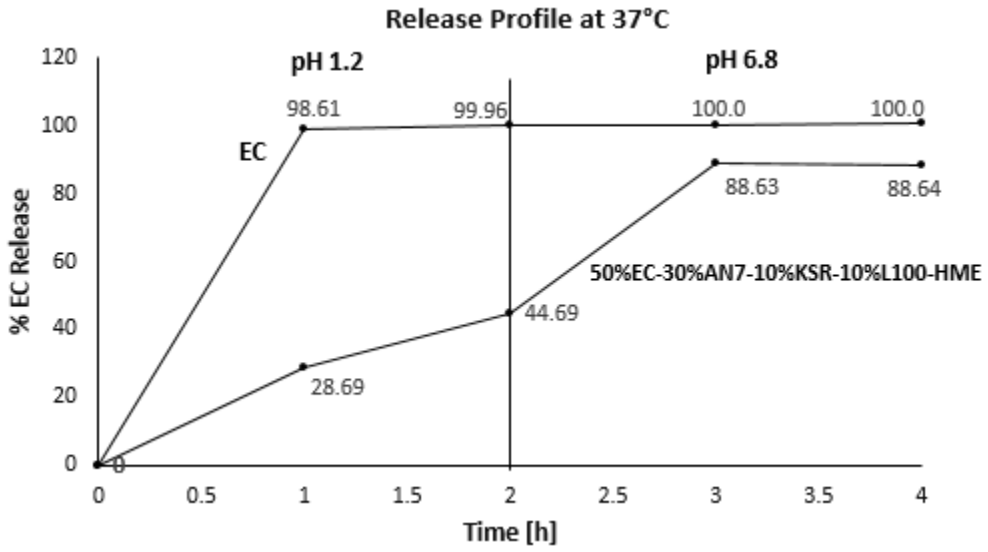

Figure 11: Release Profile in pH 1.2 (t= 1 and 2 hours) and pH 6.8 (t= 3 and 4 hours) at 37°C of PF1-HME and Pure EC

Conclusions

The criterion of 80% epicatechin release after 4 hours and the gradually and controlled release profile in gastrointestinal tract simulated conditions (*In vitro studies*) was reached.

Based on the in vitro test results, it can be concluded that HME is a suitable process to obtain an effective encapsulation of the active ingredient, (-)- epicatechin, in a polymer matrix, to enhance an efficient release in the simulated conditions, increasing the opportunity of epicatechin absorption, making this formulation suitable to be used in food applications.

Formulations prepared by BMM and HME achieved crystalline solid dispersions (CSD) that were confirmed by DSC showing a Tm corresponding to the crystalline EC and a Tg corresponding to the amorphous polymer matrix.

The CSD of PF1 prepared by HME was stable during 6 months (preliminary assessment). This result was obtained by DSC and there was no significant differences between the ΔHm values obtained right after HME processing and after 6 months of storage.

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As a further work, stability tests in chambers and XRD studies will be performed to analyze in detail the morphology of crystalline solid dispersions of PF1.

Future work

As a future work will be considered evaluations of:

a. Taste masking and sensory perceptions.
b. Thermal stability under simulated food processing conditions

Bioavailability (in-vivo studies) tests are optional and they are not required by FDA in USA and/or INVIMA in Colombia.

Current research developments at the ICIPC are focused on a. and b. items

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