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Miguel F.M. Monsanto

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ter verkrijging van de graad van doctor aan de Technische Universiteit Eindhoven,
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door

Miguel Filipe Madalena Monsanto

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Summary

Separation of Polyphenols from Aqueous Green and Black Tea

Tea is a rich source of polyphenols that can be used as a supplement in several products, to increase the health benefits. Polyphenols have a high economic value and can be applied in several areas, such as food, cosmetics and pharmaceuticals. While in green tea mostly catechins can be found, black tea is the source of several types of polyphenols, including theaflavins, which are formed by enzymatic polymerization of the catechins.

The objective of the work described in this thesis is to design a food grade process for the separation and purification of catechins and theaflavins from tea. The Product Driven Process Synthesis (PDPS) methodology is applied for the separation and recovery of target products, instead of the more common application of PDPS to structured food products. The PDPS methodology combines in a structured approach product and process synthesis principles, with an engineering overview. PDPS includes a hierarchy of 9 decision levels of increasing detail.

In a preliminary economic analysis at the Input-output level of PDPS it was found that the process output for the black tea needs to include both catechins and theaflavins. The input process streams are the output of the industrial tea leaf extraction process with 4% of total solids. The green tea output is a powder containing 90 % (wt %) catechins and the black tea output is a powder containing 60 % (wt %) theaflavins, as well as a powder containing 90 % (wt %) catechins.

At the task network level of PDPS two alternatives are presented for both green tea and black tea. The impact of tea cream formation on the polyphenols separation and the thermal degradation of the catechins and theaflavins is evaluated. Tea creaming is a natural occurring precipitation effect that occurs during cooling after tea extraction. Part of the components that are soluble in hot water, are insoluble in cold water and precipitate. The tea cream formation inhibits the polyphenols separation since it decreases the amount of available polyphenols in solution. The possible need of a solvation step is related to the process temperature and the tea cream formation. Two process temperatures were evaluated: 50 °C and 70 °C. At 50 °C the economic potential is higher than at 70 °C, as a consequence of less degradation. To prevent the thermal degradation an alternative process that applies lower temperatures was explored for green as well as for black tea.

For the green tea, precipitation (enhanced tea creaming) was tested for the separation of catechins. The objective was to recover a large amount of catechins from the cream phase without the use of toxic solvents. The process has been empirically described with polynomial models generated from a statistical analysis of the results obtained by Design of

Experiments (DoE). Four precipitation influence factors i.e. two precipitation agents, temperature and pH were analyzed to determine which factors significantly influence the responses. The models were used to optimize the conditions that maximize the catechins recovery and minimize the amount of caffeine, which is considered a contaminant.

The results show that the amount of precipitation agents (hydroxypropylmethylcellulose and polyvinylpyrrolidone) are the most significant factors for the yield of catechins, while the amount of polyvinylpyrrolidone and temperature are the most significant factors for the yield of caffeine. It has also been discovered that the gallated catechins were mainly responsible for the improved precipitation and the variation observed for the yield of catechins. The use of a tea with a high content of gallated catechins should increase the amount of green tea cream and favor precipitation as a separation method for green tea catechins. The optimal combination of factors allows the recovery of 69 % of the catechins and increases the ratio of catechins to caffeine in the cream phase by 60 %.

For the black tea case the same approach as in the green tea case was applied, i.e. intensifying the tea cream effect for the separation of polyphenols. However, the polyphenols recovery was poor. Therefore, an alternative recovery route was explored with the objective of finding the combination of factors that minimize the cream formation and maximize the amount of polyphenols in the clear phase. A new Design of Experiments was defined, where four factors i.e. temperature, amount of tea solids, pH and amount of complexing agent (ethylenediaminetetraacetic acid, EDTA) were studied to determine which factors significantly influence the yield of theaflavins and catechins.

According to the statistical analysis results, the percentage of tea solids and the temperature are the strongest effects for the yield of theaflavins. The pH and the interaction effect between the amount of solids and the temperature are the strongest effects for the yield of catechins. The results also demonstrate that by using the proper combination of factors it is possible to increase the yield of catechins and theaflavins in the clear phase up to 80-90 %.

In addition to precipitation, adsorption was applied for the separation of polyphenols from black tea. Four commercially available macroporous resins were screened for the characterization and optimization of a solvent swing packed bed adsorption. The information necessary for the adsorption process design, i.e. kinetic data, adsorption equilibrium data and adsorbent characteristics has been collected. The adsorption process has been modeled with a Langmuir multicomponent isotherm. The model shows a good fit to experimental results for the catechins and caffeine and a reasonable fit for the theaflavins.

In desorption, a solution containing 70 % of ethanol (wt %) in water was found to be the best desorption medium. The theaflavins have a higher absolute enthalpy of adsorption than the catechins. The catechins have a higher adsorption enthalpy for the Amberlite XAD7HP

(polymethacrylic acid ester) resin than for the Amberlite FPX66 (polystyrene-divinylbenzene) resin. The resin XAD7HP performs best for the sorption of catechins, with a recovery of 60 % of the catechins. The resin FPX66 performs best for sorption of theaflavins, with a recovery of 59 %. Overall, when the objective is to maximize the recovery of catechins and theaflavins and to minimize the recovery of caffeine, the FPX66 is the optimal resin choice.

Adsorption was also applied for the separation of catechins from green tea. In this case, two commercially available food grade resins are considered: the Amberlite XADHP and the Diaion HP20 (polystyrene-divinylbenzene). For the desorption step a solution containing 70 % of ethanol (wt %) in water is used.

The adsorption and desorption behavior in a packed bed column has been modeled using the one dimensional plug flow with axial dispersion concept. This concept allowed the simulation of the dynamics of the solvent swing sorption process. The linear driving force (LDF) approach has been used to describe the mass transfer. Three sensitive model parameters (overall mass transfer coefficient, maximum adsorption capacity and the Langmuir constant) were regressed from the experimental data. The four green tea catechins and the caffeine were included in the competitive sorption model and showed a good fitting to the experimental data. The HP20 resin has a much higher affinity for caffeine than for the catechins. This adsorption affinity difference makes the HP20 resin a good option to separate the caffeine from the catechins. The XAD7HP resin has a high affinity for both caffeine and catechins, allowing the separation of the modeled components from the green tea.

The adsorption model sets the basis for process design and optimization for the recovery of green tea catechins, using macroporous resins in a packed bed. Based on the column adsorption model two operating designs were simulated and optimized for the operational time of the packed bed. In Design 1 the objective was to maximize the amount of catechins and minimize the amount of caffeine. Two columns are used in Design 1 and after one operational cycle (95 minutes) the yield of catechins was 52 % and the yield of caffeine was 19 %. The relative purity of the catechins to caffeine increased from 78 % to 91 %. In Design 2 the only objective was to maximize the amount of catechins. A single column is operated during 100 minutes, achieving a yield of catechins of 89 % and a yield of caffeine of 88 %.

Ultimately, the acquired data and models are used in a conceptual process design that combines adsorption and spray drying for the production of a high purity green tea catechins dry powder. The process scheme is presented together with the operational conditions and economic evaluation, which includes operational and capital expenditures as well as total annual costs for the selected process, allowing decision making regarding the

chosen technology. The conceptual design shows that it is possible to produce a fine catechins powder with 83.5 % purity and that the process has a positive operating profit.

Therefore, it can be concluded that the combination of packed bed adsorption with spray drying is a promising process for the separation and purification of catechins from green tea.

Table of Contents

1.

Introduction

ABSTRACT: Tea is the second most consumed beverage in the world and it is produced from the Camellia sinensis plant. Tea is a rich source of polyphenols, which when purified have a high economic value as they can be used as a supplement in several products to increase their health benefits. While in green tea mostly catechins can be found, black tea is the source of several types of polyphenols formed by enzymatic polymerization of catechins, including theaflavins. Caffeine is present in relatively high amounts in the tea extract and to achieve a high polyphenols purity it is necessary to minimize the caffeine content. The polyphenols separation can be challenging due to the similarities in their physical properties and the interactions between several tea components.

1.1. Introduction

After water, tea is the most popular beverage consumed by almost two-thirds of the world population. There is a wide variety of teas from which the main types are black, green, oolong and white teas. All teas originate from the same plant (a warm weather evergreen named *Camellia sinensis*) and vary in their production processes. Tea is a rich source of polyphenols, which when purified have a high economic value as they can be used as a supplement in several products to increase their health benefits. Polyphenols are, therefore, regarded as desired components with several applications in a variety of areas, such as food, cosmetics and pharmaceuticals. The growing market of functional foods is related to the increasing consumer demand for more natural, tastier and healthier additives (Figure 1.1).

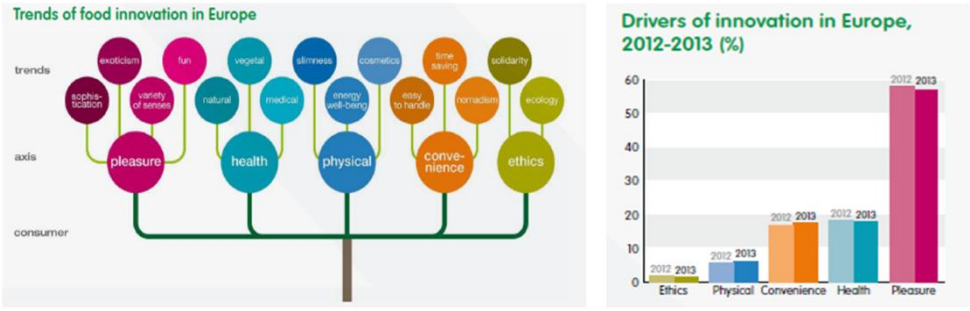

Figure 1.1. Trends and drivers of food innovation in Europe. Source: Eurostat 2011.

At present, to the best of the authors knowledge, there are no large-scale technologies for the isolation and purification of polyphenols from tea, which are cost-effective, environmentally friendly and use only food grade solvents. There is a need to develop processes that can provide these components at acceptable cost and in an environmentally friendly way.

1.2. Nutraceuticals and polyphenols

A nutraceutical is by definition any substance that may be considered "as a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease" (DeFelice, 1992). Nutraceuticals include e.g. isolated nutrients, dietary supplements and herbal products. Consumers show an increasing interest for this type of products due to their association to health benefits (section 1.3.1). In a specific case, the recent regulatory measures targeting the use of synthetic color pigments to food (warning label required by the European Food Safety Authority), has strongly contributed to the increasing application of natural food colorants.

Polyphenols are a large class of phenolic-based compounds, found as plant-derived secondary metabolites synthesized by the plant. They form one of the most numerous groups of natural products found in plants, with over 8000 phenolic structures from which 4000 are flavonoids (Harborne and Williams, 2000). Polyphenols are often produced by plants in response to various environmental stresses. Stress may be caused by diseases, insects, climate, ultraviolet radiation, etc. (Dixon and Paiva, 1995).

Other sources of variability can include cultivar, growing location, agricultural practices, processing and storage conditions, and preparation methods (Amiot et al., 1995; Hakkinen et al., 2000). Although in some cases plants can be used directly as a source of polyphenols, the concentrated and purified forms provide improved activity and allow a wide variety of product applications. There is, therefore, a need to use recovery and purification steps.

The flavonoids (Figure 1.2) are a group of organic molecules found in vascular plants, characterized by its C6-C3-C6 carbon backbone containing multiple hydroxyl groups that are hydrogen-donating antioxidants and singlet oxygen quenchers, with a strong antioxidant potential (up to five times higher that vitamin C) and metal chelating properties (Middleton et al., 2000). Flavonoids include different sub-groups of phenolic compounds such as anthocyanins, flavan-3-ols, flavones, flavanones and flavonols, which differ mainly in the connection of the B ring to the C-ring as well as in the oxidation state and the C-ring substitutions (Tang et al., 2003).

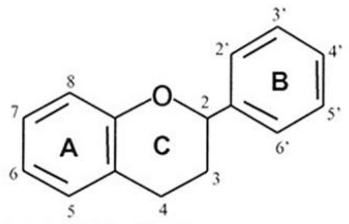

Figure 1.2. General structure of flavanoids.

In this work the focus is on flavan-3-ols (also called flavanols), which differ from most flavonoids, since there is no double bond between C2 and C3, and no C4 carbonyl in Ring C. The flavan-3-ols have two chiral centers (C2 and C3), due to the hydroxylation at C3 and as a consequence four possible diastereoisomers: catechin has a *trans* configuration and epicatechin has a *cis* configuration. During fermentation these molecules can form dimers like theaflavins (Figure 1.3).

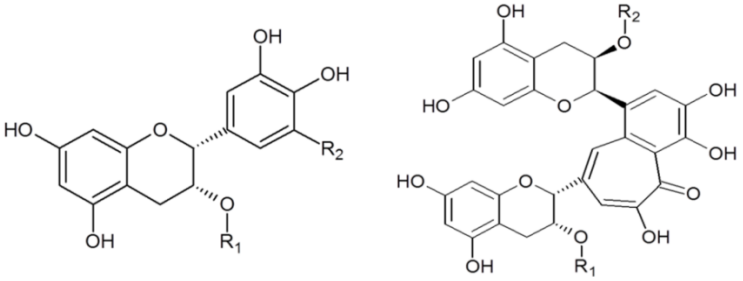

Figure 1.3. General structure of catechins (left) and theaflavins (right).

1.3. Tea and tea polyphenols

Tea is the second most consumed beverage in the world and it is produced from the *Camellia sinensis* plant, mainly cultivated at high altitude in mineral-rich soil. The largest tea producing countries are Argentina, China, India, Kenya, Indonesia and Sri Lanka. In the 17th century tea became highly popular throughout Europe and the American colonies. Tea was originally used as a medicine and later as a beverage. The four main types of tea are white, green, oolong and black tea (in increasing "degree" of fermentation: white tea is not fermented and black tea is fully fermented), see Figure 1.4. In the green tea production process the enzyme oxidase is inactivated to prevent the oxidation of catechins.

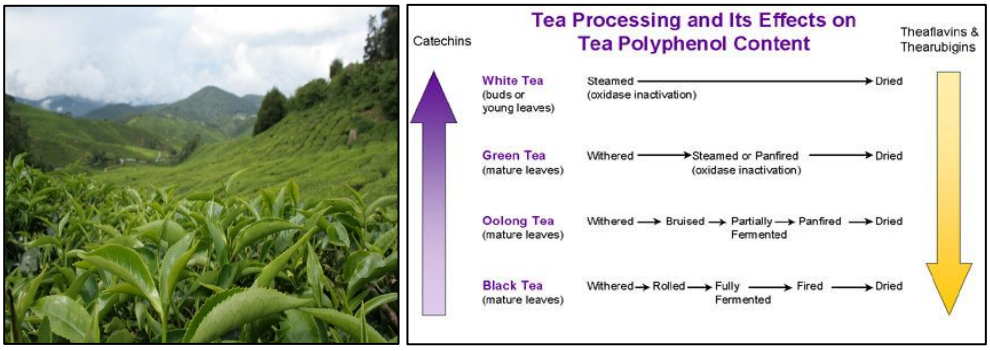

Figure 1.4. *Camellia sinensis* plants (left) and main types of teas (right).

In the green tea production the tea leaves (*Camellia sinensis*) are after withering, submitted to short time heating (firing) to inactivate enzymes. In the case of black tea, after withering the leaves are submitted to enzymatic oxidation.

Tea has a complex chemical composition with several components: proteins, amino and organic acids, polysaccharides, minerals, chlorophyll, volatile compounds, lignins, alkaloids (caffeine, theophylline, and theobromine) and polyphenols (flavan-3-ols, theaflavins, thearubigins, and proanthocyanidins) (Harbowy and Balentine, 1997). Caffeine is the most abundant alkaloid in tea (Hilal and Engelhardt, 2007).

While in green tea mostly catechins can be found, black tea is the source of several types of polyphenols formed by enzymatic polymerization of catechins, including theaflavins, which can only be found in black and oolong teas (Figure 1.4).

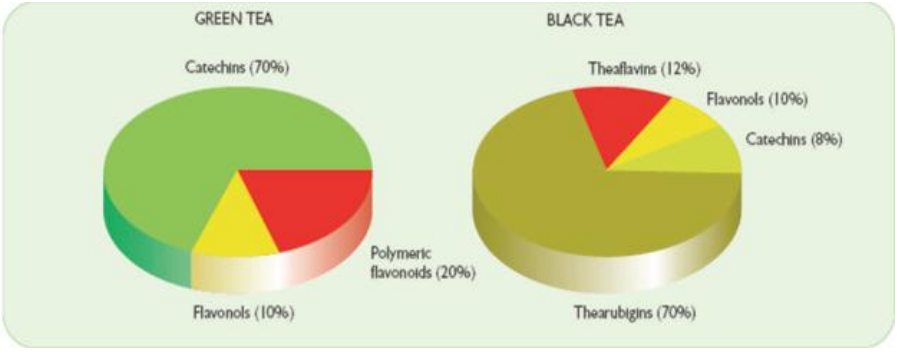

Figure 1.5. Polyphenols composition in green tea and black tea.

The monomeric flavan-3-ols undergo enzymatic oxidation and the reactive oxidized product dimerises/oligomerizes, leading to theaflavins, as well as other dimeric structures (theaflavates, theasinensins, theacitrins) and oligomeric products. The oligomeric products, e.g. thearubigins, bisflavanols and other oligomers, become increasingly hydroxylated during the process (Figure 1.5). The present work focuses on tea monomers (catechins) and on one class of dimers (theaflavins) formed in the polymerization reaction (Gogoi et al., 2010; Harbowy and Balentine, 1997; Yang et al., 2000).

Polyphenols are regularly consumed in the form of tea made from *Camellia sinensis* leaves. Although the catechins and theaflavins are only highly diluted in an average tea beverage, they are among the most abundant in the solid fraction of tea (Harbowy and Balentine, 1997; Vuong et al., 2010).

The fact that tea is such a popular drink together with all the potential health benefits, led to several publications focused on the chemical and biological properties as well as on health implications. The increasing interest in tea polyphenols has also been revealed by the

increase number of papers published in the last 20 years: in 1993 only 19 papers where published, but 10 years later that number increased to 228 and in 2013, 558 papers where produced (according to a Scopus search).

1.3.1. Health effects

Several studies demonstrate that populations with high consumption of plant-based foods have a lower incidence of cardiovascular diseases and certain types of cancer, which may be related to polyphenols present in plant-based foods. The tea polyphenols offer a wide range of claimed functional health benefits due to their antimutagenic and anticarcinogenic properties (Kuroda and Hara, 1999). The addition of catechins can prolong the shelf life and improve the color and flavor of foods (Vuong et al., 2011).

Tea health benefits have been widely investigated and several potential beneficial physiological and pharmacological effects have been identified. Several positive effects have been reported: retardation of the catabolism of catecholamines, anti-inflammatory, antioxidant and antimicrobial effects, growth inhibition of implanted malignant cells, inhibition of angiotensin-converting enzymes and hypocholesterolemic action. A limited number of studies also indicate a positive effect on bone density, dental caries and cognitive function (Hattori et al., 1990). Nevertheless, there are some contradicting studies on humans regarding the relation between tea and health, particularly the risk for cardiovascular disease and cancer (Cooper et al., 2005).

1.3.2. Catechins

Catechins are colorless molecules that contribute to the bitterness and astringency of tea and can be classified into two groups: epistructured catechins and nonepistructured catechins, see Figure 1.6. The major catechins present in tea are in decreasing order of presence: epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC). The nonepistructured catechins, which include gallocatechin gallate (GCG), gallocatechin (GC), catechin gallate (CG) and catechin (C), are only present in small amounts (Masukawa et al., 2006).

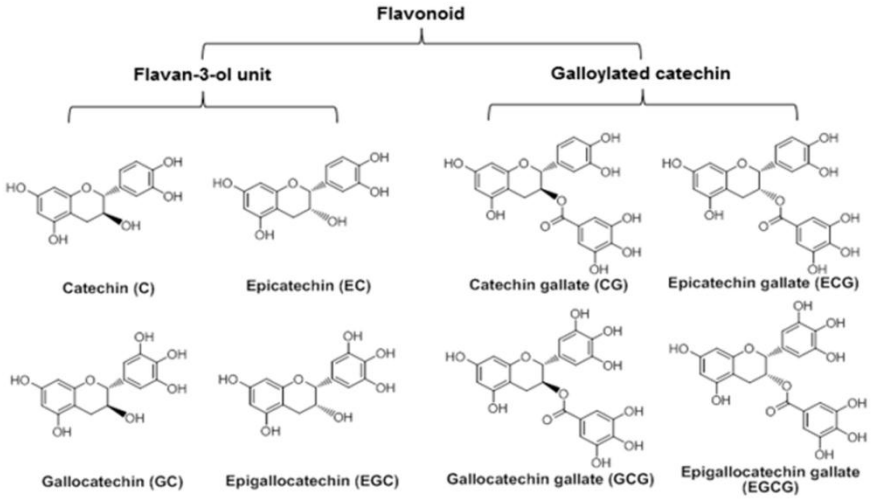

Figure 1.6. Green tea catechins: epistructured catechins (EC, EGC, EGC and EGCG) and nonepistructured catechins (C, GC, CG and GCG).

Catechins have strong antioxidative properties, because they contain hydroxyl groups in their structure and thus can scavenge reactive oxygen species. Catechins precipitate by reacting with macromolecules, which results in the formation of haze, commonly known as tea cream formation (Penders et al., 1998). Catechins are also able to inhibit the activity of different enzymes (Sekiya et al., 1984) and are not stable in the presence of oxidizing enzymes, high temperatures (>95◦C) and alkaline pH (Graham, 1992).

1.3.3. Caffeine

Caffeine (1,3,7-trimethylxanthine), see Figure 1.7, is classified as an alkaloid (nitrogen-containing ring compounds) that can be found in plants. It is a weak organic base with a bitter taste and it is the most widely consumed central nervous system stimulant (Nehlig et al., 1992). The physiological effects on human health have been widely studied including: diuretic and bronchodilator properties as well as behavior influence (Hindmarch et al., 2000).

Caffeine is present in relatively high amounts in the tea leaf (between 2 and 5% of dry weight) and also in the tea extract. It is therefore, very important to minimize the caffeine content, to be able to achieve a high polyphenols purity. Furthermore, a high consumption of tea may cause irritation of the gastrointestinal tract and sleeplessness for certain people, due to the caffeine effect. Many methods have been explored for decaffeination of tea. Usual techniques for the isolation of caffeine from tea leaves are solid–liquid and liquid–liquid extraction (Vuong et al., 2011). However, this is usually done with organic solvents,

such as ethyl acetate or dichloromethane, which may not be safe due to the potential harmful effects of these solvent residues (Perva-Uzunalic et al., 2006). More recently, the use of supercritical carbon dioxide extraction techniques for decaffeination of tea leaves have been developed which does not require harmful solvents (Chang et al., 2000). Nevertheless, the separation of caffeine and polyphenols (or other tea components) is never simple, due to the similarities in molecular size and solubility.

1.4. Tea cream effect

The tea cream effect occurs during the cooling process after tea extraction. This effect is governed by the different solubilities of the tea components in hot and cold water and the formation of complexes (Figure 1.7). The natural occurring precipitation phenomenon is caused by interaction of extracted natural polymeric molecules, like proteins or pectins and complex formation of these with smaller molecules like polyphenols (e.g. flavonol glycosides) and caffeine (Tolstoguzov, 2002).

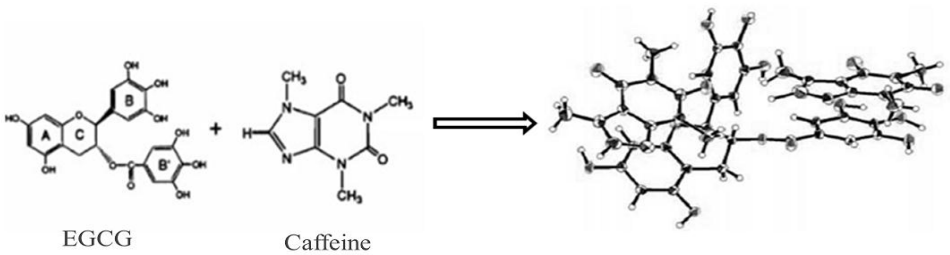

Figure 1.7. Formation of a complex between catechin (GCG) and caffeine (Ishizu et al., 2011).

Above a certain solids concentration, spontaneous demixing is predominant in the cream formation, showing the substantial insolubility of polyphenols at low temperature (Figure 1.8) and originating a cream and a clear phase in the tea (Penders et al., 1998).

Most of the research about polyphenols separation focuses on extraction (Bazinet et al., 2007; Labbe et al., 2006; Nawaz et al., 2006) and although the tea cream effect was already suggested to be the basis of a possible separation route for the tea catechins in previous publications (Sekiya et al., 1984; Vuong et al., 2011), there is to the best of the authors knowledge, no previous work reported on this process route.

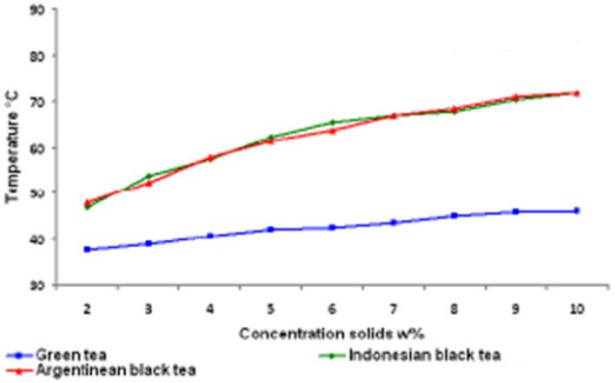

Figure 1.8. Phase diagram for green and black tea. Source: Unilever R&D.

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1.5. Separation and purification of tea polyphenols

Polyphenols separation and purification are challenging due to the similarities in physical properties and structure of tea polyphenols. In addition, the amount and diversity of components present in tea, the interaction of some of these components with the polyphenols and the similar properties of tea polyphenols with caffeine, decreases the possible separation technologies application. A list of the separation and purification methods commonly used for the tea polyphenols is presented in sections 1.5.1 to 1.5.4.

1.5.1. Membrane separation

Membranes are used for a size based separation of tea polyphenols and more specifically ultrafiltration (UF) membranes have been investigated for both separation and purification of tea polyphenols (Evans and Bird, 2006; Ramarethinam et al., 2006). Also in another investigation composite UF membranes (cellulose acetate-titanium) were used to separate polyphenols from tea (Li et al., 2005). Although the use of membranes allows the separation and concentration of the polyphenols from other tea components, it presents, however, several disadvantages such as membrane fouling and low polyphenols purities.

1.5.2. Supercritical fluid extraction (SFE) with carbon dioxide

Supercritical fluid extraction has been more recently investigated for the separation of tea components (Huang et al., 2007b) It has the advantage of preserving the polyphenols properties (absence of air), being environmentally friendly and achieving high efficiencies. However SFE shows severe limitations in the separation of caffeine from catechins, since almost 40% of the catechins are removed together with the caffeine (Park et al., 2007). SFE also requires high capital investment due to high capital costs for the high-pressure extraction equipment (Patel et al., 2006).

1.5.3. Precipitation

Precipitation was previously suggested as a separation route for the tea catechins (Sekiya et al., 1984; Vuong et al., 2011). Several components and properties can be used to achieve this separation, which is closely related to the tea cream formation. The phase diagrams for black tea cream follow the phase behavior of mixtures of simple compounds and for a black tea with up to 10 % of solids the temperature needs to be maintained above 70 °C, to prevent the tea cream formation (Penders et al., 1998). Besides the amount of solids and temperature, other factors like pH, precipitation agents and chelating agents also have the ability to influence the tea precipitation (Jobstl et al., 2005; Tolstoguzov, 2002). The addition of caffeine to green tea and consequent precipitate formation, was also reported for the isolation of EGCG from green tea (Copland et al., 1998).

1.5.4. Adsorption

Adsorption has been the most investigated method for the separation of polyphenols from tea. Various types of resin adsorbates have been used to isolate tea catechins. (Zhao et al., 2008) used macroporous resins to selectively adsorb and purify catechins from green tea. This method has the advantage of allowing the use of food grade materials and solvents as well as the possibility of fractionation of the individual polyphenols. However the reported purities are not very high, so additional purification steps are usually required.

On an analytical and preparative level, chromatography has been commonly used for the separation of tea polyphenols from green tea (Amarowicz et al., 2003) and black tea (Ozawa, 1982), by gradient elution, based on the different retention times of the different tea components. During the gradient elution there is a continuous change of the sorption equilibrium of a certain component, due to the change in the composition of the eluent during the adsorption process.

Adsorption is very promising for the separation and purification of tea polyphenols and has the advantage of being a mature separation technology already employed at an industrial scale by the food and pharma industries.

1.6. Adsorption processes

Adsorption is the process of adhesion of a molecule of a substance onto the surface of a liquid or a solid, resulting into a higher concentration of the molecules at the surface. The interactions between the molecule and the surface maybe physical (van der Waals forces, hydrogen bonds) or chemical (covalent bonds).The adsorbed molecule is referred to as the (ad)sorbate and the substance on which it is absorbed is the (ad)sorbent.

The following four consecutive steps are involved in adsorption on porous materials and when all of them need to be accounted for, it makes the modelling and design very complex (Figure 1.9):

1. Transport of the adsorbent from the bulk fluid phase to the fluid film surface of the adsorbate.

2. Transport through the film to the outer surface of the adsorbate.

3. Transport from the surface through the pores of the adsorbate.

4. Adsorption of the molecule onto the internal surface (i.e. the pore walls) of the adsorbate.

The reverse process is desorption (removal of the adsorbed substance from the surface) and is driven by a change in concentration of the adsorbate or a change in the composition of the mobile phase.

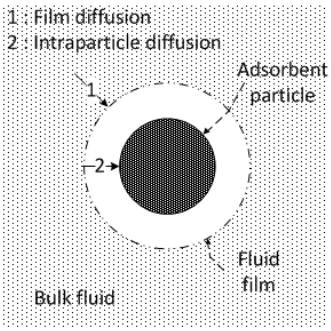


Figure 1.9. External and internal mass transfer resistances in a particle.

The role of the adsorbent is to provide the selectivity and capacity required for the separation of components in a mixture. In the adsorption process the selectivity is dictated by the adsorbing molecules and the nature of the surface, on which different substances are adsorbed with different affinities.

Previous studies on packed bed adsorption columns with macroporous resins have shown promising results for the separation and isolation of green tea polyphenols (Lai et al., 2009; Scordino et al., 2003; Silva et al., 2007; Vuong et al., 2010). However, not all the applied

resins are food grade. For the case of black tea polyphenols adsorption, almost no information is available. There is, nevertheless, a need of cost effective adsorption processes based on experimental results and (mechanistic) modeling.

Three important properties that govern the adsorption of polyphenols from tea are the hydrophobicity, polarity and ability to form hydrogen bonds between the resin and the polyphenols. An apolar (and therefore hydrophobic) resin cannot effectively adsorb a polar (and therefore hydrophilic) molecule due to the lack of specific mutual interactions.

In adsorption processes selectivity is governed by the adsorbing molecules and the nature of the surface, where different substances are adsorbed with different affinities. In literature several commercially available resins used in adsorption showed potential as adsorbents for green tea polyphenols (Gogoi et al., 2010; Zhao et al., 2008). However, for the black tea polyphenols adsorption only scarce information is available. In addition, not all resins are food grade.

Polymeric adsorbents (resins) are manufactured by polycondensation or free radical polymerization and have a permanent porosity with a high specific internal surface area. The high surface area combined with a hollow and layered structure provide a good mechanical strength (Bai et al., 2005). For example, the FPX66 resin (a copolymer of styrene and divinylbenzene) is used to adsorb hydrophobic molecules present in polar solvents or volatile organic compounds from vapor streams (Kammerer et al., 2005).

The use of adsorption processes for the recovery of valuable components from liquid streams usually requires the use of one additional step, to desorb these components. This is usually done by a temperature increase (temperature swing adsorption) or by a change in the solvent used for the desorption step (solvent swing adsorption). However, for the case of adsorption of polyphenols from tea the temperature swing cannot be applied, since the target polyphenols loose stability at high temperatures.

1.6.1. Solvent swing adsorption

In solvent swing adsorption there is a change in the bulk fluid to switch from adsorption to desorption. This change allows taking advantage from difference between the affinities of the adsorbents for the different solvents.

In the column sorption experiments a three steps process is used: the first step involves the usage of an aqueous tea feed stream, where the polyphenols are adsorbed onto the adsorbate. In the second step, water is used to wash the column to remove non adsorbed components. In the last step, an eluent solution is used for the desorption of the components that have more affinity towards the eluent.

Modeling and design of multicomponent adsorption processes is not simple due to a manifold of complex interactions between the phases, which determine sorption equilibria and the kinetics.

1.7. Product design and Product Driven Process Synthesis (PDPS)

This thesis follows the Product Driven Process Synthesis (PDPS) methodology, which proposes a structured approach for the synthesis of products in the food and drink sector. PDPS has been previously applied to industrial cases for structured products, since this type of products are difficult to design only with process synthesis (Bongers and Almeida-Rivera, 2009). Although a process synthesis approach for bulk chemicals is already very well established, it has proven inadequate for food products due to intrinsic characteristics of these products. This methodology requires a complete definition of the target product(s), raw materials and process specifications. Possible process routes are presented to achieve the final product propertie(s) and a conceptual process design is generated (Douglas, 1988).

Recently there has been a shift from a process focus to a product focus, where product design combines among others, sustainability and chemical and physical properties (Hill, 2004). Chemical product design defines the needs that the product should fulfill, generates and selects ideas to meet these needs, defines product properties and finally decides the manufacturing process (Moggridge and Cussler, 2000). There is shift from commodity based chemical products, to high value added and product performance-based products. This change is mainly driven by an ever-increasing demand from customers for products with a high functionality and well defined performance. This also puts the focus on market analysis that can accurately read the consumer needs and translate this into product attributes. Also the time to market has gained a central role as it can give companies a competitive edge. Sometimes it is more favorable to have a fast production process instead of a cheaper one. To achieve the complex end user products and to obey the social and environmental constraints of the industrial-scale processes, a multidisciplinary approach needs to be used.

1.8. Objectives

The objective of the work described in this thesis is to design a process for the separation and purification of polyphenols from aqueous green and black tea process streams with a suitable purity, high yield and acceptable costs.

The process must be environmentally friendly and hygienic. Only food grade materials (solvents, adsorption resins, etc) should be used and the product must be suitable for food applications. Mild separation techniques should be used to preserve the input tea stream properties and if possible allow a re-use of the stream.

This thesis follows the PDPS methodology and uses a structured synthesis approach for this type of food separation systems. As such, a 'toolbox' is developed that includes design 'rules' and heuristics for decision making, as well as models for application in the design of the separation process. This should result in reduced product/process development time by delivering methods and tools to design a process that can generate the target components at affordable costs.

An initial conceptual design needs to be developed for both green and black tea, including operational conditions and an economic evaluation.

1.9. Outline

At present, to the best of the authors knowledge, there are no large-scale technologies available to isolate polyphenols from tea that are cost-effective, environmentally friendly and use non-toxic solvents. In Chapter 2 the Product Driven Process Synthesis (PDPS) methodology is applied for the separation and recovery of polyphenols from liquid tea. In this particular case PDPS is applied to separation technology, where target products need to be recovered instead of the more common application to structured food products.

In Chapter 3 precipitation (enhanced tea creaming) is used for the separation of polyphenols from green tea. The yield of the separation process is described with polynomial models generated by statistical analysis based on Design of Experiments (DoE). Four precipitation influence factors have been studied: hydroxypropylmethylcellulose, polyvinylpyrrolidone, temperature and pH. Optimization is performed to maximize the polyphenols recovery.

Chapter 4 reports the use of a DoE to determine the optimal combination of factors that minimize cream formation and maximize the amount of polyphenols in the clear phase. Four factors (temperature, amount of tea solids, pH and amount of ethylenediaminetetraacetic acid) were studied to assess the impact on the polyphenols availability and statistical analysis is used to determine which factors significantly influence the responses and to generate polynomial models.

In Chapter 5 four commercially available macroporous resins are screened for the characterization and optimization of a solvent swing packed bed adsorption process to separate the polyphenols from black tea. To design the adsorption system, information about adsorption equilibrium, adsorbent characteristics and kinetics is needed.

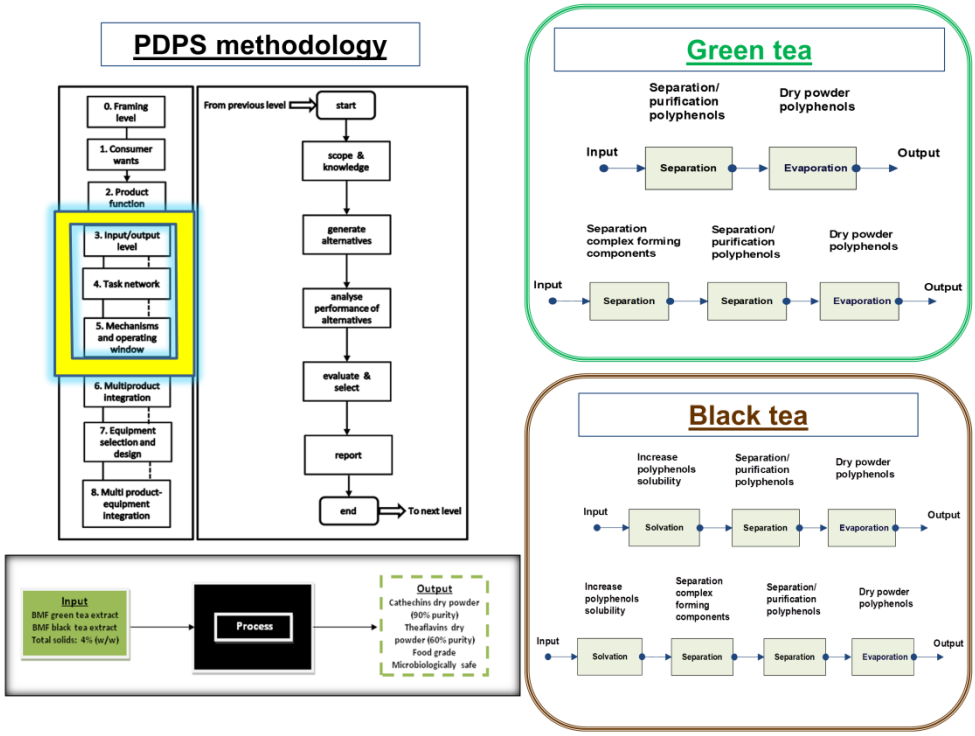
Chapter 6 describes a fixed bed column adsorption process packed with a macroporous resin, which was tested for the separation and purification of polyphenols from tea. A mathematical model that describes the sorption of tea polyphenols was developed. The highlight of this work is the modeling of a complex multicomponent system, where the different polyphenols and caffeine are competing for the adsorption sites.

In Chapter 7 the previously acquired data and models are used in a conceptual process design for the separation of catechins from green tea. Process schemes are presented together with the operational conditions and an economic evaluation, which includes operational, capital expenditures and the total annual costs for the selected processes.

The last Chapter (Chapter 8) summarizes the key findings and presents the thesis conclusions. A final outlook is also presented.

2.

Product-driven Process Synthesis methodology for the polyphenols separation from tea



ABSTRACT: The Product Driven Process Synthesis (PDPS) methodology has been applied for the separation and recovery of polyphenols from liquid tea. The preliminary economic analysis in the Input-output level of the PDPS shows that the output for the black tea needs to include both catechins and theaflavins. The green tea output is a dry powder with 90 % catechins purity. The output from the black tea is a dry powder with 90 % catechins purity and a dry powder with 60 % theaflavins purity. In the Task network level of the PDPS two alternatives were presented for both the green tea and the black tea. The impact of the tea cream formation and the thermal degradation of the catechins and theaflavins was evaluated, as well as the possible effects in the selection of the task networks.

2.1. Introduction

In more recent times there has been a shift from a process focus to a product focus, where product design combines among others, chemical and physical properties, sustainability and stability with respect to mechanical stress and thermal load (Hill, 2004). The chemical product design defines the needs that the product should fulfill, generates and selects ideas to meet these needs, defines product properties and finally decides how the product should be manufactured (Moggridge and Cussler, 2000).

This shift from a commodity based chemical industry towards a high value added and product performance-based one, allows higher profit margins than the traditional production of bulk chemicals. Performance products have a high functionality that comes not only from ingredients, but also from product structure. This change from commodities to performance products is mainly driven by an ever-increasing demand from consumers for products with a high functionality. This also raises the importance of having an accurate market analysis that can read the consumer needs and correlate it to product attributes. Also the time to market has gained a central role, as it can give companies a competitive edge. Sometimes it is more favorable to have a fast production process, instead of a cheaper one.

To achieve the complex end user products and to fulfil the social and environmental constraints of industrial-scale processes, a multidisciplinary approach is required. This approach includes physical chemistry, interfacial engineering, molecular modeling, process control, medical sciences and strategic planning.

The Product Driven Process Synthesis (PDPS) methodology has been previously applied to industrial cases for structured products, since these products are difficult to design only with process synthesis (Bongers and Almeida-Rivera, 2009). Although a process synthesis approach for bulk chemical products is already very well established, it has proven inadequate for food products due to the intrinsic characteristics of these products. The PDPS methodology requires a complete definition of the target product(s), raw materials and process specifications.

This chapter reports the application of the PDPS methodology for the separation and recovery of polyphenols from liquid tea. PDPS is a systematic procedure developed for generating flowsheet alternatives that can transform starting materials into desired products. Short-cut decisions, mathematical models and heuristics can be used at each step of the methodology for decision support, and are derived from the knowledge of physicochemical phenomena and interactions, between the product components.

In the food market sales are guided by the end-use property of a product, together with specific quality features and functions. This is related to the fact that consumers generally judge products according to quality features and sensory properties. In the case of high-margin products being the first on the market is sometimes the key factor, with the benefit of higher profit margins. This generates a need for multipurpose systems and equipment, which allow flexible production processes.

2.2. PDPS structure

The PDPS methodology combines product and process synthesis principles with an engineering overview and it structures the process into a hierarchy of 9 decision levels of increasing detail (Figure 2.1). Each of these levels follows a five steps sequence; scope and knowledge, generate alternatives, analyze performance of alternatives, evaluate and select and in the last step generate a report (Bongers and Almeida-Rivera, 2009). As it is impossible to evaluate all ideas in detail, a preliminary screening is needed. By following an iterative product design approach and by identifying the factors that influence product performance and economics, it is possible to re-analyze the product possibilities identified in the beginning of the methodology.

The 9 PDPS levels have been described by Bongers and Almeida-Rivera (2009). A summary of this description follows here:

Level 0- Framing level. This first level includes a complete description of the project background and the business context, including supply chain considerations and evaluations.

Level 1- Consumer wants. The consumer preferences (qualitative descriptions) are translated into quantifiable product attributes.

Level 2- Product function. The quantifiable product attributes are related to measurable product properties.

Level 3- Input-output level. Here the input feed and the output products are specified and characterized. Several performance parameters can be evaluated, e.g. economic potential, quality, hygienic considerations, flexibility, availability...

Level 4- Task network. The fundamental process tasks are defined, taken from a cluster of tasks and its subgroup. The tasks are sequenced and grouped into a network.

Level 5- Mechanism and operational window. The possible mechanism and principles to perform a defined task are selected. This step includes the driving forces, kinetics and operational windows.

Level 6- Multi product integration. In the case of multi product production, overlaps and possibilities to combine the production are analysed.

Level 7- Equipment selection and design. Selection of the unit operations and integration possibilities. The final flowchart with design of the units is presented.

Level 8- Multi product-equipment integration. Optimization of unit operations in the flowsheet and plant-wide control. In the case of multiple products a multi-stage scheduling is applied, based on the product demand and portfolio.

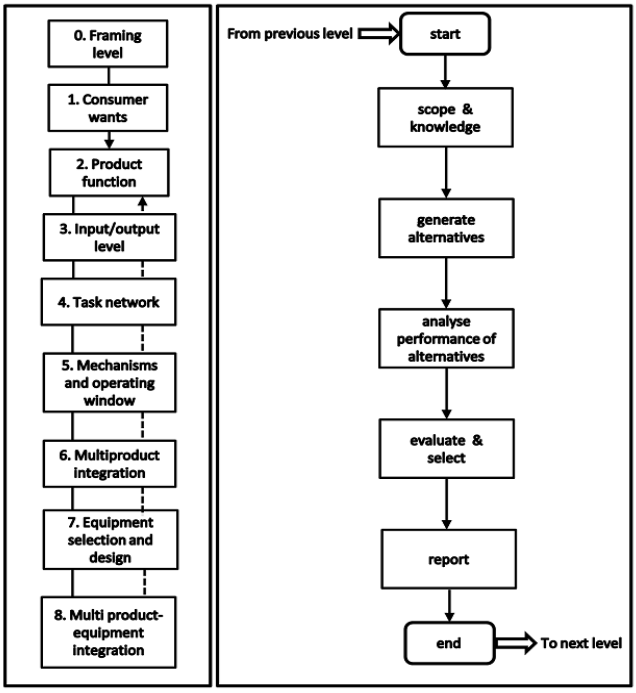


Figure 2.1. Levels of PDPS and activities at each level (Almeida-Rivera et al., 2004).

During the evaluation and selection procedure, at each level of the PDPS, not only the economic performance but also product and process characteristics as well as supply chain considerations are taken into account. This methodology results in a systematic procedure for producing chemical-based consumer products.

The first 3 levels (level 0 to level 2) of PDPS are the basis of this thesis and identify the need for the tea polyphenols. At these initial levels the business relevance and the advantages of the project are evaluated. Some of the project fundamentals include: produce consumer products with health benefits, reduce the development time by delivering methods and tools to design a process, more restrictive legislation for food additives and increase the functionality as well as reduction the costs. In this thesis the PDPS methodology is developed starting at level 3, input-output level.

2.3. PDPS application: Input-Output level (level 3)

2.3.1. Input composition (green and black tea extract)

In this level a general input-output structure is presented. It defines the specifications for the input and the output streams and determines performance parameters such as economic potential, transformation in terms of overall yield, etc.

Two input streams are considered together with two corresponding output streams. The input streams come from the industrial tea leaf extraction process with 4% of total solids. One stream contains green tea extract and the other stream contains black tea extract. The objective is to recover target polyphenols from both streams. The composition of the input streams is presented in Tables 2.1 and 2.3 for green and black tea respectively.

Most of the complete composition data found in literature regards the tea leafs. For the green tea extract only one reference source was found (Table 2.1). For the black tea extract however, four sources were found, see Table 2.3. The experimental values presented in Tables 2.1 and 2.3 are obtained for tea extractions using freeze dried BMF (Broken Mixed Fannings) tea as input material. BMF tea is made from finely broken pieces of tea leaves taken from different grades. For the extraction the freeze dried tea powder was dissolved in water at 85 °C, with stirring for 10 minutes,

The starting material of the green tea extract is dry BMF green tea and the starting material of black tea extract is dry BMF black tea. There are two input streams: one green tea extract stream and one black tea extract stream containing in both cases 4 % of total solids. The streams temperature is 70 °C. At this temperature and for this solids concentration there is no tea cream formation, see section 1.4.

Table 2.1 presents the average composition of a green tea extract. The main components are catechins, ashes, proteins, carbohydrates and caffeine (Balentine et al., 1997). The experimental values are measured by HPLC, see section 3.3.2, for a tea with 4 % of solids. As compared to the experimental results, the reported values are much higher for the catechins and much lower for the proteins. However, since the tea composition can be influenced by several factors, including the species, season, leaf age and climate

horticultural conditions (Cabrera et al., 2003), as well as the extraction procedure used, differences were already expected. The analytical method used for the measurements can also have a significant impact.

Table 2.1. Green tea extract composition (% w/w)

Component	Balentine et al.	Experimental
Catechins	30-42	17.62
Caffeine	3-6	5.31
Proteins	6	18.77
Free amino acids	6	n.a.
Carbohydrates	11	n.a.
Organic acids	2	n.a.
Ash	10-13	n.a.

n.a. not available

Table 2.2 presents the flavan 3-ols type of flavonoids present in brewed green tea. The mean values in this table are reported as mg/100 g of fresh weight of edible portion of food. All tea infusion values are standardized to 1 % infusion (1 g tea leaves/ 100 cm^3 boiling water). Values for tea are given as mg/100 g (100 cm^3) of tea infusions (as consumed) and are equivalent to one gram of dry tea. The main flavonoids in decreasing order of amount present are respectively: epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC).

Table 2.2. Flavonoid content of brewed green tea. (USDA database for the flavonoid content of green tea- release 3- 2011)

Flavonoid	Mean (mg/g dry tea)	Standard Error	Number of sources
Epigallocatechin gallate	64.15	5.02	12
Epigallocatechin	22.27	0.89	12
Epicatechin gallate	16.39	1.93	12
Epicatechin	7.36	0.31	11
Catechin	3.28	0.88	6
Gallocatechin	1.54	n.a.	1

The values in Table 2.3 show the composition of a black tea extract, from different references (Balentine et al., 1997; Harbowy and Balentine, 1997; Liang et al., 2003; Sanderson et al., 1972). The type of tea used, the extraction conditions and the analytical methods used can have a significant influence on the reported values.

Table 2.3. Black tea extract composition (% w/w)

Constituent	Harbowy, 1997	Sanderson et al., 1972	Balentine et al., 1997	Liang et al, 2003	Experimental
Catechins	4	11	3-10	3.33	4.75
Thearubigins	17	36	23	n.a	n.a.
Theaflavins	2	3	n.a.	0.85	0.67
Caffeine	7	n.a.	3-6	4.53	6.71
Proteine	11	6	6	n.a.	26.34
Free amino acids	5	7	6	2.86	n.a.
Carbohydrates	14	4	11	n.a.	n.a.
Organic acids	11	2	2	n.a.	n.a.
Ash	n.a.	10	10-13	n.a.	n.a.

Table 2.4 presents in detail the flavan 3-ols type of flavonoids present in brewed black tea. The mean values in this table are reported as mg/100 g of fresh weight of edible portion of food. All tea infusion values are standardized to 1% infusion (1g tea leaves/100 ml boiling water). Values for tea are given as mg/100 g (100 ml) of tea infusions (as consumed) and are equivalent to one gram of dry tea. The main flavonoids amounts in decreasing order are respectively: thearubigins, epigallocatechin gallate, epigallocatechin, epicatechin gallate and epicatechin.

As referred before the experimental values are obtained for extracted BMF green tea and BMF black tea. The results obtained for the polyphenols in this type of tea are lower than those reported in literature. However, when using tea from different locations the polyphenols values can be significantly different.

Table 2.4. Flavonoid content of brewed black tea.
(USDA database for the flavonoid content of black tea- release 3-2011)

Flavonoid	Mean (mg/g dry tea)	Standard Error	Number of sources
Epicatechin	2.13	0.10	10
Epicatechin gallate	5.86	0.17	10
Epigallocatechin	8.07	0.45	10
Epigallocatechin gallate	9.36	0.46	10
Catechin	1.51	0.07	5
Gallocatechin	1.25	0.22	2
Theaflavin	1.58	0.16	3
Theaflavin-3, 3'-digallate	1.75	0.21	3
Theaflavin-3'-gallate	1.51	0.16	3
Theaflavin-3-gallate	1.25	0.14	3
Thearubigins	81.30	9.76	2

2.3.2. Output composition (green and black tea extract)

For the output a first estimation for the economic potential calculation is based on the revenue of product sales and on the cost of raw materials (Moggridge and Cussler, 2000). The results are used to determine the output products for this work, using the market benchmark purities for catechins and theaflavins. Equation 2.1 is used to relate the raw materials and the products at current prices.

Economic potential= (products sales/year) ─ (raw material costs/year) (2.1)

This preliminary economic potential gives a first indication of the process profitability. Several assumptions are made for this calculation (Appendix A).

Tables 2.5 and 2.6 present the economic potential for five different scenarios. Although in this thesis the output only takes into account the production of catechins and theaflavins, an alternative scenario is included where instead of the catechins a specific catechin, the epigallocatechin gallate (EGCG) is produced. Due to the higher market price of EGCG (with 95 % purity) compared to the price of catechins (with 90 % purity), this can translate into a higher economic potential, see Appendix A. For the economic potential calculations polyphenols standard market purities are selected.

The values in Table 2.5 demonstrate that the economic potential for the green tea stream is positive for the two presented scenarios. This preliminary economic potential analysis is positive for the green tea stream for the recovery of catechins with 90 % purity as well as for the recovery of EGCG with 95 % purity.

Table 2.5. Economic potential evaluation for green tea

Scenario 1. Green tea: produce catechins (90 % purity)	
Maximum product sales (M€/year) =	3.20
Raw material costs (M€/year) =	0.36
Economic potential (M€/year) =	2.84
Scenario 2. Green tea: produce EGCG (95 % purity)	
Maximum product sales (M€/year) =	7.42
Raw material costs (M€/year) =	0.36
Economic potential (M€/year) =	7.06

For the black tea stream (Table 2.6) the economic potential is only positive if both catechins and theaflavins are produced (Scenario 4 and 5). This implies that the output for the black tea needs to include both catechins and theaflavins. Since the objective of this work is not to obtain the fractions of the individual catechins, the production of EGCG will not be considered. The EGCG possibility is only mentioned to highlight the high economic potential.

Table 2.6. Economic potential evaluation for black tea

Scenario 3. Black tea: produce theaflavins (60 % purity)	
Maximum product sales (M€/year) =	0.37
Raw material costs (M€/year) =	0.54
Economic potential (M€/year) =	-0.17
Scenario 4. Black tea: produce catechins (90 % purity) and theaflavins (60% purity)	
Maximum product sales (M€/year) =	1.23
Raw material costs (M€/year) =	0.54
Economic potential (M€/year) =	0.69
Scenario 5. Black tea: produce EGCG (95 % purity) and theaflavins (60 % purity)	
Maximum product sales (M€/year) =	0.87
Raw material costs(M€/year) =	0.54
Economic potential (M€/year) =	0.33

There are two output streams matching the two input streams: one for green tea and the other for black tea. The selected output from the green tea is a dry powder with 90 % catechins and the selected output from the black tea is a dry powder with 90 % catechins and 60 % theaflavins. The output streams have to be microbiologically safe and the process needs to be hygienic and to use a non-toxic solvent route (Figure 2.2).

Figure 2.2. Input and output level for green and black tea.

2.4. PDPS application: Task network (Level 4)

At the task network level, the process is decomposed into fundamental tasks corresponding to the necessary properties to transform the raw materials into the final products.

A general task network structure with the fundamental tasks is presented. The fundamental tasks required for process synthesis have been originally developed for application in the production of structured food products. Since in this project the output is not a structured product but a product composed of target components, mainly separation tasks will be used.

2.4.1. Mechanism and factors influencing the separation of polyphenols from tea

There are two input aqueous streams: one green tea extract and one black tea extract containing in both cases 4 % of total solids and at 70 °C. For this conditions there is no tea cream formation, see section 1.4.

Factors influencing tea cream formation include solids concentration and pH as well as the temperature and duration of the extraction. The creaming properties of teas differ greatly depending on the content of the macromolecular components, including proteins and polyphenols. Tea creaming is a phase separation caused by both interpolymer complexation and limited co-solubility of these heterogeneous complexes with one another and with other macromolecules (Tolstoguzov, 2002).

Tea cream is considered to be an inhibitor for the polyphenols separation, since it decreases the amount of polyphenols available in the clear phase. For the amount of tea solids in the input stream (4 % solids), for black tea there is only cream formation below 60 °C and for the green tea below 45 °C (Figure 1.8). Because both the input streams are at 70 °C, as long as the temperature is kept above the cream formation temperatures, no cream will be formed. The amount of total solids influences the amount of cream formed, but not the cream composition. This is probably due to the polyphenols that promote the tea cream effect.

Food products are typically structured products where the performance is determined by the internal microstructure of the product. In the case of structured products, the process usually requires less reaction and separation tasks, and more mixing and preservation tasks. However, in the work reported in this thesis where target components need to be recovered from a complex mixture, mainly separation tasks are selected.

The components present in the tea can also be divided according to their solubility in water. This division can be used for separations based on the solubility.

- Components soluble in cold water:

Polyphenols, caffeine, aminoacids, carbohydrates and organic acids.

- Components partially soluble in hot water:

Polysaccharides, proteins, ash and lipids.

- Components insoluble in water (Tea fines):

Cellulose, lignin, chlorophylls and crude fiber.

Several other properties can be used for the polyphenols separation from tea extract streams, such as: molecular size, polarity, hydrophobicity and chemical affinity. All these properties can be related to fundamental tasks and mechanisms in the PDPS methodology.

The next fundamental tasks are considered to be necessary to change the attributes and are selected for further application:

- Solvation (change the solubility of the target polyphenols in the desired phase)
- Separation of a system into two systems with different composition (allows the separation of the target polyphenols from undesired components and the purification of the target polyphenols)
- Evaporation of the solvent to get the dry powder purified polyphenols.

Although there are differences in the composition of green and black tea (including the creaming formation mentioned before), it should be possible to generate the same task network for the two types of tea after a pre-treatment step that will take into account the cream formation. Nevertheless, if necessary, a differentiation in the green and black tea processes can be included later. This will be decided in the next levels of the PDPS methodology.

The task network for the separation of polyphenols from tea is generated based on 3 main steps:

1. Separation of insoluble components
2. Separation and purification of polyphenols to a target purity
3. Obtaining the polyphenols in the form of dry powder

The second step can be decomposed into a 2 steps separation, where non target components are separated in a first step and the polyphenols are separated in a second step.

To select the most viable routes (Hill, 2004), the following heuristics based on expert knowledge (Unilever) can be applied in the process of selecting alternative process sequences :

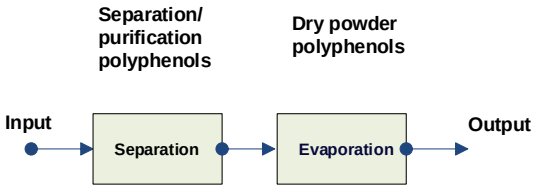
- Smaller size components should only be removed after the removal of large size components and easy separations are preferred. This means that the tea fines (insoluble components in the extract) should be removed in the first step. It would also be advantageous to have only one phase (in this case a liquid phase) for the rest of the process.
- Complex forming reactive components should be removed as soon as possible.
- Separation of the solvent from the polyphenols by evaporation can be used in the last step of the task network. To avoid polyphenol degradation, elevated temperatures must be prevented. When the heat of evaporation is fairly high and

the product is exposed to relatively high temperatures for a long period of time, the polyphenols may lose their biological activity.

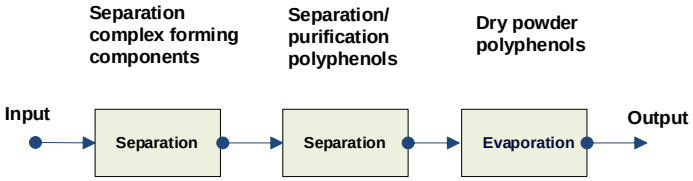
2.4.2. Task network for green tea

The following two alternatives are generated for the transformation from input into output based on the information from the previous section:

Alternative 1



Alternative 2

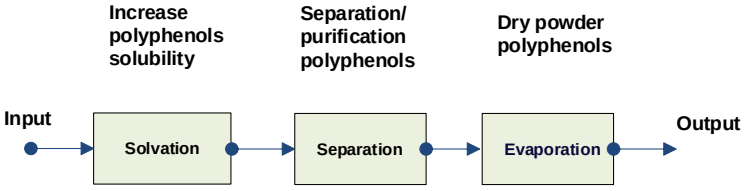


The difference between the two proposed alternatives is the existence of a first step to separate the components that can form complexes and tea cream. This step is closely related to the operational temperature and it will be accessed later.

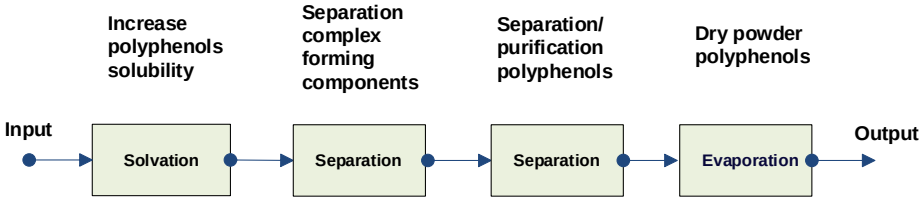
2.4.3. Task network for black tea

For the black tea two alternatives are also generated:

Alternative 1



Alternative 2



The alternatives presented can be combined in different sequences with the limitation that the third step (separation/ purification of polyphenols) and the fourth step (formation of the dry powder) must be the last two steps of the process and in this specific order.

The performance of the alternatives can be analyzed with an economic evaluation that takes into account the process temperature variation, which is directly connected to polyphenols degradation (catechins have a higher thermal stability than theaflavins) and cream formation. If the temperature is high enough to avoid tea cream formation, no solvation task will be required.

Analyze performance of alternatives (green and black tea)

Scenario 1.

In the first scenario the temperature will be set at 50 °C. At this temperature, tea creaming occurs for black tea but not for green tea. This means that losses of polyphenols due to cream formation in the black tea have to be taken into account. At 50 °C a 5 % degradation is reported for the EGCG during a 3 hours period (Wang et al., 2008). A similar degradation is assumed for all the catechins.

No information could be found in the literature about the theaflavins degradation at 50 °C. However, as the thermal stability of theaflavins is less than that of catechins, the loss by degradation is above 5 %. An approximation can be made using the same increase in the degradation rate between 50 and 70 °C as for the catechins. This means that at 50 °C the theaflavins will have 10 % degradation during a 3 hours period.

The preliminary economic evaluation is performed in the same way as in the input-output level of the PDPS and takes into consideration the above values for the polyphenols degradation.

In this scenario the economic potential is still very favorable for the green tea. For the black tea, however, it is only slightly positive. The total economic potential for scenario 1 is 3.05 M€/year (Appendix A).

Scenario 2.

In the second scenario the process temperature is 70 °C. At this temperature there is no cream formation in both green and black tea streams. To calculate the preliminary economic potential it will be assumed that the full process cycle will take around 2 hours. For this time and at this temperature up to 20 % of catechins and 42 % of theaflavins will degrade. A less favorable case will assume that the full process will take 3 hours, which can result in the degradation of up to 29 % for the catechins and 56 % for the theaflavins (Lun Su Y. et al., 2003).

For a process time of 2 hours the total economic potential is 2.56 M€/year (Appendix A). For a process time of 3 hours the total economic potential for this scenario is 2.15 M€/year the economic potential with most of the contribution coming from the green tea (Appendix A).

An alternative to avoid the polyphenols degradation is to allow the temperature to drop below the cream formation temperature. In this case, a change in the spatial distribution of the phases would occur, with separation of the components into the cream phase (including a substantial amount of polyphenols that are present in this phase). This hypothesis is further investigated for the green tea (Chapter 3) and for the black tea (Chapter 4).

Screening experiments for the black tea cream formation were conducted to confirm whether it is possible to work in a lower range of temperatures, with a reduced amount of cream and a higher amount of theaflavins and catechins in the clear phase. Four factors (% solids, temperature, pH and EDTA) are tested to see if they affect the theaflavins and catechins availability in the clear phase. The first hypothesis is that the EDTA can work as a chelating agent on the metals that induce the cream formation. The second hypothesis is that the other three effects (% solids, temperature and pH) can improve the solubility of the complexes responsible for the tea creaming.

Table 2.7. Influence factors importance on black tea polyphenols availability (cream effect).

	% solids	Temperature	pH	EDTA
Theaflavins	X X X	X X	X	
Catechins			XXX	

According to the obtained results the stronger effects for the theaflavins are mainly the percentage of solids, followed by the temperature. The pH also has some effect, although smaller.

In the case of the catechins the pH has the strongest effect, although there is also a significant interaction effect between the solids content and the temperature. The full results are presented in detail in Chapter 4.

The screening experiments show that the solubility of theaflavins forming complexes can be increased mainly by the amount of solids and temperature variation. In the case of catechins forming complexes, there is an indication that their solubility can mainly be increased by varying the pH.

2.5. Conclusions

At the input-output level of the PDPS a preliminary economic potential evaluation is used to determine the output streams. At this level it was defined that there are two input streams with 4 % of solids: one for green tea and the other for black tea. The output from the green tea is a dry powder with 90 % catechins purity. The output from the black tea is a dry powder with 90 % catechins purity and a dry powder with 60 % theaflavins purity.

At the Task network level of the PDPS methodology one of the key issues is the possible need of using a solvation step due to tea cream formation. If the temperature is high enough to prevent the tea creaming, a solvation step will not be necessary.

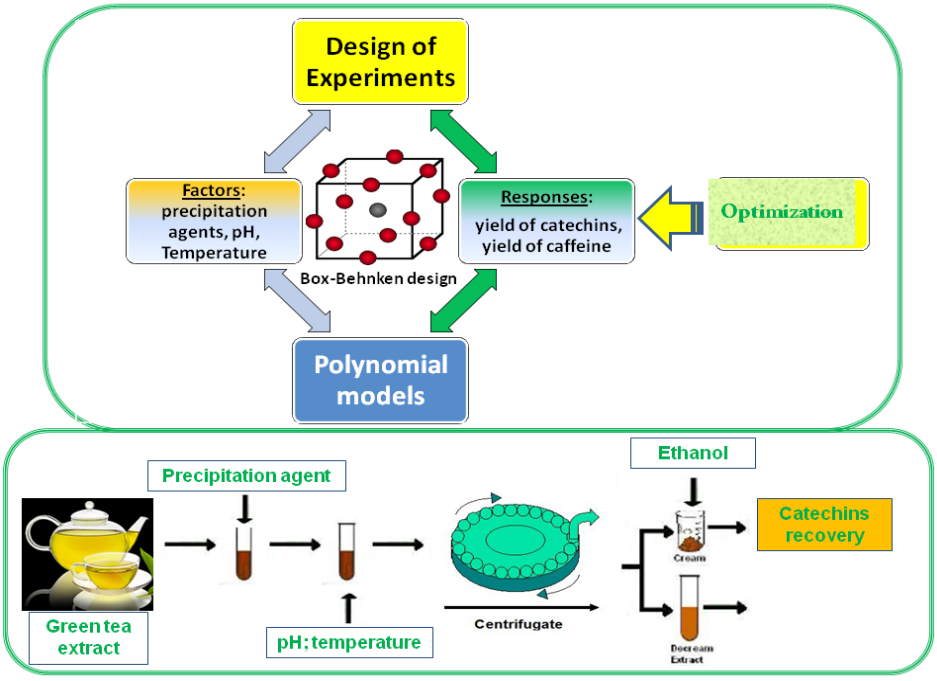
Another issue is the significant polyphenols degradation (mainly for the theaflavins) at relatively high temperatures. Two main scenarios are evaluated at 50 °C and at 70 °C. At the lower temperature the economic potential is higher when compared with scenario at 70 °C (3.05 M€/year vs 2.15 M€/year). In the preferred scenario the process temperature is 50 °C. At this temperature, tea creaming occurs for the black tea but not for the green tea. To the black tea stream a fundamental task (solvation) needs to be applied, to increase the solubility of the target polyphenols and separate the cream formed.

For this reason an alternative process that uses lower temperatures is explored in Chapter 3 (green tea) and in Chapter 4 (black tea). The outcome of this chapters will also be useful to evaluate the need of a step to separate complex forming components.

The later levels of the PDPS methodology are presented in Chapter 7 in the form of a conceptual design process.

3.

Optimization of green tea catechins precipitation



ABSTRACT: This chapter reports the use of an enhanced tea creaming effect as a phase separation via precipitation, for the recovery of catechins from green tea. The tea cream formation occurs after the extraction process upon cooling, due to the different solubilities of the tea components in hot and cold water. The precipitation process is the result of complex formation when oligomeric and polymeric molecules, like proteins and pectins interact with each other or with small molecules like catechins and caffeine.
The separation process is described with polynomial models resulting from the statistical analysis of the experimental results. The experiments are generated via a design of experiments (DoE). Four precipitation influence factors are studied: two precipitation agents (hydroxypropylmethylcellulose and polyvinylpyrrolidone), temperature and pH.
In a second step, the model is used to determine the optimal conditions that maximize the catechins recovery, while minimizing the amount of caffeine. With this process it was possible to recover a high amount of catechins from the cream phase without the use of toxic solvents.

3.1. Introduction

As previously referred green tea catechins are valuable components for their health benefits and the fact that they can be added to food for quality improvement (Vuong et al., 2011). There are, however, other components present in a tea extract (such as proteins, carbohydrates, caffeine …) which need to be separated from the catechins. The separation of some of the tea components is not a straight forward process, due to the similarities in molecular size and solubility.

Another problem is the use of organic solvents, such as dichloromethane and ethyl acetate in the separation and purification processes, which is a big disadvantage, since the solvent residues are associated with potential harmful effects (Perva-Uzunalic et al., 2006). Since the objective is to be able to label the final product as natural, only food grade solvents (water and ethanol) are considered. The use of natural ingredients brings major marketing advantages and because they are perceived as superior by the consumers, command premium prices.

3.1.1. Tea cream formation

Tea cream is formed after the extraction process upon cooling, due to the different solubilities of the tea components in hot and cold water. This natural precipitation occurs when oligomeric and polymeric molecules, like proteins and pectins interact with each other or with relatively small molecules like catechins and caffeine and form complexes (Tolstoguzov, 2002). Above a certain solids concentration or below a certain temperature, spontaneous macroscopic phase separation occurs (cream formation) in the tea. This shows

the substantial insolubility of polyphenols at high solids concentration and/or low temperature (Penders et al., 1998).

Most of the research about polyphenols separation focuses on the extraction steps (Bazinet et al., 2007; Labbe et al., 2006; Nawaz et al., 2006) and although the tea cream effect was already suggested as a separation route for the tea catechins in previous publications (Sekiya et al., 1984; Vuong et al., 2011), there is no reported research using multiple precipitation mechanisms that can be characterized with mathematical models, for the optimized separation of catechins from green tea. Precipitation also has the advantage of being a simpler and more economic than other processes like membrane separation or adsorption chromatography (Su et al., 2003).

3.1.2. Literature

The distribution of catechins and caffeine between the cream and clear phase, can be influenced mainly by the following mechanisms:

- Addition of a salt.

By adding aluminium chloride or zinc chloride to a green tea aqueous solution, it was found that the green tea catechins precipitate and that salt addition could be used for their separation (Chen et al., 2001; Wu and Bird, 2010). Inorganic salts like ammonium sulfate can also be used for green tea precipitation, since it is one of the best known salts for precipitation by salting out (Chan et al., 1986).

- Addition of a precipitation agent.

Polyvinylpyrrolidone (PVP) is a commonly used precipitating agent for specific polyphenolic compounds, due to the hydrophobic interactions and the formation of intermolecular hydrogen bonds between the hydroxyl group (–OH) of the polyphenol and the carbonyl group (–C=O) of PVP (Leiper et al., 2005). PVP also has the advantage that catechins rather than caffeine tend to form hydrogen bonds with the carbonyl group of PVP, together with the hydrophobic associations between the aromatic ring of the catechins and the pyrrolidone ring (Dong et al., 2011).

Methylcellulose (MCL) or other cellulose derivates are used for precipitation of polyphenols and for several precipitation-based assays, with the additional advantage of being non-toxic and food-grade (Cortes et al., 2010; Sarneckis et al., 2006). The polyphenols interactions with cellulose are also characterized and found to be positively correlated to the molecular size, the number of galloyl groups and the hydrophobicity of the polyphenols (Tang et al., 2003). This indicates that for the green tea catechins, the gallated forms have more interactions with cellulose.

- Temperature

Temperature can also influence green tea cream formation due to the inherent change in the solubility of the compounds (Penders et al., 1998; Xu et al., 2012). These authors found that the phase diagrams for tea have some analogy with the phase behavior of classical simple mixtures. These types of mixtures dissolve at high temperatures but separate into immiscible phases below an upper critical solution temperature, see Figure 1.8.

- pH

pH can also influence the amount of cream formation and the amount of polyphenols in each phase. However due to the low stability of green tea catechins in solution with pH ≥7, in this work only the acidic pH range is considered (Zhu et al., 1997).

3.2. Design of Experiments and statistical analysis

Green tea aqueous solutions and cream formation phenomena are very complex, in terms of the amount/diversity of components and interactions/associations of these components. This makes it very difficult to fully characterize and model such a system.

The use of design of experiments (DoE) coupled with statistical analysis is a very efficient way to model such a complex system and also produces a higher level of knowledge (including interaction effects), when compared to the more usual approach of studying several factors at a time.

A response surface methodology (RSM) includes several mathematical and statistical tools that can model and optimize a response of interest of an arbitrary experiment as a function of a selected set of variables:

$$y = f(d_1, d_2, ...d_i) \tag{3.1}$$

where y is the quantity of interest, called the response, d_i are the variables (factors) that affect the outcome of the experiment and f is the mathematical function that describes the influence of each factor on the final response.

The objective of the RSM is to find the best expression for the function *f*, while minimizing the number of experiments. The function f is a polynomial series that can be represented as follows (Montgomery, 1997):

$$f = a_0 + \sum a_i d_i + \sum a_{ij} d_i d_j + \sum a_{ii} d_i^2 + ... \tag{3.2}$$

where d_i, d_j are the independent variables and a_0, a_i, a_{ij} and a_{ii} are constants.

In this work, a response surface methodology (RSM) with Box-Behnken design (BBD) is used to find the best expression for a mathematical function with a minimal number of

experiments. This type of multivariate statistical analysis deals with two types of variables: responses and factors. The responses are the dependent variables, since they depend on the levels of the factors.

The BBD is a rotatable or nearly rotatable second-order design based on three-level incomplete factorial designs and has a complex confounding of interaction, where the experimental points are placed on the edges of an *N*- dimensional hypercube. This design allows multivariate optimization and it is based on mathematical models, which evaluate the relevance and statistical significance of the factors effects and also the interaction effects between factors. The BBD has the advantage of being more efficient than other response surface designs, like central composite or three-level full factorial design (Ferreira et al., 2007). The number of experiments (*N*) required for the BBD is defined as $N = 2k(k-1) + C_0$, where k is number of factors and C_0 is the number of central points.

A total of 27 experiments (4 factors and 3 center-points replications) are generated using the Statgraphics software in a randomized way (Table B.1- Appendix B). The factors are varied at 3 levels, representing the low, intermediate and high values of the selected range. Center-points are additional experimental runs in a midway point between the low and high level of every factor. The centerpoints are mostly the only replicated experiments in an experimental design and provide valuable information about the reproducibility.

The significance of the experimental data is evaluated via the Student's t value, in the form of Pareto charts (Montgomery, 2012; Souza et al., 2007; Zondervan et al., 2007). The parameters and combinations of parameters that significantly influence the selected responses were determined and fitted by a second order model that correlates it with the independent variables (factors).

3.3. Materials and methods

3.3.1. Reagents and equipment

In all the experiments demineralized water was used. Acetonitrile, and citric acid (analytical grade) were obtained from Sigma-Aldrich, The Netherlands.

Polyvinylpyrrolidone (PVP) with an average molecular weight of the polymer of 10,000, methylcellulose (MCL) with a viscosity of 0.4 Pa.s, hydroxypropylmethylcellulose (HPMCL) with a viscosity range of 0.08-0.1 Pa.s, ammonium sulfate (≥ 99 % purity) and polyvinylalcohol (PVA) were also obtained from Sigma-Aldrich, The Netherlands. Glacial acetic acid (HPLC grade), ethanol absolute and NaOH solutions (0.5 $mol.dm^{-3}$) were purchased from Merck KGaA, Germany. Zinc chloride and aluminium chloride (≥ 99 % purity) where from Fluka analytical, The Netherlands.

The individual polyphenols standards and the freeze-dried dry green tea powder were kindly supplied by Unilever R&D, The Netherlands. The water used in the HPLC analysis was Milli-Q gradient (Millipore).

The HPLC system is an Agilent 1220 Infinity LC gradient system equipped with a variable wavelength detector and a Luna 5µm Phenyl-Hexyl column (Phenomenex, The Netherlands), the ultracentrifuge is from Beckman Coulter (Optima L-90K) and the pH meter is from Inolab (WTW series-pH 730).

3.3.2. Analytical methods: HPLC analysis

Several techniques can be used for the analysis of tea components, including liquid chromatography LC–MS or high-performance capillary electrophoresis (HPCE). More recent methods include ultra-high performance liquid chromatography (UHPLC) and high performance liquid chromatography (HPLC) (Guillarme et al., 2010; Sharma et al., 2005). In this thesis HPLC methods are used for the analysis of tea polyphenols and caffeine.

The concentrations of caffeine and individual catechins were determined by HPLC analysis. The wavelength detector is set at 278 nm and a gradient elution is performed at 30 °C, using 2 % (v/v) acetic acid in water (Eluent A) and 2 % (v/v) acetic acid in acetonitrile (Eluent B) as mobile phases. The solvent flow rate is set at 1 ml/min with following gradient cycle: 5 % of B for 40 min, 18 % of B for 10 min, 18 % of B to 50 % of B over 0.1 min, 50 % of B for 5 min, 50 % of B to 5% of B over 0.1 min and 5 % of B for 20 min. For the HPLC analysis a series of three injections per sample was made. An analytical error of 3 % was considered, based on the calculated maximum relative standard deviation (standard deviation value divided by the average value).

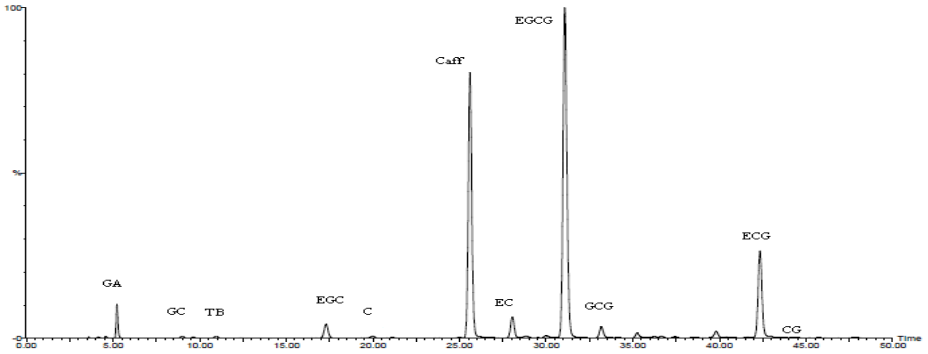


Figure 3.1. HPLC chromatogram for green tea.

A total amount of catechins is calculated by adding the amounts of the four main catechins present in green tea, i.e.: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate

(ECG) and epigallocatechin gallate (EGCG). An HPLC chromatogram for green tea is presented in Figure 3.1.

3.3.3. Precipitation experiments

Green freeze dried tea powder was dissolved in 30 ml of water at 85 °C in a 100 ml glass Erlenmeyer, while stirring for 10 min, to prepare an aqueous green tea solution with a solids content of 4% (w/w). The solid precipitation agents were then added to the tea solution (with stirring), followed by a pH adjustment. The pH adjustment was done either with citric acid (to lower the pH) or with NaOH solution (to increase the pH).

The tea solution was cooled at an average rate of 1.5 °C/min to the target temperature (see Table 3.3) and afterwards centrifuged for 25 min at 20,000 rpm at the same temperature. The clear phase was then decanted from the cream fraction. The resulting cream phase was extracted with 20 ml of ethanol at 20 °C for 2 hours (while stirring). Ethanol was used as extraction solvent, mainly because of the ability to form hydrogen bonds between its electronegative oxygen and the catechins hydroxyl groups (Hu et al., 2009). In the last step, the green tea extract, the clear phase and the cream phase were analyzed by HPLC to quantify the catechins and caffeine content.

3.4. Results and discussion

3.4.1. Screening experiments

With the objective of determining the relevant factors and an adequate design space for the experiments, screening experiments were performed. A total of three salts, i.e. aluminium chloride, zinc chloride and ammonium sulphate, and four precipitation agents were tested: methylcellulose (MCL), hydroxypropylmethylcellulose (HPMCL), polyvinylpyrrolidone (PVP) and polyvinylalcohol (PVA). The best results were achieved with the addition of the precipitation agents. The addition of salts resulted in a rather low yield of catechins (Table 3.2).

Precipitation agents were found to have a strong influence on the yield of catechins (variation between 25 to 63 %). From the tested precipitation agents, PVP and HPMCL leaded to the highest yields for the catechins. But the highlight result from the screening experiments is that the combination of these two precipitation agents leads to catechins yields up to 63 %. The PVP and HPMCL together with the two other selected factors (temperature and pH), add up to a total of four influence factors.

Table 3.2. Results of the screening experiments for precipitation with salts and with precipitation agents.

Screening factor	Yield of catechins (Y_{cat}) *
Control sample (no addition)	15-20
Zinc chloride	21
Aluminium chloride	22
Ammonium sulfate	25
PVP	39
MCL	27
PVA	25
HPMCL	31
MCL + PVP	45
HPMCL + PVP	63

***** see Equation 3.4

3.4.2. Design of experiments- factors and responses

Besides selecting the four influence factors it is also necessary to define the DoE design space, by choosing a variation range for each factor (Table 3.3). The justifications for the selected ranges are presented below.

- Hydroxypropylmethylcellulose (HPMCL): concentration range between 0 and 40 g/dm^3. In the screening experiments the value in the middle of this range, i.e. 20 g/dm^3 had the best results for the responses.
- Polyvinylpyrrolidone (PVP): range between 0 and 30 g/dm^3. In the screening experiments the value in the middle of this range, i.e. 15 g/dm^3 had the best results for the responses.
- Temperature (°C): range between 5 and 20 °C. At low temperatures tea creaming formation increases and green tea catechins have a higher stability (Jobstl et al., 2005).
- pH: range between 2 and 5. Below pH 5, green tea catechins are considered to be stable and the lower the pH, the higher the stability. At neutral and basic pH, considerable catechins degradation occurs after a two hours period. The stability progressively decreases with an increase in pH (Su et al., 2003; Zhu et al., 1997).

Table 3.3. Range for the DoE influence factors.

Factors	HPMCL (g/dm^3)	PVP (g/dm^3)	T (°C)	pH
Connotation	A	B	C	D
Low (-1)	0	0	5	2
Middle (0)	20	15	12.5	3.5
High (1)	40	30	20	5

To evaluate the DoE objectives (maximize the amount of catechins and minimize the amount of caffeine in the cream phase) two responses are selected: the yield of caffeine (Y_{caff}) and the yield of catechins (Y_{cat}). The responses are defined as follow:

$$\text{Yield of caffeine: } Y_{caff}(\%) = \frac{mass\,caffeine\,in\,cream\,phase}{mass\,caffeine\,in\,extract\,phase} * 100 \quad (3.3)$$

$$\text{Yield of catechins: } Y_{cat}(\%) = \frac{mass\,catechins\,in\,cream\,phase}{mass\,catechins\,in\,extract\,phase} * 100 \quad (3.4)$$

3.4.3. Design of experiments- statistical analysis

Figure 3.2 shows the standardized Pareto charts for the yield of caffeine (a) and the yield of catechins (b). In the y-axis the factors and interaction between two factors are presented. The single letters represent the factors: HPMCL (A), PVP (B), temperature (C) and pH (D). The interaction between two factors is represented by the combination of two different letters (ex. AB, BC, etc) and a second order effect for the factor is represented by a double letter (ex. AA, BB, etc). The effects are shown in decreasing order of significance (from top to bottom) and all the effects that cross the vertical line to the right side are considered statistically significant (for a 10 % level of significance). The bar length is proportional to the value of a t-statistic of the corresponding effect. The plus bars (grey colour) indicate a positive impact on the response, while minus bars (blue colour) indicate a negative impact.

In this work there are three statistically significant effects that influence the yield of caffeine (see Figure 3.2.a) and four statistically significant effects that influence the yield of catechins (see Figure 3.2.b). In both cases the strongest effect is the addition of PVP. The yield of caffeine is influenced by a first and second order effect of PVP addition and a second order effect of the temperature. The yield of catechins is influenced by a first and second order effect in both the HPMCL and PVP concentration. In this case there are no significant interaction effects. All the other studied effects are considered to be not statistically relevant.

The main effects plots (Figure 3.3) represents the effect over the responses when a factor is varied between the low and the high level, while all the other factors are kept constant at a midpoint. Figure 3.3 provides a clear representation of the linear or quadratic effect of the factors on the responses. In our case we only have quadratic effects. Both the HPMCL and the PVP have a maximum point, for the yield of catechins and the yield of caffeine. The temperature has a minimum point for the yield of caffeine and a maximum point for the yield of catechins.

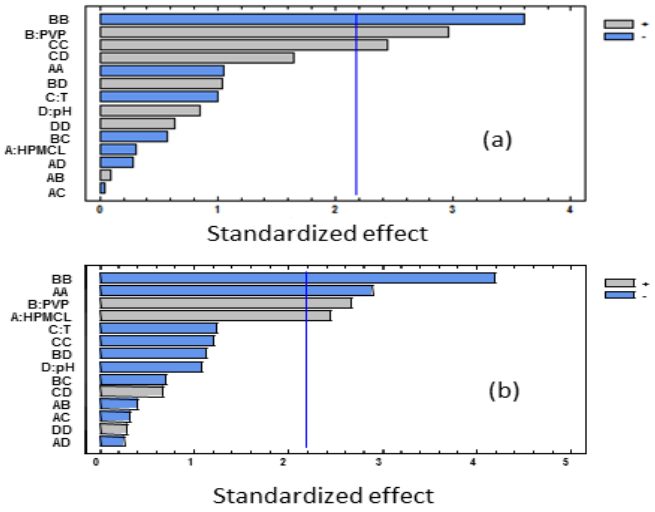


Figure 3.2. Standardized Pareto charts for yield of caffeine (a) and yield of catechins (b).

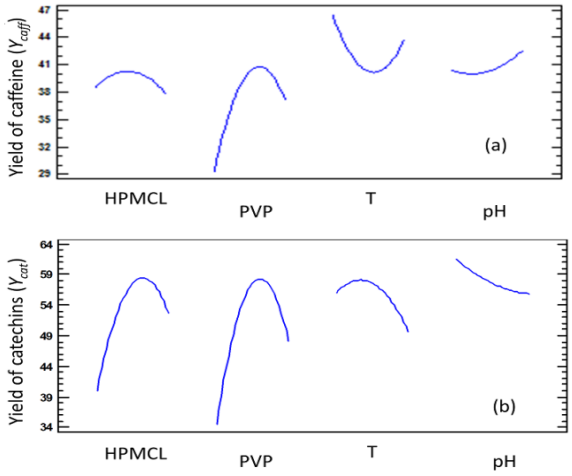


Figure 3.3 Main effect plots for the yield of caffeine (a) and the yield of catechins (b).

The screening experiments also indicated that HPMCL and PVP have an effect mainly for the gallated catechins, i.e. it indicates that it is important to also analyze the effects for the individual catechins.

The Pareto charts for the yield of individual catechins (Figure 3.4) demonstrate that PVP is the main factor for the yield of catechins. There is, however, a very significant difference between the gallated and the non-gallated catechins: while the PVP has a positive and linear effect for the non-gallated forms (EGC and EC), when it comes to the gallated forms, the

effect is negative and second order, see Figures 3.4 and 3.5. In addition, the effect of HPMCL is only relevant for the EGC (Figure 3.4.a) and it has a positive impact.

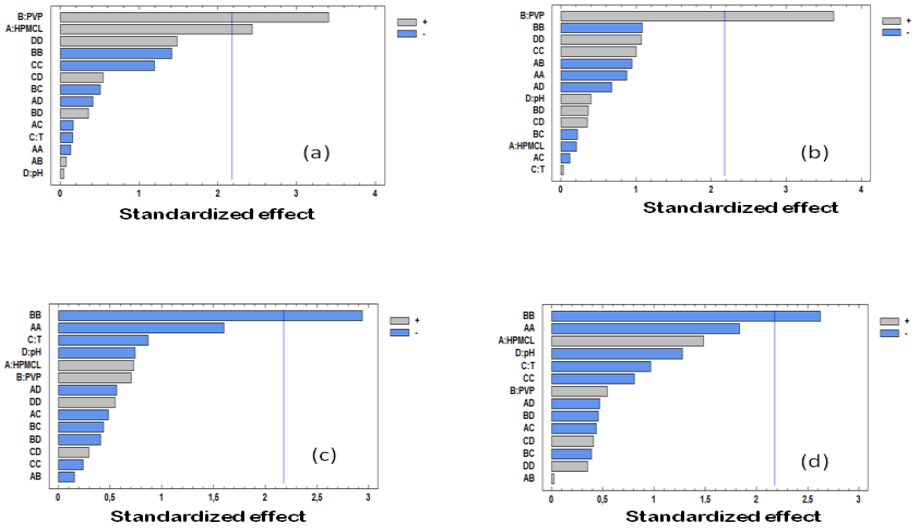


Figure 3.4. Standardized Pareto charts for the yield of individual catechins in cream: (a) EGC yield, (b) EC yield, (c) EGCG yield, (d) ECG yield.

The effects over the gallated catechins forms (EGCG and ECG) follow a very similar trend, while the non-gallated forms are affected in a different way by the HPMCL and the PVP. It can also be observed that the yields of the gallated catechins are significantly higher than the non-gallated forms, and that they are mainly responsible for the variation in the yield of catechins observed during the experiments (Figure 3.5).

There are several studies stating that the gallated catechins are predominant in the green tea cream. This might be related to the galloyl group and the hydroxylphenyl B ring (which behaves like a claw) and to the higher amount of hydroxyl groups that are available to form hydrogen bonds (Chao and Chiang, 1999; Liang et al., 2007; Mcmanus et al., 1985; Yin et al., 2009). The obtained results are consistent with those studies, where the gallated catechins account for 50– 80 % of the total catechins in green tea cream. Therefore, EGCG and ECG are considered to be the crucial catechins which participated in the tea cream formation (Yin et al., 2009). This is in line with the results that indicate that the catechin monomers with higher molecular weight and a high number of hydroxyl groups (EGCG > ECG > EGC > EC), bind more easily and strongly to the precipitation agent (Dong et al., 2011).

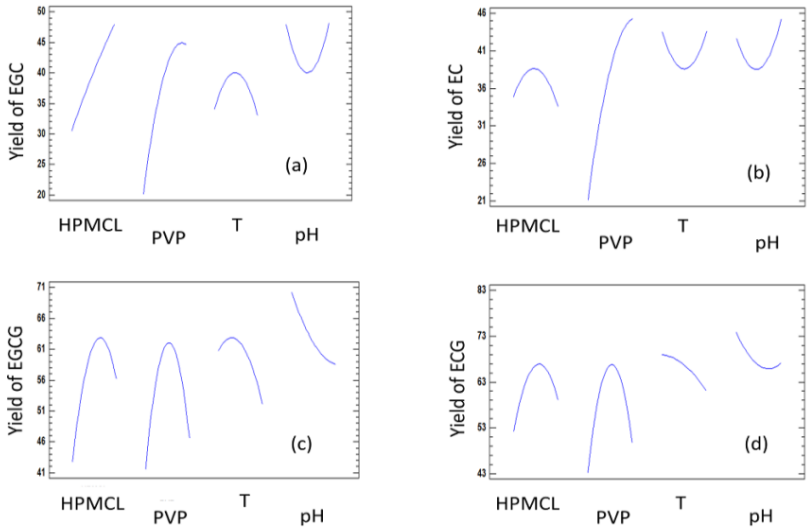


Figure 3.5. Main effect plots for the yield of individual catechins: (a) yield of EGC, (b) yield of EC, (c) yield of EGCG, (d) yield of ECG.

These results are also reinforced by a study comparing the formation of complexes between catechins and caffeine that form a precipitate, where it has been found that while EC forms a 1:1 complex with caffeine, ECG forms a 2:4 complex with caffeine. The caffeine-ECG complex is more hydrophobic than the caffeine-EC complex, indicating that the 2:4 complex between ECG and caffeine is predominant in an aqueous solution (Sato et al., 2012).

Based on the statistically relevant influence factors (Figure 3.3), for each response a second order polynomial model has been regressed (Table 3.4). The quadratic relationships link the influence factors to the responses and by testing the significance of the factors, it is possible to optimize the process by finding the parameters for the factors that maximize the amount of catechins and minimize the yield of caffeine.

Table 3.4. Fitted regression models for the selected responses.

Response	Fitted regression model
Yield of caffeine	$Y_{caff} = 45.07 + 1.17 * \mathrm{PVP} - 2.40 * \mathrm{T} - 0.0306 * \mathrm{PVP}^2 + 0.0891 * \mathrm{T}^2$
Yield of catechins	$Y_{cat} = 13.43 + 1.44 * \mathrm{HPMCL} + 2.63 * \mathrm{PVP} - 0.0275 * \mathrm{HPMCL}^2 - 0.0700 * \mathrm{PVP}^2$

The fitting and the distribution of the models can be evaluated with a parity plot. Figure 3.6 represents the parity plots for the two selected responses which indicate that the models

have reasonably good fitting, with the R^2 values above 0.84. The points show a good distribution and are randomly scattered around the diagonal line.

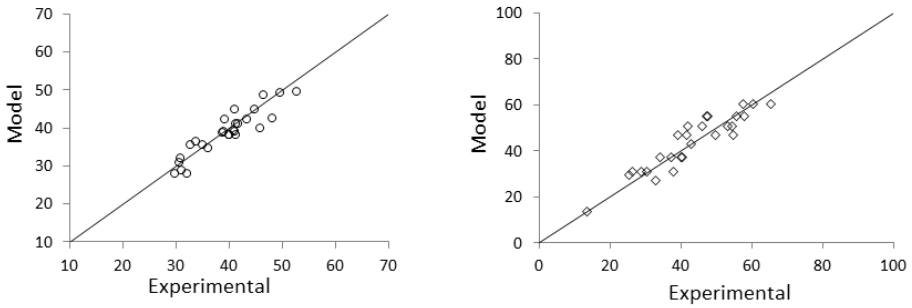


Figure 3.6. Parity plots for the yield of caffeine (left) and the yield of catechins (right).

3.4.4. Design of experiments- Response surface plots

The response surface plots (Figure 3.7) for the two selected responses show a representation of their variation over the entire design space, with the non-significant factors fixed at the intermediate value.

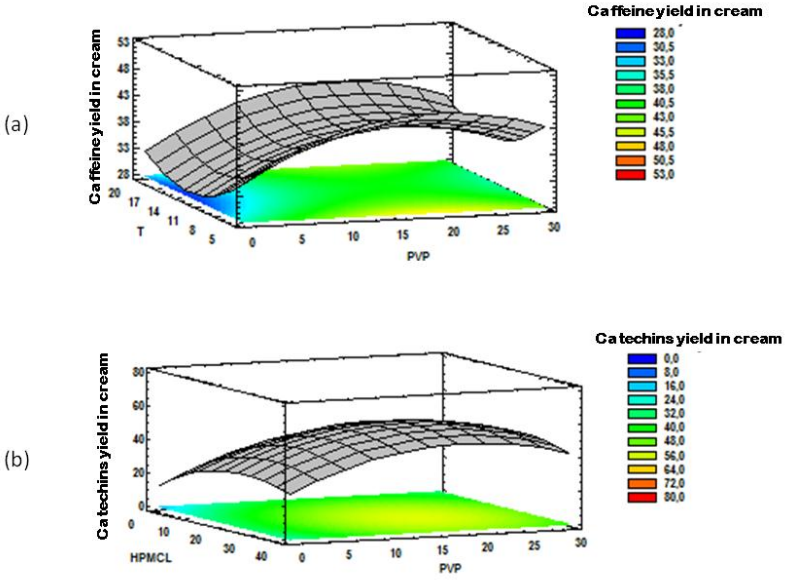


Figure 3.7. Response surface plots for yield of caffeine (a) (fixed HPMCL and pH value at the central point) and yield of catechins (b) (fixed T and pH value at the central point).

The lowest values for the caffeine yield are observed in the region with a low amount of PVP. According to the yield of catechins response surface plot, the best results are obtained

for the middle range of HPMCL and PVP (center of the design space), which can also be observed by the parachute shape type of surface that represents the variation for the yield of catechins. According to the model in Table 3.4, the maximum theoretical yield obtained for the catechins is 67 % (see Figure 3.7.b). This value compares with a yield in the range of 15-20 % for the green tea catechins, without any precipitation enhancement, see Table 3.2.

The results presented in Table 3.5 are in the form of a desirability function (D), where the predicted values from each response surface are transformed into a dimensionless scale d_i. The scale of the desirability function ranges between d=0 (for an unacceptable response value) and d=1 (for a completely desirable one). The desirability function value is the geometric mean of the combined individual desirability values for a given set of m responses: $D = (d_1 \times d_2 \times \ldots d_m)1/m$ (Bezerra et al., 2008). The desirability function is then maximized via an algorithm that determines the set of optimal values for the variables and it can be presented in the form of a desirability plot (see Figure 3.8).

Recalling that the objective of this DoE is to maximize the yield of catechins and minimize the yield of caffeine, a graphical representation of this objective function (desirability function) is obtained in Figure 3.8. The desirability plot for the two responses is presented as a function of the three significant influence factors: HPMCL, PVP and temperature. In a scale from 1 to 5, a weight factor of 5 is attributed to the more important response (yield of catechins) and a weight of 2 is attributed to the less important response (yield of caffeine).

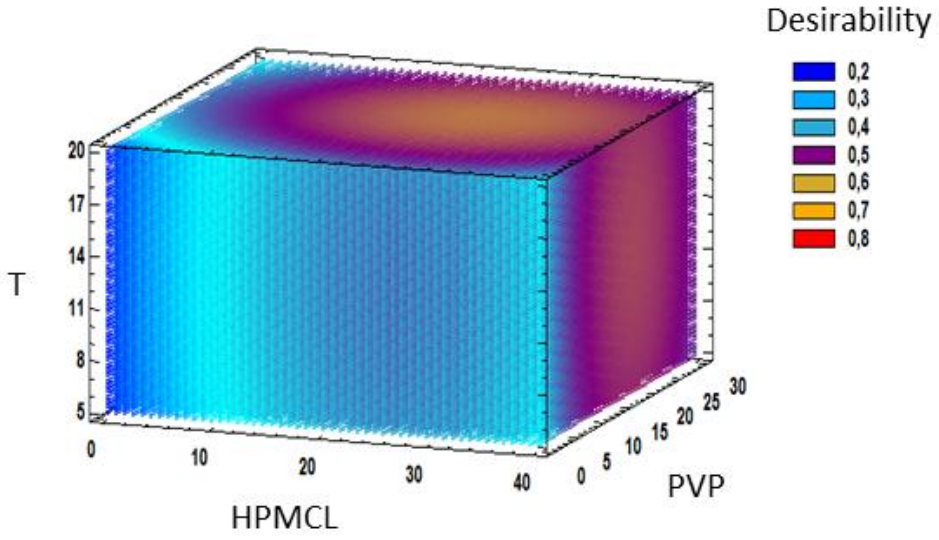


Figure 3.8. Desirability plot for the DoE (fixed pH =3.5).

The higher desirability values are obtained in the region matching the intermediate or high values of HPMCL and PVP. Temperature does not have a strong influence on the desirability optimal region.

The maximum optimal overall desirability value obtained 0.66 and this corresponds to a Y_{caff} = 40 %, a Y_{cat} = 64 % and to the following combination of factors: 26 g/dm^3 of HPMCL, 18 g/dm^3 of PVP, T of 13.5 °C and a pH of 2.4 (Table 3.5). This optimum also results into a 60 % increase in the ratio of catechins/caffeine, when compared to the values of the initial green tea extract.

Table 3.5. Model response values and overall desirability at the optimum

Responses	**Optimum**	
	Prediction (%)	**Overall desirability (D)**
Yield of caffeine (Y_{caff})	40.1	0.66
Yield of catechins (Y_{cat})	63.8	

3.5. Conclusions

This work demonstrates that the naturally occurring tea cream precipitation can be intensified to maximize the amount of catechins recovered, while minimizing the amount of caffeine (considered a contaminant) and using only non-toxic food grade solvents. This precipitation process is much simpler from an operational point of view, than other processes like membrane separation or column chromatography.

The statistical analysis indicated that the amounts of precipitation agent (PVP and HPMCL) are the most significant factors that affect the yield of catechins, while the amount of PVP and temperature are the most significant factors for the yield of caffeine. pH is the only non-significant factor and PVP is overall the highest process impacting factor.

The gallated and non-gallated forms of the catechins behave differently during the precipitation process. The yields of the catechins gallated forms were much higher than the yields of the non-gallated forms, meaning that gallated forms were mainly responsible for the improved precipitation and the consequent variation observed in the yield of catechins. This suggests that it is beneficial to use a tea with a high content of gallated catechins, since it will increase the amount of green tea cream and favor precipitation as a separation method for green tea catechins.

According to the developed model the process achieved a maximum theoretical yield of 67 % for the green tea catechins. This compares with a yield in the range of 15-20 % for the green tea catechins, whiteout any precipitation optimization. The achieved increase in the amount of catechins in the cream phase proves that there is a change in the phase distribution of the catechins between the clear and cream phases. The best experimentally

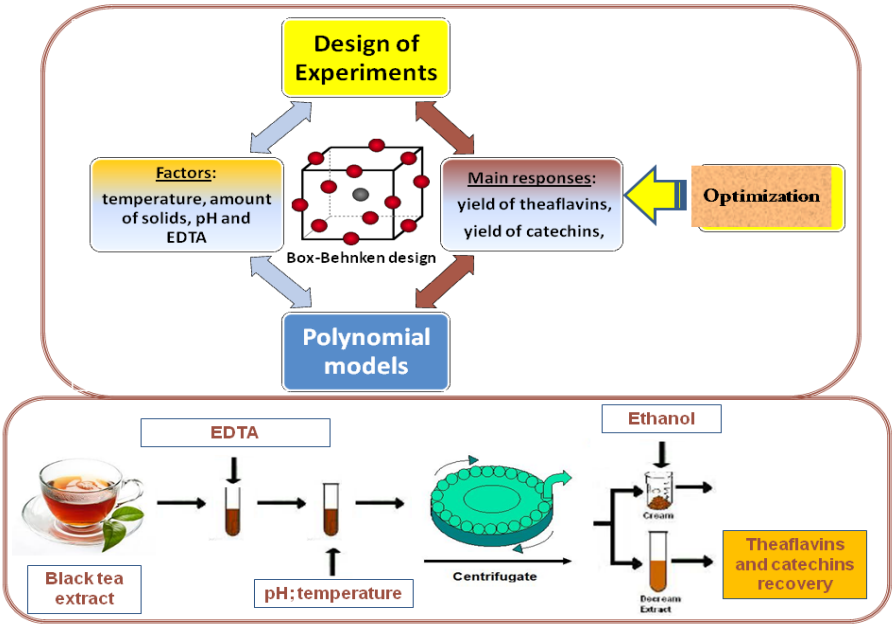
achieved yield of catechins was 69 %. It should be further noted that it is possible to increase the ratio catechins/caffeine in the cream phase by 60 %.

3.6. Nomenclature

BBD	Box-Behnken Design
Caff	Caffeine
Cat	Catechins
C_0	Number of central points in BBD
DoE	Design of experiments
EDTA	EDTA
EC	Epicatechin
EGC	Epigallocatechin
ECG	Epicatechin gallate
EGCG	Epigallocatechin gallate
GA	Gallic acid
HPMCL	Hydroxypropylmethylcellulose
k	Number of factors in the BBD
MCL	Methylcellulose
PVA	Polyvinylalcohol
PVP	Polyvinylpyrrolidone
RSM	Response surface methodology
T	Temperature

4.

Black tea cream effect on polyphenols optimization using statistical analysis



ABSTRACT: Black tea cream formation occurs naturally during the tea extraction process, as part of the compounds that are soluble in hot water turn out to be insoluble in cold water and form a precipitate, known as tea cream. The cream formation is an inhibitor for the polyphenols separation since it decreases the amount of available polyphenols. Four factors (temperature, amount of tea solids, pH and amount of EDTA) are studied to assess the impact in the polyphenols availability and in the amount of tea cream formed. The objective of this work is to determine the optimum combination of factors that minimize the cream formation and maximize the amount of polyphenols available in the clear phase.

By using a design of experiments instead of a one-factor-at-a-time, additional information such as interaction effects can be obtained. Statistical analysis is used to determine which factors significantly influence the responses. The outcomes are further used to generate polynomial models. This is a very effective strategy and indicates that EDTA is the only non-relevant factor. By using the right combination of factors it is possible to strongly increase the yield of polyphenols in the clear phase to the range between 80 and 90 %.

4.1. Introduction

Black tea polyphenols are formed during the fermentation step (controlled enzymatic reactions) of green tea leaves. Tea cream formation occurs after the tea extraction process, when the tea cooling process starts and part of the compounds that are soluble in hot water, turn out to be insoluble in cold water and precipitate.

4.1.1. Black tea cream formation

The tea cream formation is strongly dependent on temperature and amount of tea solids, which affect the solubility and it is particularly relevant in the case of black tea (due to the high amount of tea cream formed), when compared to other types of teas (Figure 1.8).

Although the exact tea cream formation mechanism is not known, it is considered to be caused by interpolymer complexation. The cream formation occurs due to the change in molecular weight and solubility of phenolic polymers upon complexation with several other components, e.g. proteins, polysaccharides, lipids and metal cations. The composition of the two formed phases is different: while the tea cream phase contains mainly polyphenol-protein complexes, the clear phase contains the pectin-polyphenol complexes (Tolstoguzov, 2002).

Typically for a black tea with up to 10 % of solids the temperature needs to be maintained above 70 °C to prevent the tea cream formation (Penders et al., 1998). The authors found that the phase diagrams for black tea cream have some analogy with the phase behavior of mixtures of simple compounds. These types of mixtures have one phase at high temperatures, but separate into immiscible phases below an upper critical solution

temperature. There are, however, other factors like pH and chelating agents that can also influence the cream formation and the ratio of polyphenols in the clear and cream phases (Jobstl et al., 2005; Tolstoguzov, 2002; Wu and Bird, 2010).

4.2. Design of Experiments and statistical analysis

Similarly to the previous chapter a Design of Experiments (DoE) has been setup to fully characterize the selected design space with polynomial models, with the objective of minimizing the cream formation and maximize the amount of polyphenols in the clear phase. The resulting models can be used to optimize the responses and can also be extrapolated to the outside of the design space.

The DoE has several advantages over the more conventional approach of using one-factor-at-a-time (OFAT), including a better relative efficiency, since it quantifies interactions between the different factors and covers a broader design space, resulting in a higher process knowledge.

The variation interval for the four selected influence factors; temperature (T), amount of solids, pH and concentration of chelating agent (EDTA) is detailed in section 4.3.1. The influence of these factors is pre-tested with screening experiments, to assess the significance of the effects on the amounts of polyphenols distributed over the two phases and to select the variation ranges for pH and EDTA.

The DoE objectives are evaluated with four selected responses: cream split factor (C_{sf}), yield of solids (Y_s), yield of theaflavins (Y_{tf}) and yield of catechins (Y_{cat}). As it is not known whether the influence factors have linear or nonlinear behaviour, a response surface methodology (RSM) and a Box-Behnken design (BBD) are proposed for modeling and optimization of the influence of some operating variables (see section 3.2). Statistical tests for significance and the development of quadratic relationships that link the influence factors to the responses, are used to optimize the process, i.e. to find the settings that minimize the cream formation and maximize the amount of polyphenols recovered.

4.3. Theory

In this work, the responses are fitted to a second order model that represents the correlation with the independent variables. The selected RSM design is the Box-Behnken design. The DoE experiments are generated using the Statgraphics software. A total of 27 experiments (4 factors varied in 3 levels (-1, 0, 1) and 3 center-points replication) are collected in Table B.2, see Appendix B, for the four experimental factors (A: amount of solids, B: pH, C: temperature and D: EDTA).

4.3.1. Factors

Besides selecting the four influence factors it is also necessary to set the DoE design space, by choosing a variation range for each factor (Table 4.1).

Table 4.1. Range for the DoE influence factors.

Factors	% solids	pH	Temperature (°C)	EDTA (g/dm^3)
Connotation	A	B	C	D
Low (-1)	2	3	20	0.02
Middle (0)	6	4.5	30	0.21
High (1)	10	6	40	0.4

The justification for the selected ranges is present below:

- *% solids (2-10 % solids)*: the selected range includes the typical concentrations for aqueous black tea industrial production. Above 10 % of solids, depending on the temperature, serious solubility issues may occur.

- *pH (3-6)*: black tea polyphenols are unstable at neutral and alkaline conditions. At very low pH some polyphenols degrade and there is an increase in the amount of tea cream (Tolstoguzov, 2002; Wu et al.,2010). At the proposed pH range (3-6) there is high stability for the complexes of EDTA with metals (Jhoo et al., 2005; Liang and Xu, 2003).

- *Temperature (20-40 °C)*: this is the range of temperatures where tea cream is formed for the corresponding range of amount of solids tested (Astill et al., 2001; Penders et al., 1998).

- *EDTA (0.02-0.4 g/dm^3):* The addition of a chelating agent allows the chelation of the metals that induce the cream formation. Theaflavins and calcium are known to form complexes that enhance cream formation, due to charge compensation, since theaflavins are acidic and carry a negative charge, leading to electrostatic repulsion between the charged surfaces of the cream particles. Positively charged ions have the ability to form complexes and compensate these negative charges, promoting aggregation and precipitation (Jobstl et al., 2005). EDTA was chosen as chelating agent since it has a high formation constant for several metal chelates (Howard and Wilson, 2003; Yamada et al., 2007).

4.3.2. Responses

To evaluate the DoE objectives, i.e. minimizing the cream formation and maximizing the amount of catechins and theaflavins in the clear phase, four responses are selected: cream split factor (C_{sf}), yield of solids (Y_s), yield of theaflavins (Y_{tf}) and yield of catechins (Y_{cat}). The responses are defined as follows:

Cream split factor: $C_{sf}\,(\%) = \frac{mass\,cream}{mass\,tea\,extract} * 100$ (4.1)

Yield of solids: $Y_s\,(\%) = \frac{mass\,solids\,in\,clear\ phase}{mass\,solids\,in\,extract\ phase} * 100$ (4.2)

Yield of theaflavins: $Y_{tf}\,(\%) = \frac{mass\,theaflavins\,in\,clear\ phase}{mass\,theaflavins\,in\,extract\ phase} * 100$ (4.3)

Yield of catechins: $Y_{cat}\,(\%) = \frac{mass\,catechins\,in\,clear\ phase}{mass\,catechins\,in\,extract\ phase} * 100$ (4.4)

4.4. Experimental

4.4.1. Reagents and equipment

Acetonitrile, EDTA and citric acid are analytical grade and obtained from Sigma-Aldrich, The Netherlands. Glacial acetic acid (HPLC grade) and NaOH solution (0.5 N) were purchased from Merck KGaA, Germany. Individual polyphenols standards were obtained from Unilever R&D. The water used in the HPLC analysis was Milli-Q gradient (Millipore). Freeze-dried dry black tea powder was supplied by Unilever R&D.

The ultracentrifuge is from Beckman Coulter (Optima L-90K) and the pH meter is from Inolab (WTW series-pH 730). For the HPLC analysis an Agilent 1220 Infinity LC gradient system equipped with a variable wavelength detector was used.

4.4.2. Analytical methods: HPLC analysis

The theaflavins analysis is performed in a C18 column (Hyperclone 3 μ m,) from Phenomenex.Inc, by isocratic elution with a solution of 80 % of acetic acid in water (2 % v/v) and a solution of 20 % of acetic acid in acetonitrile (2 % v/v). The concentrations in the eluent are determined by measuring the absorbance at a wavelength of 274 nm with a variable wavelength detector. Based on the calculated maximum relative standard deviation an analytical error of 4 % was considered. Total theaflavins are determined by the summation of the four theaflavins: theaflavin (TF), theaflavin 3 monogallate (TFMG), theaflavin 3' monogallate (TF3MG) and theaflavin digallate (TFDG).

The caffeine and individual catechins analysis are described in Section 3.3.2. The HPLC chromatograms for green tea and black tea are presented in Figure 4.1.

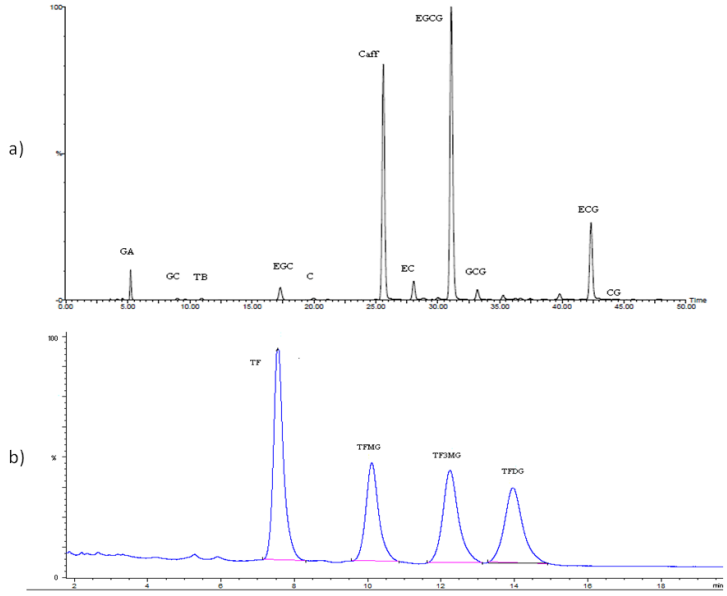


Figure 4.1. HPLC chromatograms for the polyphenols: a) catechins, b) theaflavins.

4.4.3. Sample preparation

Black freeze dry tea powder is dissolved in 50 ml of water at 85 °C in an Erlenmeyer, while stirring for 10 minutes to prepare a black tea extract with the target concentration. The EDTA is added to the tea extract immediately after extraction and the pH is adjusted while the tea is still at a temperature above 65 °C. The pH adjustment is made with the addition of citric acid (to pH=3) or a NaOH solution (to pH=6).

The tea extract is then cooled at a constant rate (average rate: 1.5 °C/min) to allow cream formation, until it reaches the target temperature of the experiment and kept at that temperature for the remaining time of the experiment (1 hour). The tea is afterwards centrifuged for 15 minutes at 20000 rpm at the same temperature. The clear phase is then decanted from the cream fraction and both fractions are weighted, to determine the cream split factor. Finally, tea extract, clear phase and cream phase are analyzed separately for catechins and theaflavins content.

4.5. Results and discussion

4.5.1. Design of experiments- statistical analysis

The statistical analysis is performed by the software Statgraphics and the selected Box-Behnken design is used to determine the optimal settings of the experimental factors. To

test the significance of the variability in the responses, for each of the selected effects, the results are plotted as standardized Pareto charts to allow a better visualization of the statistically significant effects (Figure 4.2).

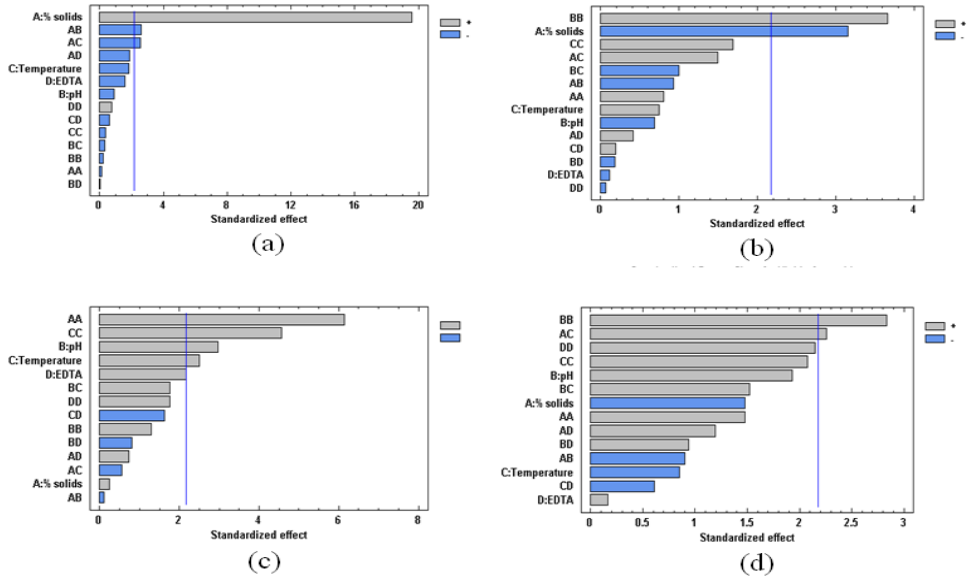


Figure 4.2. Standardized Pareto charts for the selected responses. (a) cream split factor, (b) yield of solids, (c) yield of theaflavins, (d) yield of catechins.

The factors are: % solids (A), pH (B), temperature (C) and EDTA (D). Similar to Chapter 3, when two different letters appear combined (ex. AB, BC, …) it represents the interaction between two factors and when the same latter appears twice (ex. AA, BB, …) it represents a second order effect for that factor. In Figure 4.2 the effects are shown in decreasing order of significance (from top to bottom). Any bars crossing the vertical line are statistically significant at the selected significance level (5 %). The plus bars means that the effect has a positive impact on the response and the minus bars represents a negative impact.

The results in Figure 4.2.a show that there are three statistically significant effects for the cream split factor (C_{sf}); the percentage of solids (A) and two interaction effects: percentage of solids with pH (AB) and percentage solids with temperature (AC). The results also clearly show that the percentage of solids has by far the strongest effect.

For the response yield of solids (Y_s) (Figure 4.2.b), only two effects are significant: a second order effect of the pH (BB) and the percentage of solids (A). Figure 4.2.c shows the results for the response yield of theaflavins (Y_{tf}), where four significant effects are found: two quadratic effects of percentage of solids (AA) and temperature (CC), one effect from pH (B) and one from the temperature (C). The yield of catechins (Y_{cat}) only has two

significant effects: a quadratic effect of the pH (BB) and an interaction effect between the percentage of solids and the temperature (AC) (Figure 4.2.d).

In the main effects plot (Figure 4.3) the lines indicate the estimated change in the response as each factor is changed from its low level to its high level, with all other factors kept constant at a value midway between their lows and their highs. This plot also provides a clear representation of the linear or quadratic effect of the factors on the responses. By analyzing Figure 4.3 it can be observed that the only linear effect is the percentage of solids for two of the responses (cream split factor and yield of solids). All the other effects have some degree of curvature in the selected variation range, meaning that they are second order effects.

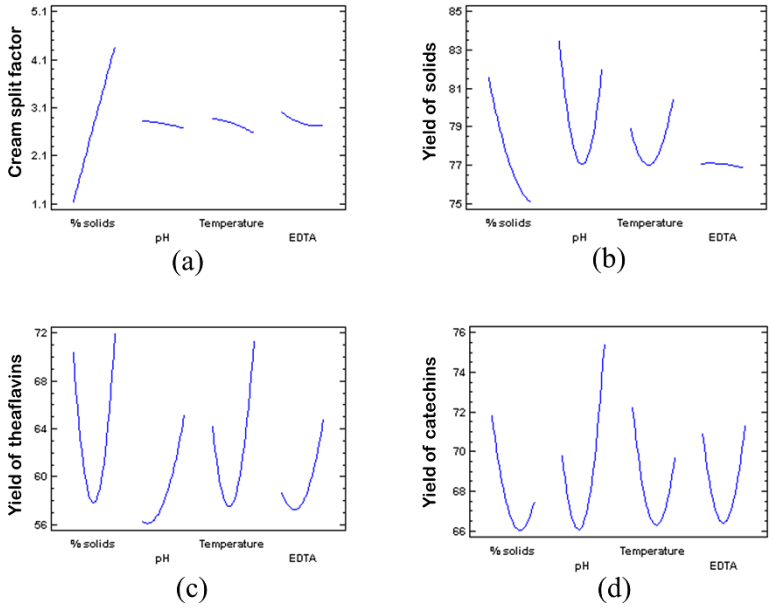


Figure 4.3. Main effects plots charts for the selected responses. (a) cream split factor, (b) yield of solids, (c) yield of theaflavins, (d) yield of catechins.

By excluding any insignificant two-factor interactions it is possible to represent the interaction plots (Figure 4.4) for the responses that have this type of interactions in a significant way. The interaction plots show the cream split factor variation when the percentage of solids is changed, for each level of pH (Figure 4.4.a) and for each level of temperature (Figure 4.4.b). For this particular response, it can be seen that at a pH of 3 the percentage of solids is more relevant than at a pH of 6, while at 20 °C the percentage of solids is more important than at 40 °C. For the response yield of catechins, at a temperature of 20 °C the percentage of solids is more important than at 40 °C (Figure 4.4.c).

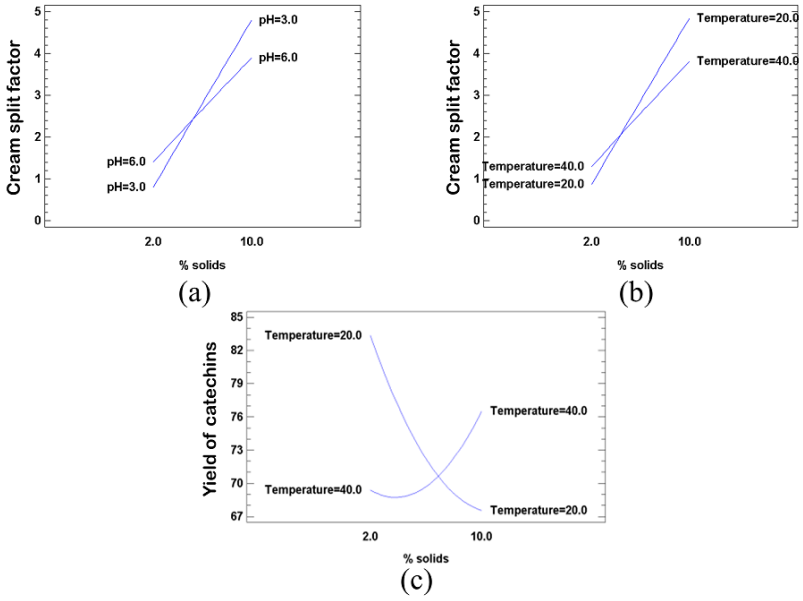


Figure 4.4. Interaction plots for the selected responses. (a), (b) cream split factor, (c) yield of catechins.

4.5.2. Regression model

For each of the DoE selected responses a model is regressed on the basis of the influence factors (A: amount of solids, B: pH, C: temperature and D: EDTA). The results are collected in Table 4.2. Calculations with these models demonstrate that for a black tea with 4 % of solids, a total of 80 % of theaflavins and 75 % of catechins can be kept in the clear phase.

Table 4.2. Fitted regression models for the selected responses.

Response	Fitted regression model
Cream split factor	$-2.318 + 0.963 * A + 0.327 * B + 0.0395 * C - 0.0629 * A * B - 0.00913 * A * C$
Yield of solids	$130.31 - 0.815 * A - 20.13 * B + 2.184 * B^2$
Yield of theaflavins	$137.89 - 8.542 * A + 2.974 * B - 4.619 * C + 0.729 * A^2 + 0.0830 * C^2$
Yield of catechins	$130.94 - 4.848 * A - 13.002 * B - 0.986 * C + 0.144 * A * C + 1.654 * B^2$

Parity plots for the responses are presented in Figure 4.5. It can be observed that the models have good fitting, with a coefficient of determination (R^2) above 0.85.

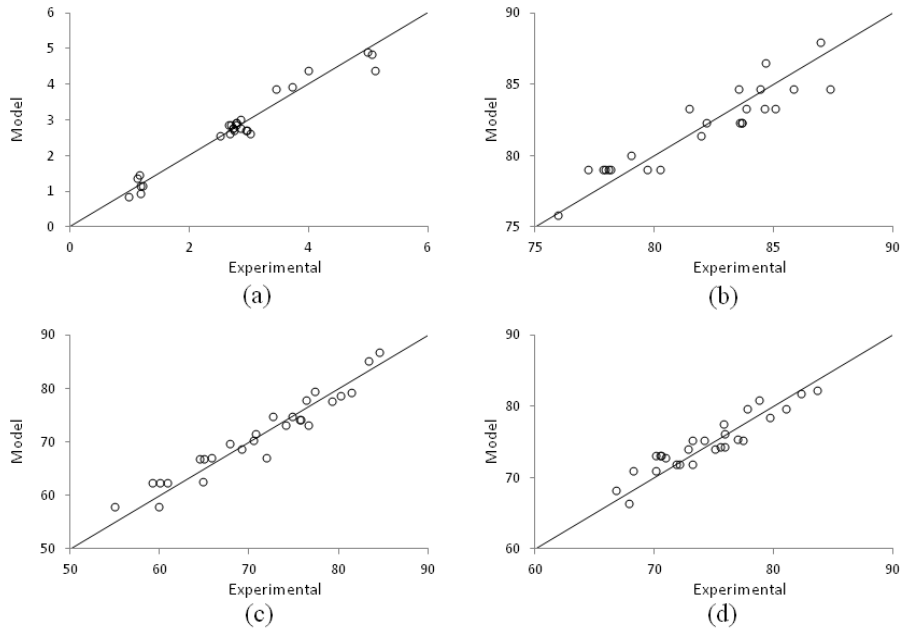


Figure 4.5. Parity plots with the fit of regression model vs experimental values, for the selected responses. (a) cream split factor, (b) yield of solids, (c) yield of theaflavins, (d) yield of catechins. For equations, see Table 4.2.

4.5.3. Response surface plots

Figure 4.6 presents the variation of the responses along the design space. To obtain a 3D representation, one of the factors needs to be fixed. In this case the EDTA amount is chosen as the fixed factor, since it is not a relevant factor. For the cream split factor (C_{sf}), as shown previously, the most influential factor is the amount of solids and the highest results for the C_{sf} correspond to a high amount of solids. In the case of yield of solids (Y_s), the higher values are found for a low percentage of solids and for the extremes of the pH interval.

According to the results, the stronger effects for the yield of theaflavins (Y_{tf}) are mainly the percentage of solids and the temperature. The higher values for the Y_{tf} are found for high temperatures combined with low or high amount of solids. The pH also has an effect, although smaller than the others.

For the yield of catechins (Y_{cat}) a maximum is observed for low values of pH, amount of solids and temperature.

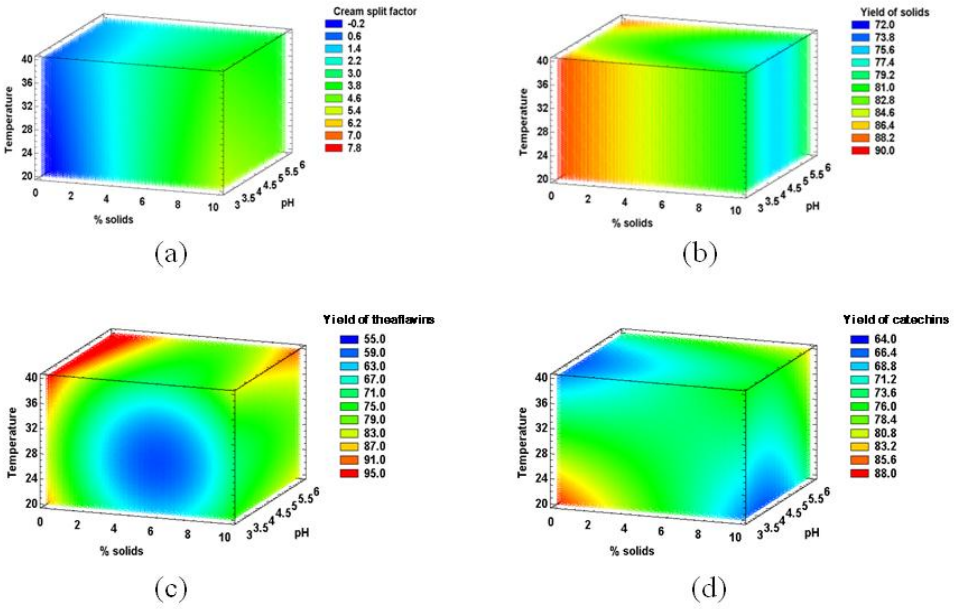


Figure 4.6. Response surface plots for the selected responses (fixed EDTA value at the central point). (a) cream split factor, (b) yield of solids, (c) yield of theaflavins, (d) yield of catechins.

4.5.4. Optimization and extrapolation

For the optimization only two of the four responses are taken into consideration: the yield of theaflavins in the clear phase and the yield of catechins in the clear phase. These yields are considered to be the two key responses of the DoE and the objective is to maximize both of them.

This optimization is evaluated with a desirability function, which is the relationship between predicted responses on a certain variable and the desirability of responses. This is done by combining the individual desirability into a single number and then search for the greatest overall desirability. The desirability ranges from zero to one for any given response. A value of one represents the ideal case. This method transforms predicted values for multiple dependent variables, into one unique overall desirability score. The overall desirability function (D) is calculated by raising the desirability of each response (d) to the power of each impact, multiplying the results together and raising this product to a power of one divided by the sum of impacts. For a given set of m responses: $D = (d_1 \times d_2 \times \ldots d_m)^{1/m}$. This function is maximized via an algorithm to find a set of optimal variable values.

A plot of the overall desirability is presented in Figure 4.7. As it can be checked there are two regions in the design space with high desirability values (low left front corner and high

right back corner), indicating that we have two possible optimal points in our design space. It also indicates that the central area of the design space has a very low desirability.

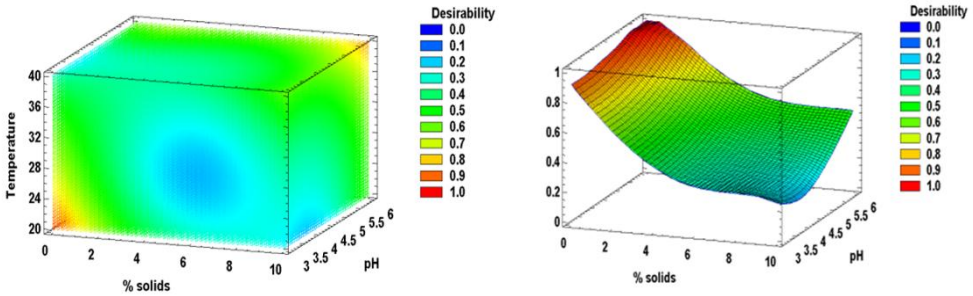


Figure 4.7. Desirability plots. Left; fixed EDTA value of 0.32 g/dm^3. Right; fixed values: EDTA = 0.32 g/dm^3 and T = 20°C.

The first optimal point corresponds to a low percentage of solids, high pH and low temperature, while the second optimum is found at a high percentage solids, pH and temperature (Figure 4.7). For the first optimum, the two selected responses individual desirability are as follow: the yield of theaflavins has a desirability of 0.76 and the yield of catechins has a desirability of 0.99 (overall desirability is 0.87). For the second optimum a slightly lower overall desirability of 0.85 is obtained.

Because the optimal points (higher desirability scores) are located in the border of the design space, this also indicates that results might be improved with extrapolation. That is confirmed by following the method of steepest ascent (procedure for moving sequentially along the path of steepest ascent, in the direction of maximum increase in the response), which allows maximizing the obtained responses (Tables 4.3 and 4.4).

Table 4.3. Optimization and extrapolation data (optimum 1).

	Optimization		**Extrapolation (path of steepest ascent)**	
	Predicted	Experimental	Predicted (Start at derived optimum)	Experimental
Yield TF	82.4	80.7	91.3	86.2
Yield Cat	88.8	84.9	92.6	85.4
% solids	2.0		0.96	
pH	6.0		6.1	
Temperature (°C)	20.0		18.5	
EDTA (g/dm^3)	0.32		0.30	

Table 4.4. Optimization and extrapolation data (optimum 2).

	Optimization		Extrapolation (path of steepest ascent)	
	Predicted	Experimental	Predicted (Start at best predicted vertex)	Experimental
Yield TF	91.2	87.5	100.0	89.2
Yield Cat	82.0	82.4	88.9	84.3
% solids	10.0		10.8	
pH	6.0		6.6	
Temperature (°C)	40.0		42.3	
EDTA (g/dm^3)	0.40		0.40	

In both cases it can be observed that the predicted extrapolation points have better results than the predicted optimization points. Nevertheless, the deviation between the experimental points and the predicted points is higher for the extrapolation points then for the optimization points. Therefore, this indicates that the generated polynomial models can only be used outside the design space (extrapolated) if corrections are applied. It should also be taken into consideration that outside the design space several issues regarding polyphenols degradation and components solubility may occur, see section 4.3.1.

It should also be noted that the temperature and pH variations can cause degradation of the catechins as well as the theaflavins. It was reported that at 50 °C, a 5 % degradation occurs for a catechin during a 3 hours period (Wang et al., 2008). Since in the experiments the maximum temperature is 40°C and the time of the experiments is less than 80 minutes, only some slight degradation of catechins can be expected. The theaflavins, however, have a lower thermal stability then of catechins. Therefore, some degradation might have occurred during the time of the experiment (Lun Su Y. et al., 2003).

Penders et al. (1998) reported that theaflavins have a preference for the cream phase, while the catechin (C) and the epigallocatechin gallate (EGCG) are preferentially partitioned into the clear phase. The results obtained in the DoE demonstrate that both the theaflavins and catechins are mainly partitioned in the clear phase. Although there is a difference in our results and those of Penders et al. (1998) regarding the theaflavins, this is possibly related to the fact that in this work we did not achieve full creaming conditions and that the measured individual theaflavins are different.

4.6. Conclusions

By using a response surface model and subsequent statistical analysis it is possible to determine which factors influence the black tea cream formation and the polyphenols partition over the clear and cream phases. It is also possible to generate second order

polynomial models that describe the system. The analysis indicates that the amount of the chelating agent EDTA is not a statistically relevant factor.

The increase in the amount of polyphenols in the clear phase (37 % increase in the yield of theaflavins and a 20 % increase in the yield of catechins) is obtained while keeping the amount of solids approximately constant. This indicates that the polyphenols are binding less strongly to the solids and more to the water. There is therefore a change in the phase distribution of polyphenols.

According to the results, the stronger effects over the amount of theaflavins are the percentage of solids, followed by the temperature. The pH also has some effect, although smaller than the other effects. For the amount of catechins, the pH is the factor with the strongest effect, although there is also a significant interaction effect between the percentage of solids and the temperature. This indicates that the solubility of theaflavins complexes can be increased by varying the amount of solids and the temperature. In the case of complexes of catechins, their solubility can be mainly increased by adjusting the pH.

For the selected design space two optimal points are found and they are located in the border of the design space. The two main responses selected (yield of theaflavins and yield of catechins) have slightly better results in the extrapolated results (outside the design space) than in the optimal results. This shows that if possible, it would be advantageous to work at low % solids (0.96 %) combined with low temperature (18 °C) or high % solids (11 %) combined with high temperature (43 °C). However, the obtained experimental values are lower than what the extrapolated model predicts.

It was also demonstrated that when using the proper combination of factors it is possible to increase the yield of catechins and theaflavins in the clear phase to a value between 80 and 90 %.

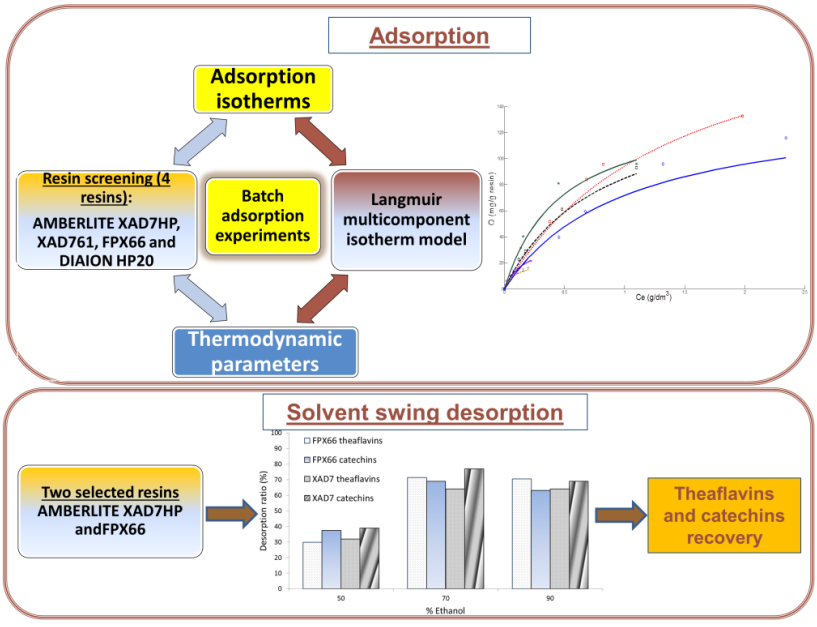
4.7. Nomenclature

BBD	Box-Behnken Design
Caff	Caffeine
C_0	Number of central points in BBD
DoE	Design of experiments
EDTA	EDTA
EC	Epicatechin
EGC	Epigallocatechin
ECG	Epicatechin gallate
EGCG	Epigallocatechin gallate
GA	Gallic acid

HPMCL	Hydroxypropylmethylcellulose
k	Number of factors in the BBD
MCL	Methylcellulose
OFAT	One-factor-at-a-time
PVA	Polyvinylalcohol
PVP	Polyvinylpyrrolidone
RSM	Response surface methodology
T	Temperature
TF	Theaflavin
TFMG	Theaflavin 3 monogallate
TF3MG	Theaflavin 3’ monogallate
TFDG	Theaflavin digallate

5.

Solvent swing adsorption for the recovery of polyphenols from black tea



ABSTRACT: A systematic resin screening study for the adsorption and desorption of catechins and theaflavins from black tea has been presented. Four food grade commercial macroporous resins are screened as a starting point for characterization and optimization of a solvent swing adsorption process, to separate the polyphenols from black tea.
It has been shown that the resin Amberlite XAD7HP has the best performance for the sorption of catechins, allowing the recovery of 60 % of the catechins. The resin Amberlite FPX66 is the best for sorption of theaflavins, with a recovery rate of 59 %. The adsorption process has been modeled with a Langmuir multicomponent isotherm. In desorption, a solution containing 70 % of ethanol (wt %) in water is found to be the best desorption medium.

5.1. Introduction

Adsorption is a surface phenomenon where the molecules from a mobile phase adhere onto a solid surface (stationary phase). The interactions between the adsorbate and the solid surface are either physical (van der Waals forces) or chemical (covalent bonds). For the adsorption macroporous adsorption resins can be used. These non-functionalized polymeric adsorbents have an high surface area combined with a hollow and layered structure, which provides good mechanical strength (Bai et al., 2005). The production is made either by polymerization or polycondensation. During the polymerization process acrylic acid, methacrylic acid or styrene, are cross-linked with divinylbenzene or alternative divinyl monomers (Kammerer et al., 2005).

Four commercially available food grade non-functionalized macroporous resins (Amberlite XAD7HP, FPX66 and XAD761 and Diaion HP20) are selected to be further investigated in a systematic resin screening. The best resin is the one that gives the optimal combined capacity and adsorption/desorption rates, resulting in a good performance for black tea polyphenols separation. For the best resin(s) further characterization is done, e.g. measurement of the adsorption isotherms, optimal ethanol content range for desorption. A solvent swing system is used to shift the adsorption equilibrium of the sorbates by changing the solvent from water based (adsorption) to ethanol based (desorption). Only these two food grade solvent systems were considered in this study.

The adsorption capacity as well as the adsorption and desorption rates in batch experiments are calculated for the polyphenols. The adsorption data are fitted to the Langmuir multicomponent isotherm model. In this study it is important to achieve a good reversibility for the adsorption in order to achieve a high yield of polyphenols recovery. The results obtained in this study will be used as a base for a packed column design and operational optimization for the polyphenols separation from black tea.

5.2. Experimental

5.2.1. Reagents and adsorbents

Acetonitrile, EDTA and citric acid are analytical grade and obtained from Sigma-Aldrich. Glacial acetic acid (HPLC grade), ethanol absolute were purchased from Merck KGaA. Individual polyphenols standards and freeze-dried tea powder were obtained from Unilever R&D, Vlaardingen, The Netherlands. The water used in the experiments was Milli-Q gradient (Millipore).

AMBERLITE™ XAD7HP, XAD761, and FPX66, and DIAION™ HP20 were obtained from Sigma-Aldrich. Before the sorption experiments, all resins were pre-treated to remove preservative agents, by rinsing with water and ethanol.

AMBERLITE™ XAD7HP is a trimethylolpropane trimethacrylate based resin crosslinked with a polyacrylic acid ester. AMBERLITE™ FPX66 resin is macroreticular polymer of styrene and divinylbenzene. AMBERLITE™ XAD761 has a highly porous crosslinked phenol-formaldehyde polycondensate matrix. DIAION™ HP20 is an aromatic macroporous copolymer of styrene and divinylbenzene. The resins main properties are presented in Table 5.1.

Table 5.1. Macroporous resins physical properties.

Resin	Structure	Surface area (m^2/g)	Particle size (mm)	Polarity
XAD7HP	Polymethacrylic acid ester	450	0.25-0.84	Moderate polar
FPX66	Aromatic divinylbenzene copolymer	700	0.6-0.75	Non polar
HP20	polystyrene-divinylbenzene	600	0.25-0.85	Non polar
XAD761	Formophenolic	150-250	0.56-0.76	Polar

The ultracentrifuge is from Beckman Coulter (Optima L-90K), the ultrasonic bath is from Branson (ultrasonic benchtop cleaner, model 3510) and the shaker with temperature control is from IKA (KS 4000i Control Orbital Shaker).

5.2.2. Black tea preparation

Freeze dried black tea was used in the preparation of a tea aqueous solution. The solution was stirred and sonicated in an ultrasonic bath until the freeze dry tea was completely dissolved.

5.2.3. HPLC analytical methods

The HPLC analytical methods used for the quantification of theaflavins, catechins and caffeine are described in Section 4.4.2.

The total theaflavins concentration was determined by adding the concentrations of four theaflavins: theaflavins, theaflavin 3 monogallate, theaflavin 3' monogallate and theaflavin digallate. A total concentration of catechins was calculated by adding the concentrations of the four main catechins components in tea, i.e.: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG).

5.2.4. Static sorption experiments

The batch adsorption tests were performed in 50 ml glass Erlenmeyers at different temperatures. Fixed hydrated resin amounts were used and 25 ml of black tea solution was added to the resin. Subsequently, the Erlenmeyer was shaken at 100 rpm in a temperature controlled shaker, for a period of 4 hours. The resin particles were separated from the surrounding liquid by rinsing with water. The separated resin particles were transferred to another Erlenmeyer. Afterwards, the desorption of the polyphenols was performed by adding 25 ml of an aqueous ethanol mixture. The solution was again shaken in the same conditions as in the adsorption.

Adsorption capacity, adsorption and desorption ratio were calculated according to the following equations:

$$Q_e = \frac{(C_0 - C_e)V_i}{W} \tag{5.1}$$

$$E = \frac{(C_0 - C_e)V_i}{C_0 V_0} \times 100\% \tag{5.2}$$

where Q_e (mg/g) is the adsorption capacity, E (%) is the adsorption ratio, C_0 (mg/dm^3) and C_e (mg/dm^3) are the initial and equilibrium concentration of the solute in solution, V_i (dm^3) is the volume of initial solution and W (g) is the weight of dry adsorbent.

$$D = \left[\frac{C_d V_d}{(C_0 - C_e)V_i}\right] \times 100\% \tag{5.3}$$

where D (%) is the desorption ratio, C_d (mg/dm^3) is the concentration of the solute (desorbed component) in the desorption solution and V_d (dm^3) is the volume of the desorption solution.

5.3. Results and discussion

An example of the components composition data for the studied black tea extract is presented in Table 5.2. The reported values are obtained for tea extractions with 4 % of solids and freeze dried tea as starting material.

Table 5.2. Black tea extract composition (dry weight %).

Constituent	(dry weight %)
Catechins	4.8
Theaflavins	0.7
Caffeine	6.7

The results presented in Figures 5.1 and 5.2 clearly show that for the black tea extract, the amount of respectively theaflavins and catechins adsorbed initially (first hour) has a steep increase. Afterwards, a slower increase in the adsorption was observed, until the equilibrium is reached after approximately 120 minutes. The amount of catechins adsorbed is higher than the amount of theaflavins.

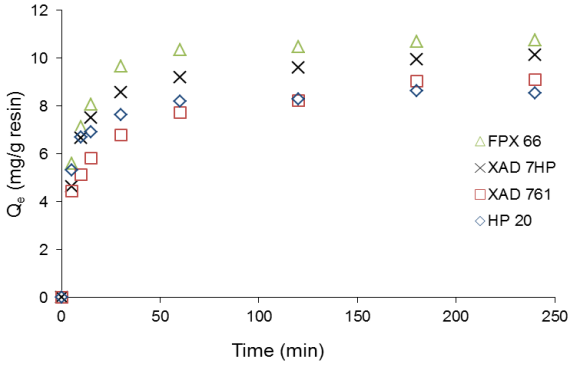


Figure 5.1. Adsorption for 0.4 g/dm^3 of total theaflavins in 60 g/dm^3 of tea extract, at 20 °C.

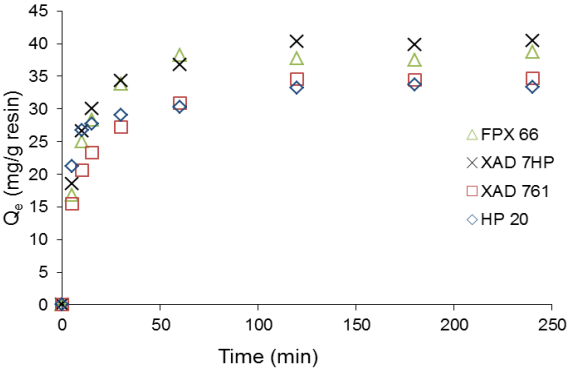


Figure 5.2. Adsorption for 1.8 g/dm^3 of total catechins in 60 g/dm^3 of tea extract, at 20 °C.

5.3.1. Adsorption Isotherms

The experimental adsorption isotherms relate the adsorption capacity of the tested resins with the concentration of the adsorbates in the solution at equilibrium for a given temperature. Figures 5.3, 5.4 and 5.5 present the adsorption isotherms respectively for catechins, theaflavins and caffeine, for a temperature of 20 °C.

The results in Figure 5.3 show that the FPX66 resin has the highest adsorption capacity and the XAD7HP has the highest affinity (steeper initial slope of the adsorption isotherm) for the catechins. The resin HP20 has the lowest adsorption capacity and lowest affinity for the catechins.

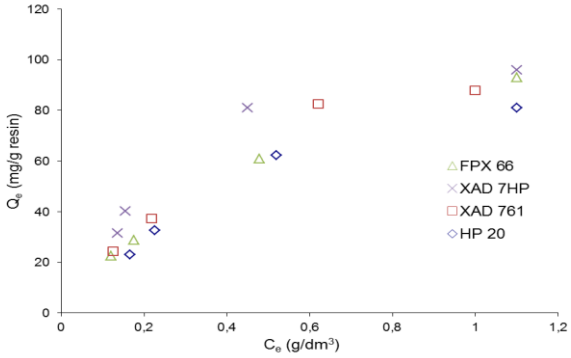


Figure 5.3. Adsorption isotherms of catechins on tested resins at 20 °C.

According to the results in Figure 5.4 the resins FPX66 and XAD7HP have a higher adsorption capacity for the theaflavins, than the resins XAD761 and HP20. In addition the FPX66 resin is the one with the highest affinity. The resin HP20 has the lowest adsorption capacity.

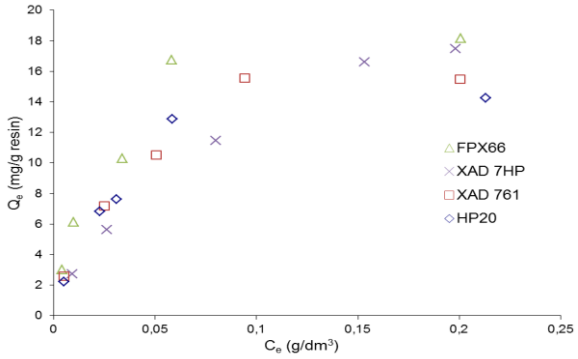


Figure 5.4. Adsorption isotherms of theaflavins on tested resins at 20 °C.

The results in Figure 5.5 show that the resins HP20 and FPX66 have the higher adsorption capacity and affinity for the caffeine, than the resins XAD7HP and XAD761. The resin XAD7HP has the lowest adsorption capacity. The adsorption isotherms data indicate that to maximize the amount of catechins and theaflavins adsorbed and to minimize the amount of caffeine, the XAD7HP seems to be the optimal resin.

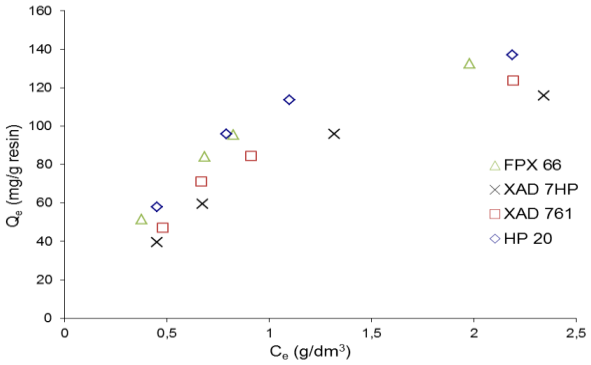


Figure 5.5. Adsorption isotherms of caffeine on tested resins at 20 °C.

According to Table 5.3 the resin with the best combined adsorption and desorption for the theaflavins is resin Amberlite FPX66, together with a high adsorption capacity. For the same reasons the best resin for the separation of catechins is the Amberlite XAD7HP (Table 5.4). For the desorption experiments a 70 % (wt %) ethanol eluent solution is used.

The two selected resins (Amberlite FPX66 and XAD7HP) are further characterized, by measuring the adsorption isotherms at three different temperatures and by solvent swing sorption with different ethanol-water eluent solutions.

Table 5.3. Adsorption capacity (Q_e), adsorption ratio (E) and desorption ratio (D) for theaflavins on the tested resins at 20 °C.

Resin	Q_e (mg/g)	E (%)	D (%)
XAD7HP	18	81	63
FPX66	18	83	74
HP20	14	71	61
XAD761	16	74	67

Table 5.4. Adsorption capacity (Q_e), adsorption ratio (E) and desorption ratio (D) for catechins on the tested resins at 20 °C.

Resin	Q_e (mg/g)	E (%)	D (%)
XAD7HP	96	78	79
FPX66	93	72	72
HP20	81	60	67
XAD761	88	67	63

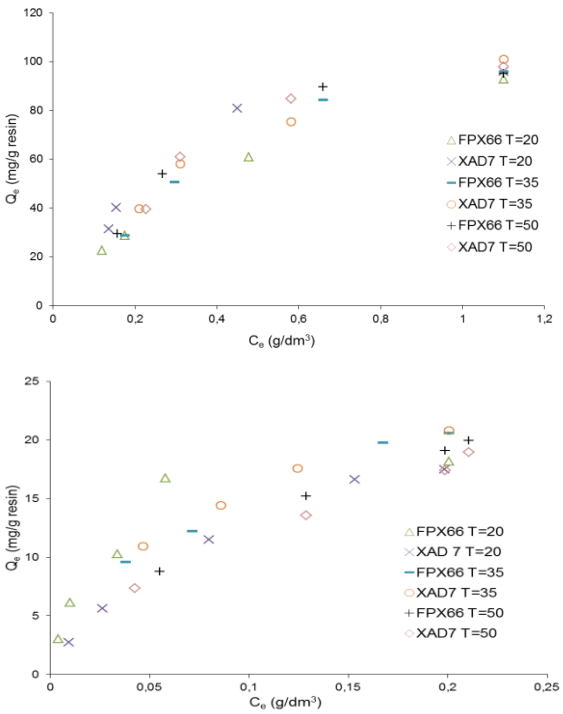


Figure 5.6. Adsorption isotherms of catechins (upper) and theaflavins (lower) on the Amberlite FPX66 and XAD7HP resins at 20, 35 and 50 °C.

An adsorption isotherm represents the balance between adsorbent and adsorbate for a given set of experimental conditions. Figure 5.6 shows the adsorption isotherms for the polyphenols on the resins FPX66 and XAD7HP at three different temperatures, representing the relation between the adsorption capacity and the concentration of polyphenols in solution, at equilibrium. The fact that only small variations on the isotherms are observed with the increase in temperature (from 20 to 50 °C), points to a small adsorption enthalpy change of the process and a dominant physisorption. Physisorption is characterized by a fast and reversible sorption, where weak interactions like van der Waals, dipole or dipole-dipole are usually involved (Kammerer et al., 2005).

5.3.2. Langmuir Isotherm

The Langmuir adsorption model is one of the most applied isotherm models for the description of physisorption of neutral molecules onto the adsorption sites (Du et al., 2007). The Langmuir adsorption is represented by Equation 5.4:

$$Q_e = \frac{Q_m b C_e}{1 + b C_e} \tag{5.4}$$

where C_e (mg/dm^3) and Q_e (mg/g) are the adsorbate concentration in solution and the amount of adsorbate adsorbed (both at equilibrium), b (dm^3/mg) is a Langmuir equilibrium constant that represents the ratio between the adsorption and desorption rate constants (measure of the affinity of the component for the resin) and Q_m (mg/g) represents the maximum adsorption capacity.

Equation 5.4 can be rewritten in a linear form, see Equation 5.5, which allows the calculation of the model parameters, b and Q_m:

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{Q_m b} \tag{5.5}$$

5.3.3. Thermodynamic parameters

The adsorption equilibrium experimental data which represent the affinity between the polyphenols and the resin, can be related to the adsorption enthalpy for the tested resin.

Thermodynamic parameters are used to evaluate the temperature dependent adsorption properties (Q_m and b) for the FPX66 and XAD7HP resins. The Gibbs free energy changes can be calculated with Equation 5.6:

$$\Delta G^0 = -RT \ln K \tag{5.6}$$

where ΔG^0 (kJ/mol) is the standard Gibbs free energy of adsorption, R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T (K) is the absolute temperature and K is the thermodynamic equilibrium constant for the adsorption process.

For the calculation of ΔG^0 the standard equilibrium constant K needs to be dimensionless. According to Zhou and Zhou (2014) the following approximation relating K with b (Langmuir equilibrium constant) can be used:

$$K = b.M_{adsorbate} \times C_{H_2O} \tag{5.7}$$

where K is the standard equilibrium constant, dimensionless, b (dm^3/g) is the experimental equilibrium constant, $M_{adsorbate}$ (g/mol) is the molecular weight of the adsorbate and C_{H_2O} is the molar concentration of water (55.5 mol/dm^3) at standard temperature (298.15 K). This approximation is valid for very diluted solutions and the adsorbed phase is considered an ideal solution (Zhou and Zhou, 2014). For the calculation of the molecular weight of the adsorbate for the catechins and theaflavins a pondered average molecular weight was used (based on the molar fractions of the adsorbates present in the black tea solution).

By using the values of the Langmuir isotherm from experiments performed at different temperatures, the enthalpy of adsorption can be calculated with the Van't Hoff equation (Atkins and de Paula, 2006).

$$\Delta_{ads}G^0 = \Delta_{ads}H^0 - T \times \Delta_{ads}S^0 \quad (5.8)$$

$$-RT\ ln\ K = \Delta_{ads}H^0 - T \times \Delta_{ads}S^0 \quad (5.9)$$

$$ln\ K = \frac{-\Delta_{ads}H^0}{R} \times \frac{1}{T} + \frac{\Delta_{ads}S^0}{R} \quad (5.10)$$

where T (K) is the temperature, $\Delta_{ads}H^0$ (KJ/mol) and $\Delta_{ads}S^0$ (KJ/mol) are the standard enthalpy and entropy changes of adsorption, respectively.

Using the K values from the adsorption isotherm, the plot of $ln\ K$ versus $1/T$ from Equation 5.10 gives approximately a linear relationship, see Figure 5.7. The slope is equal to $-\Delta_{ads}H^0/R$, so the adsorption enthalpy can be calculated under the assumption that $\Delta_{ads}H^0$ and $\Delta_{ads}S^0$ are constant over the temperature range of study. The coefficients of determination (R^2 values) obtained from the data in Figure 5.7 are above 0.94. However, it should be pointed out that only three points (from the three tested temperatures) are used for the linear regression.

For the resins Amberlite FPX66 and XAD7HP the temperature effect over the adsorption process was evaluated with adsorption measurements at three different temperatures (20, 35 and 50 °C). It was found that the enthalpy of adsorption is small (Table 5.5), in line with the observed small temperature dependence of the sorption equilibrium. The adsorption of catechins onto the XAD7HP resin has a higher adsorption enthalpy than for the FPX66 resin. The absolute enthalpy of adsorption for the theaflavins is higher than for the catechins, showing that the temperature effect on the adsorption is stronger for the theaflavins.

Table 5.5. Thermodynamic parameters for the adsorption of catechins and theaflavins by FPX66 and XAD7HP.

Resin	Temperature (K)	$\Delta_{ads}G^0$ catechins (kJ/mol)	$\Delta_{ads}G^0$ theaflavins (kJ/mol)	$\Delta_{ads}H^0$ catechins (kJ/mol)	$\Delta_{ads}H^0$ theaflavins (kJ/mol)
FPX66	293.15	-25.8	-30.2	-9.4	48.8
	308.15	-26.5	-33.7		
	323.15	-27.5	-38.3		
XAD7HP	293.15	-26.7	-30.7	-12.4	18.9
	308.15	-27.3	-33.6		
	323.15	-28.2	-35.8		

The acrylic ester based resins, like XAD7HP, are used to adsorb polyphenols from aqueous solutions onto the resin, via van der Waals forces and through the intermolecular hydrogen bond formed between the phenolic hydroxyl group and the carbonyl groups in the polymeric resin.

For physisorption the typical absolute enthalpy of adsorption values are around 20 kJ mol^{-1}, while for chemisorption the range is between 80 kJ mol^{-1} and 400 kJ mol^{-1} (Atkins and de Paula, 2006; Ye et al., 2011). The values of $\Delta_{ads}H^0$ obtained for the adsorption of catechins and theaflavins onto the resins FPX66 and XAD7HP (Table 5.5), for the studied temperature range, show that the adsorption is physical. The small heat of adsorption is not sufficient to break the molecular bonds. The surface interactions, however, might lead to molecule distortion. This is in agreement with the fact that adsorption of polyphenols onto resins with amine groups are mainly driven by hydrophobic interactions and hydrogen bonds (Huang et al., 2007a; Liu et al., 2002).

The negative value of $\Delta_{ads}G^0$ reveals that the adsorption of black tea polyphenols onto Amberlite FPX66 and XAD7HP resins is a spontaneous process.

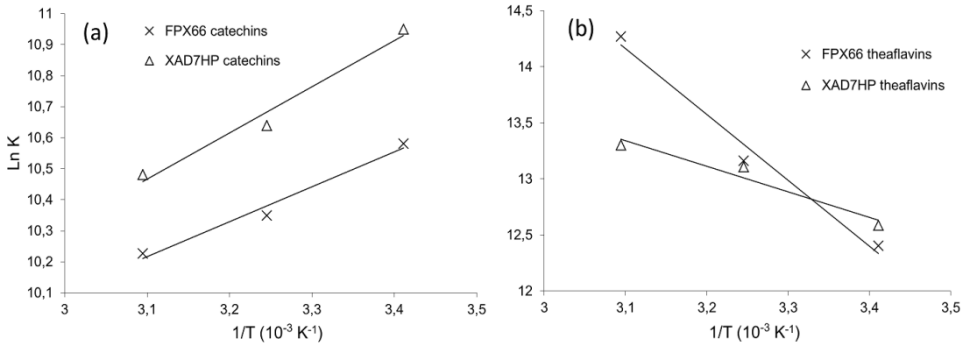


Figure 5.7. Van't Hoff plots for the adsorption of catechins (a) and theaflavins (b) onto FPX66 and XAD7HP resins.

The negative value for $\Delta_{ads}H^0$ (Table 5.5) in the case of the adsorption of catechins reveals exothermic adsorption. These results are in agreement with previously reported results, where the adsorption of tea catechins using the polymer poly(acrylamide-co-ethylene glycol dimethylacrylate) was a spontaneous and exothermic process (Lu et al., 2010). For the theaflavins, however, the adsorption enthalpy has a positive value, pointing out that the adsorption process is endothermic. This difference in thermal adsorption behavior may originate from the structural difference between catechins and theaflavins. The larger size and the less favorable steric and conformational effect of the theaflavins, when compared to the catechins, indicates the need to supply energy for the adsorption process to occur.

5.3.4. Langmuir multicomponent isotherm

To describe the adsorption of a mixture of solutes an expansion of the monocomponent Langmuir isotherm is used. Due to the multicomponent nature of the tea, the multicomponent Langmuir equation (Markham and Benton, 1931) is applied to model the adsorption behavior of the studied species. The multicomponent Langmuir isotherm model

(Equation 5.11) is widely applied due to its simplicity in predicting multicomponent isotherms. This model should only be applied in the case that all the components follow the Langmuir monocomponent model.

$$Q_i = \frac{Q_{m,i} b_i C_i}{1 + \sum b_j C_j} \tag{5.11}$$

where Q is the amount of adsorbate adsorbed, C is the adsorbate concentration in solution, b is the Langmuir constant and Q_m is the maximum adsorption capacity.

The Langmuir multicomponent model is based in a monolayer adsorption, with no interactions between adsorbed molecules, an equal availability of adsorption sites for all the adsorbates, a homogeneous distribution of adsorption energy and a reversible type of adsorption. The desorption rate from each site is considered to be independent from the occupancy of the surrounding sites (Ho and Mckay, 2000).

Figure 5.8 shows the fitting of the multicomponent Langmuir model to the experimental data for the previously selected resins: Amberlite FPX66 and XAD7HP.

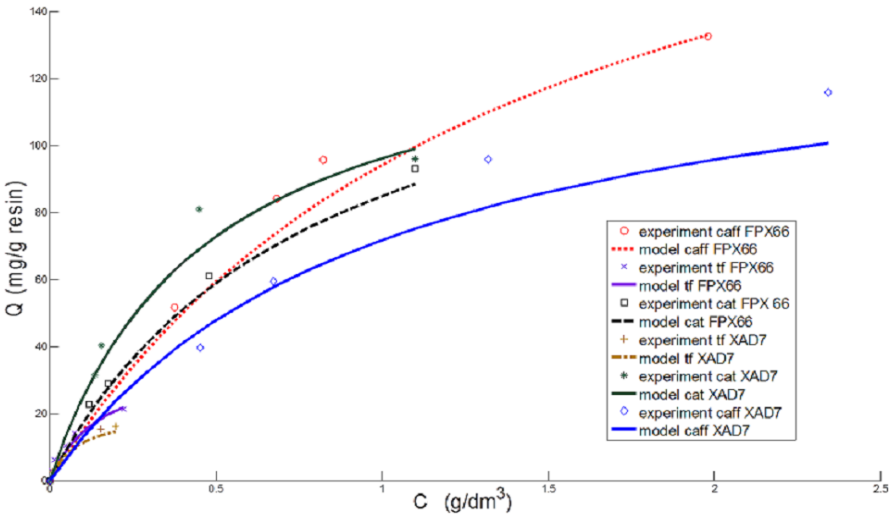


Figure 5.8. Langmuir multicomponent isotherm model fitting for the FPX66 and XAD7HP resins.

The Matlab function 'fminsearch' that uses the Nelder-Mead simplex (direct search) method (Lagarias et al., 1998) was used for the fitting. The error minimization is done with the 2-norm (finds the prediction coefficients by minimizing the 2-norm of the prediction error).

The multicomponent Langmuir isotherm model shows a good fitting for the catechins and caffeine and a reasonable fitting for the theaflavins. The parity plots (Figure 5.9) show that the points have an uniform distribution and are randomly scattered around the diagonal.

A higher Langmuir constant (b) indicates a higher affinity of the sorbent for the resin, while the maximum adsorption capacity (Q_m) is a measure of the total number of binding sites that are available for adsorption.

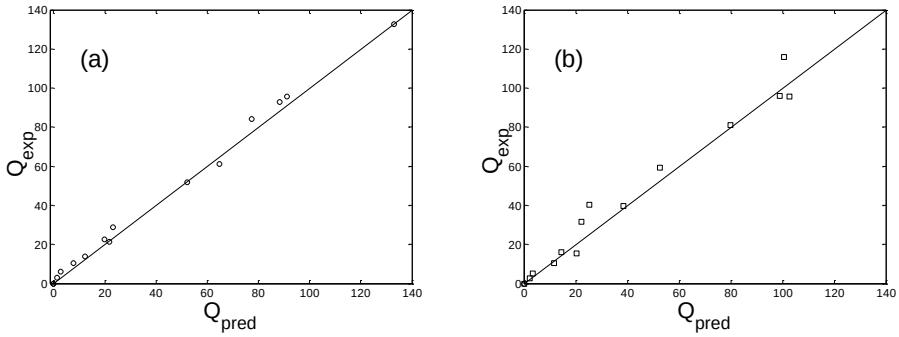


Figure 5.9. Parity plot (experimental vs predicted) for the Langmuir multicomponent isotherm model for the FPX66 (a) and XAD7HP (b) resins.

According to the data in Table 5.6, the catechins have an higher affinity for the XAD7HP than for the FPX66, the theaflavins have the highest affinity for the FPX 66 and the caffeine has the lowest affinity for the FPX66. The model results are in agreement with the experimental results presented in Tables 5.3 and 5.4.

Table 5.6. Langmuir constant, b (dm^3/g), maximum adsorption capacity, Q_m (mg/g) and coefficient of determination, R^2, for the selected resins.

	Caffeine			**Catechins**			**Theaflavins**		
Resin	b	Q_m	R^2	b	Q_m	R^2	b	Q_m	R^2
FPX66	0.21	772	0.99	0.27	700	0.99	3.01	78	0.92
XAD7HP	0.53	271	0.97	0.53	565	0.96	2.58	95	0.86

The resins performance can be compared on the basis of both Q_m and b. However, in the case of black tea adsorption, since we are working at low concentration ranges, it is more important to have a high affinity of the polyphenols towards the resin, which reflects a good uptake and is characterized by the steep rise of the isotherm curve at low values of C_e.

Based on the multicomponent Langmuir model, in a scenario where the objective is to maximize the recovery of catechins and theaflavins and to minimize the recovery of caffeine, the FPX66 resin is expected to overall have the optimal performance.

5.3.5. Static desorption

Catechins and theaflavins are soluble in several solvents including water, ethanol, methanol, acetone and ethyl acetate. Although in previous studies of polyphenol adsorption it was found that methanol and acetone could, in some cases, achieve higher desorption

yields than ethanol, the last is preferred because it is a food grade solvent (Juang and Shiau, 1999).

In this study the desorption of theaflavins and catechins from the FPX66 resin with different ethanol-water mixtures was measured. Figure 5.10 shows that for the FPX66 resin, the desorption ratio of polyphenols increases with increasing ethanol concentrations until it reaches a maximum at 70-80 % (wt %) of ethanol, where the polarity and hydrophobicity of the eluent matches the one from the polyphenols. At higher ethanol percentages the desorption ratio decreases slightly. Below 20 % (wt %) of ethanol almost no desorption of polyphenols occurs. This is due to the high hydrophilic nature of the desorbing medium. The polyphenols are moderately hydrophobic and tend to remain in the resin if the eluent is water. By increasing the amount of ethanol in the solvent, the solvent becomes less hydrophilic, matching the polarity of the polyphenols, which will then desorb into the solvent.

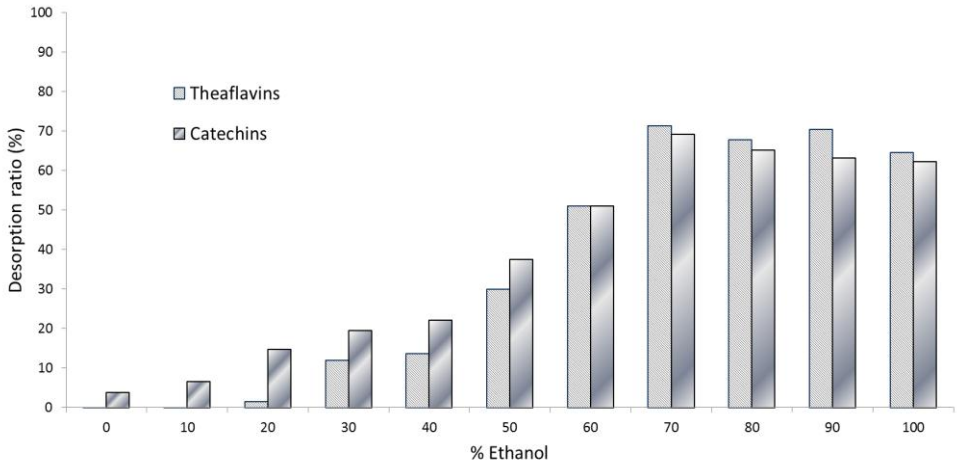


Figure 5.10. Static desorption ratios (see Equation 5.3) of theaflavins and catechins on FPX66 resin in various ethanol–water mixtures.

When analyzing the theaflavins and catechins separately, it is observed that at low ethanol fractions the catechins (less hydrophobic) are more desorbed. Above 60 % (wt %) ethanol the theaflavins (more hydrophobic) are preferably desorbed (Figure 5.10). This ethanol-water desorption ratio is in accordance with the result reported by Xu for eluting purified theaflavins (Xu et al., 2010). The results are explained by the higher hydrophobicity of theaflavins when comparing to other components present in black tea, like catechins or caffeine, which have higher desorption ratios at lower ethanol fractions. For the catechins the highest desorption is at 80 % (wt %) ethanol, which is in accordance to previously reported (Ye et al., 2011; Zhang et al., 2013).

The results for the two previously selected resins (FPX66 and XAD7HP) in Figure 5.11 demonstrate that the 70 % ethanol solution is the best choice. The FPX66 is the best resin for the desorption of theaflavins, while the XAD7HP is the best resin for the desorption of catechins. A recovery rate of 59 % is obtained for the theaflavins with the FPX66 resin and a recovery rate of 60 % is obtained for the catechins with de XAD7HP resin.

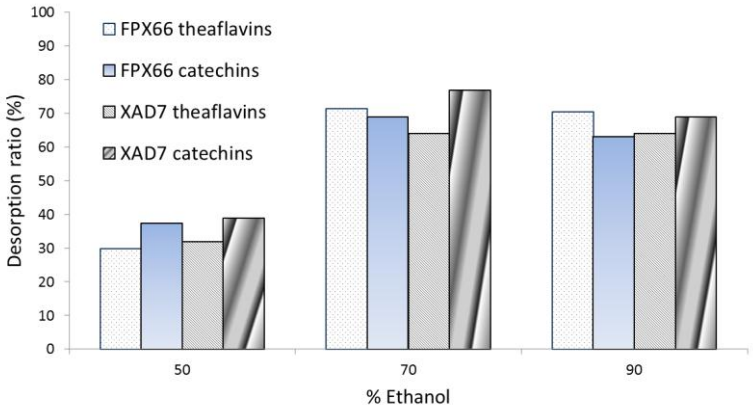


Figure 5.11. Static desorption ratios of polyphenols on FPX66 and XAD7HP resins at 50, 70 and 90 % ethanol (wt %)

5.4. Conclusions

Four commercial non-functionalized macroporous resins were tested for the separation of catechins and theaflavins from black tea. The resins with the better performance have non-polar (Amberlite FPX66) or moderately polar (Amberlite XAD7HP) properties. For the tested polyphenols concentration range, polarity is a more important factor than specific surface area.

The resin with the best results for the sorption process of theaflavins is the Amberlite FPX66, with a recovery rate of 59 %. For the sorption of catechins the best results are achieved when using the Amberlite XAD7HP resin, with a recovery rate of 60 %.

The catechins have a higher adsorption enthalpy for the XAD7HP resin than for the FPX66 resin. The temperature effect on the adsorption is stronger for the theaflavins than for the catechins (higher absolute enthalpy of adsorption for the theaflavins).

The multicomponent Langmuir isotherm model shows a good fit to experimental results for the catechins and caffeine. For the theaflavins a reasonable fit was obtained.

The ethanol/water ratio proves to be a key factor in the theaflavins selective desorption. The choice of solvent is influenced by the resin polarity and hydrophobicity, together with

the polyphenols solubility in the ethanol/water mixtures applied. In this work the maximum desorption ratio was achieved for ethanol-water mixtures above 70 % (wt %) ethanol.

The overall results demonstrate that a solvent swing adsorption is a good choice for the separation of black tea polyphenols. For the adsorption step the polyphenols are adsorbed from water onto the macroporous resin and in a second step the solvent with the highest affinity for the polyphenols (70 % ethanol) is used for the desorption.

Overall, when the objective is to maximize the recovery of catechins and theaflavins and minimize the recovery of caffeine, the FPX66 is the optimal resin, confirming the potential of macroporous commercial resins to selectively recover polyphenols from black tea solutions.

The work in this chapter provides the knowledge for the design and modeling of a solvent swing adsorption process for the separation of black tea polyphenols.

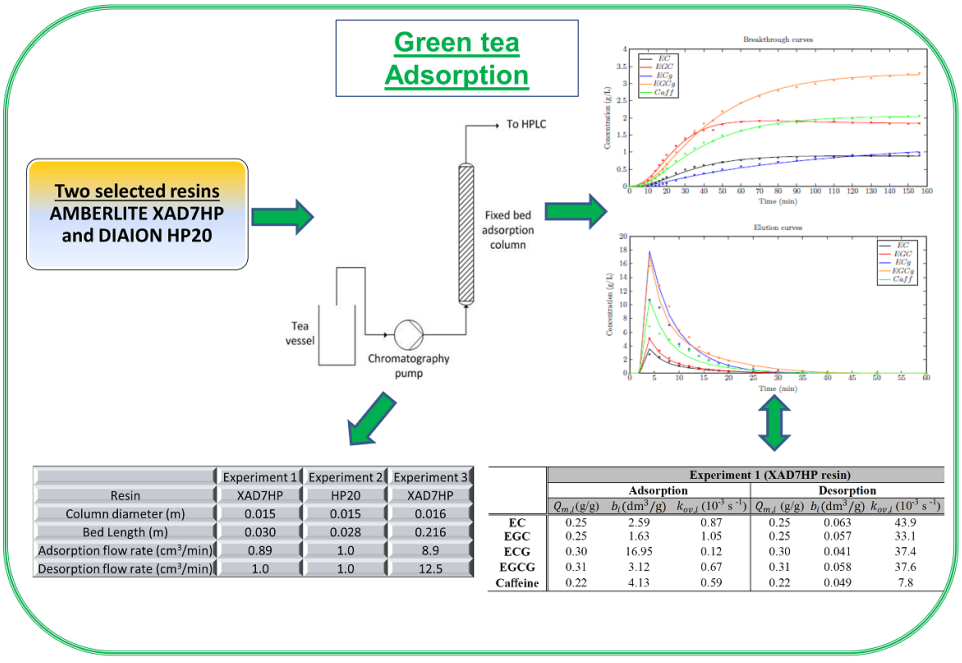
5.5. List of symbols

C_0	Initial concentration of solute in solution
C_d	Concentration of solute in the desorption solution
C_e	Equilibrium concentration of solute in solution
C_{H_2O}	Molar weight of water at standard temperature
R^2	Coefficient of determination
D	Desorption ratio
E	Adsorption ratio
EC	Epicatechin
EGC	Epigallocatechin
ECG	Epicatechin gallate
$EGCG$	Epigallocatechin gallate
K	Thermodynamic equilibrium constant
b	Langmuir equilibrium constant
$M_{adsorbate}$	Molecular weight of the adsorbate
R	Universal gas constant
Q_e	Adsorption capacity
Q_m	Maximum adsorption capacity
T	Temperature
V_d	Volume of desorption solution
V_i	Volume of initial solution
W	Weight of dry adsorbent
ΔG^0	Standard Gibbs free energy of adsorption

$\Delta_{ads}H^0$	Standard enthalpy change of adsorption
$\Delta_{ads}S^0$	Standard entropy change of adsorption

6.

Solvent swing adsorption for the recovery of green tea catechins



	Experiment 1	Experiment 2	Experiment 3
Resin	XAD7HP	HP20	XAD7HP
Column diameter (m)	0.015	0.015	0.016
Bed Length (m)	0.030	0.028	0.216
Adsorption flow rate (cm^3/min)	0.89	1.0	8.9
Desorption flow rate (cm^3/min)	1.0	1.0	12.5

	Experiment 1 (XAD7HP resin)					
	Adsorption			Desorption		
	$Q_{m,1}$(g/g)	b_1(dm^3/g)	$k_{ov,1}$ (10^{-3} s^{-1})	$Q_{m,1}$ (g/g)	b_1(dm^3/g)	$k_{ov,1}$ (10^{-3} s^{-1})
EC	0.25	2.59	0.87	0.25	0.063	43.9
EGC	0.25	1.63	1.05	0.25	0.057	33.1
ECG	0.30	16.95	0.12	0.30	0.041	37.4
EGCG	0.31	3.12	0.67	0.31	0.058	37.6
Caffeine	0.22	4.13	0.59	0.22	0.049	7.8

ABSTRACT: In this chapter a multicomponent sorption model has been developed for the separation of catechins from liquid tea streams. Five components are modeled in the competitive sorption: the four main catechins and caffeine present in green tea. The adsorption and desorption behavior is mathematically described with a one dimensional axial dispersed plug flow model, which can accurately simulate the dynamics of the solvent swing sorption columns. The model parameters were regressed from experimental data. Two commercially available food grade resins were considered: the Amberlite XADHP and the Diaion HP20. For the desorption step two food grade solvents are used: water and ethanol. The adsorption model sets the basis for the process design and optimization for the recovery of green tea catechins, using macroporous resins in a packed bed column.

6.1. Introduction

In a packed bed adsorption column nearly all solutes can be adsorbed for a limited amount of time. The adsorption process starts with an unsaturated bed of adsorbent and in an ideal fixed bed adsorption with plug flow conditions (very small internal and external mass-transfer resistances and negligible axial dispersion), equilibrium is reached almost instantaneously.

By using a solvent swing operation there is a change in the sorption equilibrium of the components, due to the change in the composition of solvent during the sorption process (Wegmann et al., 2011). In this chapter a solvent swing adsorption technique is applied and different commercially available macroporous resins (Amberlite XAD7HP and Diaion HP20) are evaluated. The resins selection is based on the work from (Sevillano et al., 2014), where the resin XAD7HP is preferred for the adsorption of catechins and the resin HP20 is the best resin for caffeine adsorption.

The objective of this work is to develop a mathematical model that can describe the sorption of green tea polyphenols on a packed bed column with a macroporous resin. The input data for the model are the kinetics, adsorption equilibrium and adsorbent characteristics. As an output, the model computes breakthrough and elution curves for catechins and caffeine (for the adsorption cycles), which can be used for process optimization, avoiding time consuming and costly experiments.

The adsorption kinetics for the mass transfer can be described by adsorption on local sites and mass transport in series, with two types of models: either based on a local adsorption assumption between the solid and bulk phase or based on the mass transfer resistance between adsorbent particle and fluid phase (Siahpoosh et al., 2009).

The first class of models (local adsorption) are used for high mass transfer rates, where the effect of mass transfer resistance to the particle can be neglected and include local equilibrium or local kinetic theories. The second class (mass transfer resistance) includes

either a rigorous or an approximated approach. Rigorous approaches are time consuming as they account for the mass transfer inside the particle along its radius, while approximated models are less time consuming. The most widely used approximated method is the linear driving force (LDF) concept (Glueckauf and Coates, 1947).

To reduce the calculation time and describe the adsorption kinetics, the linear driving force concept is used with a lumped parameter for the overall mass transfer resistance. The thermodynamic equilibrium is modeled with the multicomponent Langmuir isotherm (Fournel et al., 2010; Gogoi et al., 2010; Siahpoosh et al., 2009).

The resulting model can be used for process design and optimization (column dimensions and operational parameters) to separate the catechins from green tea. The dynamic column process model predicts the outlet concentrations of the adsorption column(s) with a solvent swing adsorption, where the feed is green tea extract and the eluent is a water-ethanol solution.

6.2. Experimental

6.2.1. Adsorbents

Two non-functionalized food grade polymeric adsorbents are used: Amberlite XAD7HP and Diaion HP20, both obtained from Sigma-Aldrich. The XAD7HP is a non-ionic aliphatic acrylic polymer and the HP20 has a polystyrenic matrix crosslinked with divinylbenzene. The adsorbents are pre-treated in three steps to remove the monomers and preservative agents: first washed with water, then with ethanol and afterwards again with water.

6.2.2. Reagents and equipment

For the HPLC (High Performance Liquid Chromatography) analysis: glacial acetic acid (from Merck KGaA), analytical grade acetonitrile (from Sigma–Aldrich) and Milli-Q water were used.

Catechins standards and freeze-dried green tea powder were supplied by Unilever R&D, Vlaardingen, The Netherlands.

The HPLC pump used in the column experiments is from Knauer (Smartline Pump 1000) and the glass column (1.5 cm diameter and variable bed height) is from Omnifit (Distrilab BV, The Netherlands). The ultracentrifuge is a Optima L-90K from Beckman Coulter.

The HPLC equipment is an Agilent 1220 Infinity LC gradient system with a variable wavelength detector and the column is a phenyl-hexyl (Luna 5µm Phenyl-Hexyl), from

Phenomenex. The HPLC analysis for the quantification of catechins and caffeine is described in section 3.3.2.

6.2.3. Sample preparation

Green tea was prepared by extraction of the freeze dried tea powder with water at 85 °C in an Erlenmeyer. The tea extract was afterwards centrifuged for 20 minutes at 20000 rpm and the two resulting phases were separated by decanting.

6.2.4. Dynamic sorption experiments

The glass column (Omnifit) was packed with the wet resin up to the target bed height (see Table 6.1). The column was initially flushed with water, after which the clear tea extract was pumped via the bottom of the column with an HPLC pump. Samples were then taken at defined intervals at the outlet of the column and analyzed via HPLC (Figure 6.1). In the next step, the column was again washed with water to remove non adsorbed components. In the last step, a 70 % (wt %) ethanol-water eluent was used for the desorption. All the experiments were performed at room temperature (20 °C).

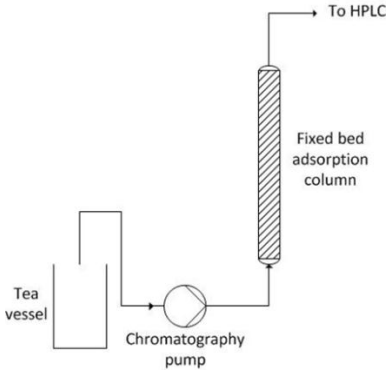


Figure 6.1. Experimental set-up for the dynamic sorption experiments.

Three packed bed column experiments were performed at similar conditions using two resins: Amberlite XAD7HP and Diaion HP20 (Table 6.1). In Experiment 3 a much longer column was used to evaluate the effect of the column dimensions.

Table 6.1. Packed bed column parameters for the XAD7HP and HP20 experiments.

	Experiment 1	Experiment 2	Experiment 3
Resin	XAD7HP	HP20	XAD7HP
Column diameter (m)	0.015	0.015	0.016
Bed Length (m)	0.030	0.028	0.216
Adsorption flow rate (cm^3/min)	0.89	1.0	8.9
Desorption flow rate (cm^3/min)	1.0	1.0	12.5

6.3. Model development

The use of mathematical models which describe the column dynamics of adsorption process allows for the prediction of breakthrough and elution curves (Xu et al., 2013). For the process design it is possible to simulate the adsorption/desorption cycles, which can be used for optimization of design and operation.

6.3.1. Multicomponent Langmuir isotherm

Similarly to chapter 5, the multicomponent Langmuir equation (Equation 6.1) is applied for modeling the adsorption behavior of the studied species based on a monolayer adsorption and considering no interactions of the adsorbates (Markham and Benton, 1931).

$$Q_i = \frac{Q_{m,i} b_i C_i}{1 + \sum_i b_i C_i} \tag{6.1}$$

where Q_i is the amount of component i adsorbed and $Q_{m,i}$ is the maximum adsorption capacity of component i, b_i is the Langmuir constant and C_i is the adsorbate concentration of component i.

The adsorption isotherms are used to characterize the amount of adsorbate adsorbed on the adsorbent. The adsorption isotherm parameters values for each component and resin are shown in Table 6.2.

Table 6.2. Isotherm parameters values.

	Q_m (g/g)		b (dm^3/g)	
	XAD7HP	HP20	XAD7HP	HP20
EC	0.2530	0.1506	0.0937	0.0747
EGC	0.2526	0.0385	0.0706	0.0446
ECG	0.3019	0.9673	0.2110	0.3653
EGCG	0.3069	0.5151	0.1462	0.0768
Caffeine	0.2162	0.3299	0.0779	0.2971

For the desorption, isotherm parameters are calculated from the partition coefficients reported by (Sevillano et al., 2014). The reported partition coefficients are calculated assuming that the elution isotherms are linear.

6.3.2. Column adsorption model

To model the dynamic behaviour of an adsorbent particle on the column, a mass balance is set up across a slice of the packed bed column. The flow is represented by using Ruthven's

axially dispersed plug flow (Ruthven, 1984), which includes both the convection and axial dispersion effects. The differential fluid phase mass balance is given by Equation 6.2.

The following assumptions are taken into consideration for this model: instantaneous and isothermic adsorption, negligible bed pressure drop, spherical adsorbent particles and perfect radial mixing.

The linear driving force (LDF) approximation (see Equation 6.3) was used for the adsorption kinetics (Glueckauf, 1955). The LDF parameters can be easily regressed to fit the experimental data.

$$\frac{\partial C_i}{\partial t} = -v\frac{\partial C_i}{\partial z} + D_{az,i}\frac{\partial^2 C_i}{\partial z^2} - \frac{(1-\varepsilon_b)}{\varepsilon_b}\rho_p\frac{\partial Q_i}{\partial t} \tag{6.2}$$

$$\frac{\partial Q_i}{\partial t} = k_{ov,i}\left(q_{s,i} - q_i\right) \tag{6.3}$$

where ε_b is the bed porosity, C_i is the concentration of the component i, $D_{az,i}$ is the axial dispersion coefficient, ρ_p is the density of the adsorbent, Q_i is the concentration of component *i* on the adsorbent surface, and $k_{ov,i}$ is the overall mass transfer resistance.

For the model calculations the Equations 6.2 and 6.3 are set up for the individual components (four catechins and caffeine) and solved simultaneously. The coupled partial differential equations (PDEs) are discretized using upwind finite differences and solved in MATLAB with implicit ode solvers.

To calculate the overall mass transfer coefficient $k_{ov,i}$ a lumped parameter is used, representing the intraparticle diffusion and the external mass transfer resistances (see Equation 6.4).

$$\frac{1}{k_{ov,i}} = \frac{1}{k_{int,i}} + \frac{1}{k_{ext,i}} \tag{6.4}$$

where $k_{ext,i}$ is the external mass transfer resistance and $k_{int,i}$ is the internal mass transfer resistance.

The external mass transfer resistance is found using Equation 6.5, where the liquid film mass transfer coefficient is obtained by the Sherwood number and calculated using the correlation given by (Wakao and Funazkri, 1978), in Equation 6.6. The internal mass transfer resistance is obtained using the expression presented by (Glueckauf, 1955), see Equation 6.7.

$$k_i^{ext} = \frac{3k_{f,i}}{R_p} \tag{6.5}$$

$$Sh = 2 + 1.1Sc^{1/3}Re^{0.6} \qquad Sh = \frac{k_f d_p}{D_{LP}} \tag{6.6}$$

$$k_i^{int} = \frac{15 D_{p,i}^e \varepsilon_p}{R_p^2} \tag{6.7}$$

The axial dispersion coefficient (D_{az}) is calculated with the correlation of (Gunn, 1987), 1987), in Equation 6.8. This correlation neglects the variance of the distribution of interstitial velocity in the packed bed, where $\tau = 1.4$ (for spheres).

$$\frac{vd}{D_{az}} = \left[\frac{ReSc(1-p)^2}{\varepsilon_b \Gamma} + \frac{Re^2 Sc^2 p(1-p)^3}{\varepsilon_b^2 \Gamma^2}\left(exp\left(\frac{-23.1(1-\varepsilon_b)}{p(1-p)ReSc}\right) - 1\right)\right] + \frac{\varepsilon_b}{\tau ReSc} \tag{6.8}$$

$$p = 0.17 + 0.33\, exp\left(-\frac{24}{Re}\right) \tag{6.9}$$

The initial condition for starting the column operation (first step) is a packed column filled with water. A step response at the inlet of the column is then applied, where the initial conditions of one step match the final conditions of the previous step.

The Danckwerts boundary conditions are used at the inlet (z=0) and at the outlet (z=L) of the column (Equation 6.10) and the initial conditions are given by Equation 6.11 (Wehner and Wilhelm, 1995).

$$\left.\frac{\partial C_i}{\partial z}\right|_{z=0} = \frac{v}{D_{az,i}}\left(C_i - C_i^{feed}\right); \quad \left.\frac{\partial C_i}{\partial z}\right|_{z=L} = 0 \tag{6.10}$$

$$C_i(z,t) = 0 \tag{6.11}$$

where *v* is the superficial fluid velocity.

To model the breakthrough curves, the coupled partial differential equations (PDEs) with initial and boundary conditions are discretized into ordinary differential equation (ODEs), using finite differences (Equations 6.12 and 6.13), to represent the flow of an adsorbate through a column. The system of ODEs was solved in MATLAB using an implicit solver (ode15s) for stiff differential equations, based on numerical differentiation formulas (NDFs).

$$\frac{dC_i}{dz} \approx \frac{C_i(z_n) - C_i(z_{n-1})}{\Delta z} \tag{6.12}$$

$$\frac{d^2 C_i}{dz^2} \approx \frac{C_i(z_{n-1}) - 2C_i(z_n) + C_i(z_{n+1})}{\Delta z^2} \tag{6.13}$$

6.4. Results and discussion

6.4.1. Model validation

The values for the transport parameters (superficial fluid velocity and Reynolds numbers) used in the model calculations are showed in Table 6.3.

Table 6.3. Superficial fluid velocity and Reynolds numbers for Experiments 1, 2 and 3.

	Experiment 1	Experiment 2	Experiment 3
	Adsorption		
v (m/s)	8.40e-05	9.43e-05	7.38e-04
Re	0.302	0.029	2.652
	Desorption		
v (m/s)	9.43e-05	9.43e-05	10.37e-4
Re	0.154	0.013	1.697

The values for the mass transfer resistance used in the model calculations of the breakthrough and elution curves are presented in Tables 6.4 to 6.6, for Experiments 1 to 3 respectively. The values for the external, internal and overall mass transfer resistance (see Equation 6.4) for each component are showed.

Table 6.4. Model parameters for the mass transfer resistance for Experiment 1.

	Experiment 1 (XAD7HP resin)					
	Adsorption			Desorption		
	$k_{ext}(10^{-3}\ s^{-1})$	$k_{int}(10^{-3}\ s^{-1})$	$k_{ov}(10^{-3}\ s^{-1})$	$k_{ext}(10^{-3}\ s^{-1})$	$k_{int}(10^{-3}\ s^{-1})$	$k_{ov}(10^{-3}\ s^{-1})$
EC	1.91	1.11	0.70	1.21	0.69	0.44
EGC	1.92	1.12	0.71	1.21	0.70	0.44
ECG	1.68	0.94	0.60	1.06	0.58	0.38
EGCG	1.69	0.94	0.60	1.07	0.58	0.38
Caffeine	2.20	1.34	0.83	1.39	0.83	0.52

Table 6.5. Model parameters for the mass transfer resistance for Experiment 2.

	Experiment 2 (HP20 resin)					
	Adsorption			Desorption		
	$k_{ext}(10^{-3}\ s^{-1})$	$k_{int}(10^{-3}\ s^{-1})$	$k_{ov}(10^{-3}\ s^{-1})$	$k_{ext}(10^{-3}\ s^{-1})$	$k_{int}(10^{-3}\ s^{-1})$	$k_{ov}(10^{-3}\ s^{-1})$
EC	111.58	160.60	65.84	67.96	100.07	40.48
EGC	112.09	161.46	66.16	68.27	100.61	40.67
ECG	96.08	134.65	56.07	58.48	83.91	34.46
EGCG	96.73	135.72	56.48	58.87	84.58	34.71
Caffeine	130.90	193.64	78.11	79.79	120.66	48.03

The overall mass transfer coefficient (k_{ov}) is higher in the adsorption than in the desorption, for the three experiments and it is also higher for the HP20 resin than for the

XAD7HP resin. From the five components, caffeine is the one with the higher mass transfer.

When comparing the values in Table 6.4 and 6.6 (the two experiments with the XAD7HP resin) it is possible to observe that the increase in Experiment 3 for the overall mass transfer coefficient is due to the correspondent increase in the external mass transfer resistance. This result was already expected, since the rate of film diffusion is controlled by the flow rate and the intra-particle diffusion is an inherent property of the adsorbent particle and not dependent on the design parameters of the column. The rate of film diffusion is higher than the intra-particle diffusion and hence not the rate limiting parameter.

Table 6.6. Model parameters for the mass transfer resistance for Experiment 3.

	Experiment 3 (XAD7HP resin)					
	Adsorption			**Desorption**		
	$k_{ext}(10^{-3}\ s^{-1})$	$k_{int}(10^{-3}\ s^{-1})$	$k_{ov}\ (10^{-3}\ s^{-1})$	$k_{ext}(10^{-3}\ s^{-1})$	$k_{int}(10^{-3}\ s^{-1})$	$k_{ov}(10^{-3}\ s^{-1})$
EC	5.85	1.11	0.93	4.20	0.69	0.59
EGC	5.88	1.12	0.94	4.21	0.70	0.60
ECG	5.18	0.93	0.79	3.72	0.58	0.50
EGCG	5.21	0.94	0.79	3.74	0.58	0.51
Caffeine	6.66	1.34	1.11	4.78	0.83	0.71

For the calculation of the mass transfer lumped parameters, molecular diffusivity values from (Sevillano et al., 2014) are used. Due to the unavailability of elution partition coefficients for the Diaion HP20 resin, the desorption comparison is not shown in Figure 6.2.

For the model validation the results obtained from the model are compared with the experimental results, see Figure 6.2. The modeled adsorption breakthrough curves for Experiment 1 and Experiment 3 have a reasonable fitting to the experiments. For Experiment 2 the fitting is not good, mainly in the specific cases of ECG and caffeine.

For the three experiments the model breakthrough curves are able to describe the saturation of each component, but are unable to describe the shape of the curve with good accuracy. The modeled elution curves do not describe the experimental results, which can be attributed to the inaccuracies in the mass transfer and isotherm parameters. For this reason, the overall mass transfer and the isotherm parameters were regressed from the experimental data. A sensitivity analysis is performed to determine the degree of influence over the model for each of these parameters.

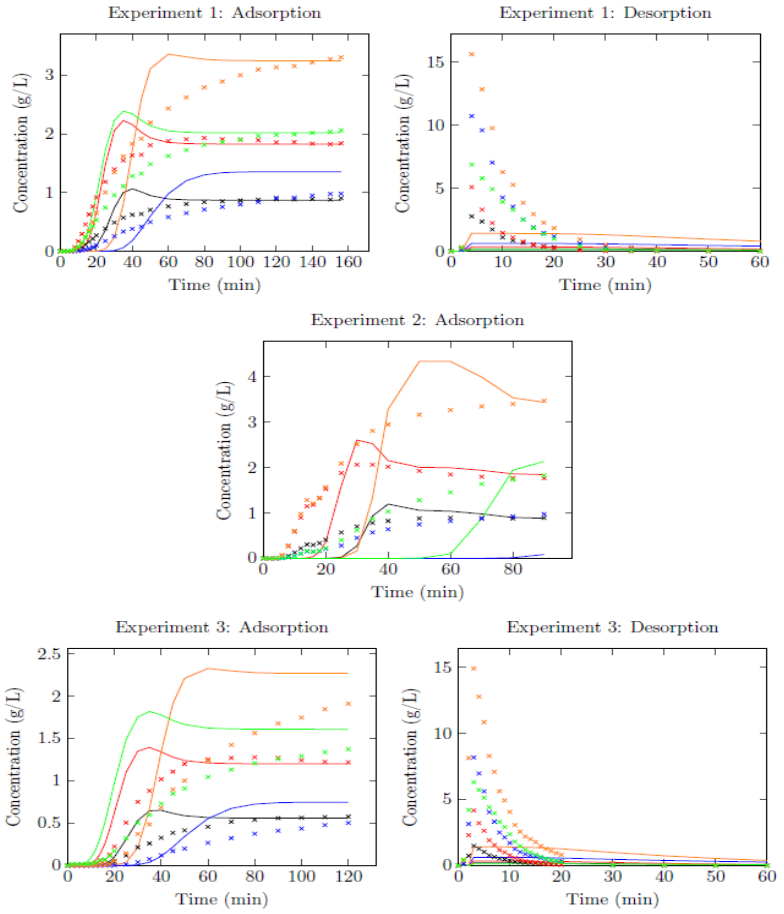


Figure 6.2. Breakthrough curves and elution curves for Experiments 1, 2 and 3 ('x' for experimental and '—-' for model. Legend: EC, EGC, ECG, EGCG and Caff.

6.4.2. Sensitivity analysis

The sensitivity of four parameters ($D_{az,i}$, $k_{ov,i}$, $Q_{m,i}$ and b_i) from the model was tested (Figure 6.3). The analysis of the axial dispersion coefficient $D_{az,i}$ show that a value increase of 200 % has no influence on the model output (Figure 6.3). This indicates that the mass transfer and not the axial dispersion is the main responsible factor for the *apparent* dispersion.

The results in Figure 6.3 also show that when the value of the overall mass transfer coefficient ($k_{ov,i}$) is reduced by 10 % the model results are strongly influenced. This was already expected since the mass transfer is the limiting factor for the resin saturation rate.

For the isotherm parameters ($Q_{m,i}$ and b_i) an increase of respectively 10 % and 100 %, show a strong influence on the shape of the breakthrough curves and on the calculated results.

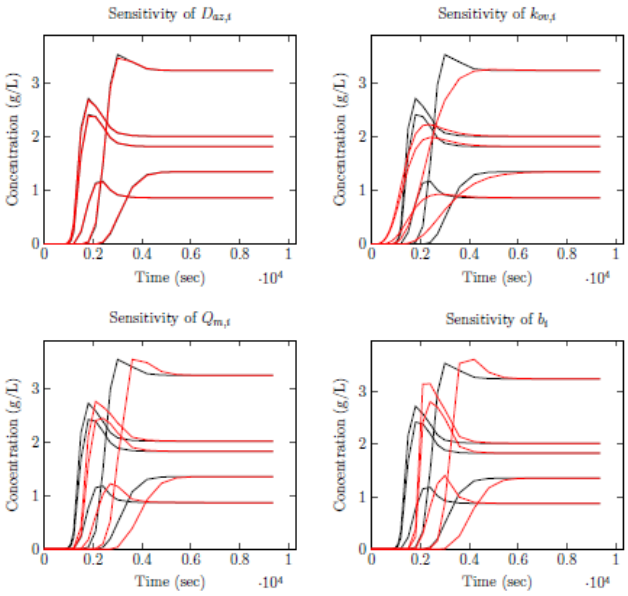


Figure 6.3. Sensitivity analysis results for the adsorbed components: EC, EGC, ECG, EGCG and Caff (original '—' and modified '—') for the selected parameters: $D_{az,i}$,$k_{ov,i}$, $Q_{m,i}$ *and* b_i.

The analysis shows that three ($k_{ov,i}$, $Q_{m,i}$ and b_i) of the four tested parameters strongly influence the column model and should be regressed from the experimental data.

6.4.3. Parameter regression

The built-in MATLAB function fsolve was used as an optimization routine to regress the parameters $k_{ov,i}$, $Q_{m,i}$ and b_i. This function uses the Levenberg-Marquardt (LM) algorithm to minimize the residual between the numerical and experimental data (Fan, 2003). The LM algorithm is an iterative technique for solving nonlinear least-squares problems. It finds the minimum in a multivariate function that can be expressed as the sum of squares of non-linear functions.

The breakthrough and elution curves comparing the model and experimental results are shown in Figures 6.4 to 6.6, for the Experimental runs 1to 3 respectively.

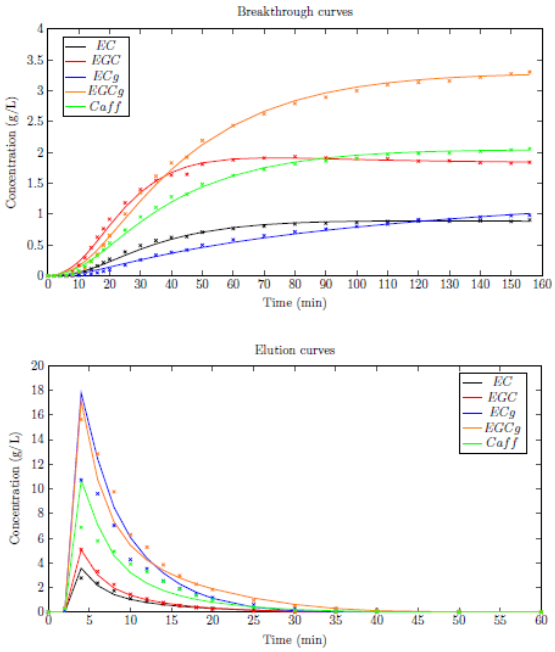


Figure 6.4. Model '—' and experimental 'x' breakthrough and elution curves for Experiment 1 (XAD7HP resin), for experimental conditions, see Table 6.1.

The results in Figure 6.4 demonstrate that the model based on the regressed parameters for the XAD7HP resin correspond well with experimental data. The EGC is the fastest component to reach saturated bonding, while the ECG still did not reach saturation at 150 min. Upon elution the different adsorption capacities of the catechins can be observed. The ratio of the compounds in the eluent is different from the ratio of the compounds in the feed. While EGC and caffeine have similar amounts in the feed, in the eluent only half the amount of caffeine to EGC is found. In the specific case of the elution curves for ECG and caffeine, the model overpredicts the maximum concentration value. Nevertheless, the model follows the trend for all the components.

The results in Figure 6.5 show that the model is able to accurately predict the behaviour of all the components, in both the breakthrough curves and the elution curves, for the HP20 resin. The breakthrough curves in Figures 6.4 and 6.5 also show some overshoot for the smaller components (EGC and caffeine), which is related to the components different rates of diffusion and affinities for the resin. In this case the smaller components are more adsorbed at an initial stage and afterwards partially desorbed, when the bulkier components with more affinity arrive to the same adsorption sites.

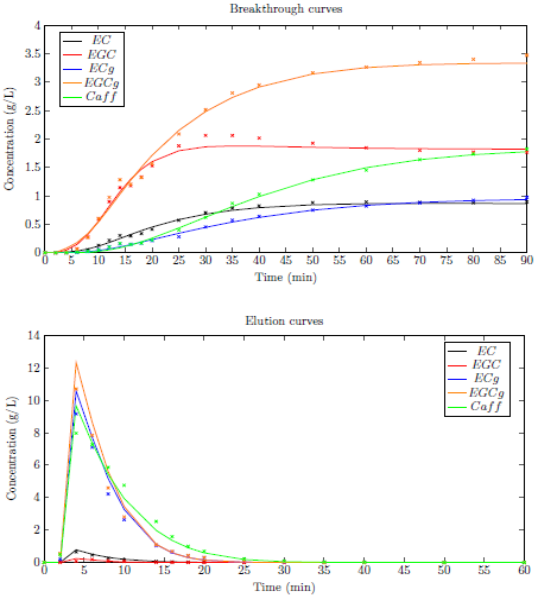


Figure 6.5. Model '—' and experimental 'x' breakthrough and elution curves for Experiment 2 (HP20 resin), for experimental conditions, see Table 6.1.

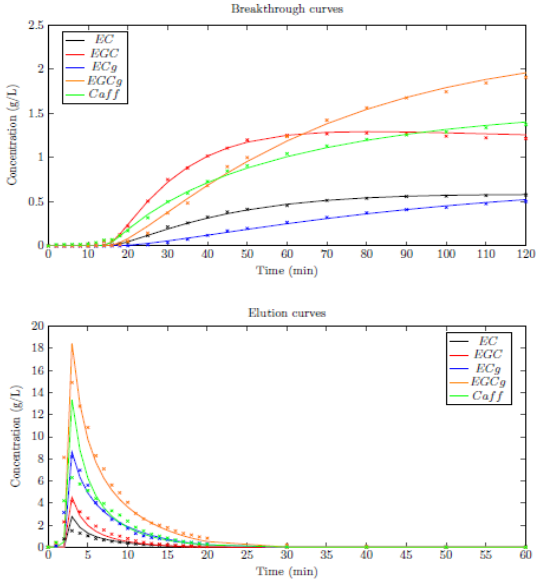


Figure 6.6. Model '—' and experimental 'x' breakthrough and elution curves for Experiment 3 (XAD7HP resin), for experimental conditions, see Table 6.1.

The results in Figure 6.6 also show a good fitting to the model. Like in the two previous experiments, EGC is the fastest component to reach saturated bonding and after 120 min

caffeine, EGCG and ECG did not reach saturation. For the elution curves it is observed that for caffeine the model overpredicts the maximum concentration value. The model residual plots are presented in Appendix C.

The regressed parameters values are presented in Tables 6.7 to 6.9, for the three experiments. There is a clear difference between the values of the Langmuir equilibrium constants ($Q_{m,i}$, b_i) for the 2 resins. The XAD7HP resin has a somewhat higher Q_m for the gallated catechins (ECG and EGCG), while the HP20 resin has a higher Q_m for the gallated catechins (ECG and EGCG) and caffeine.

Table 6.7. Regressed parameters values for Experiment 1.

	Experiment 1 (XAD7HP resin)					
	Adsorption			**Desorption**		
	$Q_{m,i}$(g/g)	b_i(dm^3/g)	$k_{ov,i}$ (10^{-3} s^{-1})	$Q_{m,i}$ (g/g)	b_i(dm^3/g)	$k_{ov,i}$ (10^{-3} s^{-1})
EC	0.25	2.59	0.87	0.25	0.063	43.9
EGC	0.25	1.63	1.05	0.25	0.057	33.1
ECG	0.30	16.95	0.12	0.30	0.041	37.4
EGCG	0.31	3.12	0.67	0.31	0.058	37.6
Caffeine	0.22	4.13	0.59	0.22	0.049	7.8

Table 6.8. Regressed parameters values for Experiment 2.

	Experiment 2 (HP20 resin)					
	Adsorption			**Desorption**		
	$Q_{m,i}$ (g/g)	b_i(dm^3/g)	$k_{ov,i}$ (10^{-3} s^{-1})	$Q_{m,i}$ (g/g)	b_i(dm^3/g)	$k_{ov,i}$ (10^{-3} s^{-1})
EC	0.10	0.047	17.3	0.10	0.011	11.5
EGC	0.02	0.026	169.1	0.02	0.011	41.6
ECG	0.96	0.069	1.2	0.96	0.012	19.6
EGCG	0.52	0.041	3.4	0.52	0.011	17.8
Caffeine	0.33	0.119	2.3	0.33	0.021	10.9

Table 6.9. Regressed parameters values for Experiment 3.

	Experiment 3 (XAD7HP resin)					
	Adsorption			**Desorption**		
	$Q_{m,i}$ (g/g)	b_i(dm^3/g)	$k_{ov,i}$ (10^{-3} s^{-1})	$Q_{m,i}$ (g/g)	b_i(dm^3/g)	$k_{ov,i}$ (10^{-3} s^{-1})
EC	0.25	10.54	0.74	0.25	0.063	66.7
EGC	0.25	6.21	1.02	0.25	0.057	50.3
ECG	0.30	26.51	0.36	0.30	0.056	56.8
EGCG	0.31	17.69	0.40	0.31	0.058	57,2
Caffeine	0.22	23.07	0.27	0.22	0.049	11.9

The XAD7HP resin has an higher affinity for all the components when compared to the HP20 resin. The HP20 resin has a much higher affinity for the caffeine than for the catechins, indicating that it is a good option to use the HP20 resin in a first operation to separate caffeine from the other components.

6.5. Conclusions

In this chapter the dynamics of multicomponent solvent swing adsorption packed bed columns have been described with an axial dispersed column model. Three sensitive model parameters, overall mass transfer coefficient $(k_{ov,i})$, maximum adsorption capacity $(Q_{m,i})$ and the Langmuir constant (b_i) were regressed from the experimental data.

Five green tea components (four catechins present in green tea and caffeine) were modeled in the competitive sorption and show a good fitting with the experimental data. The modeled breakthrough and elution curves could be used to predict the competitive adsorption between all the components.

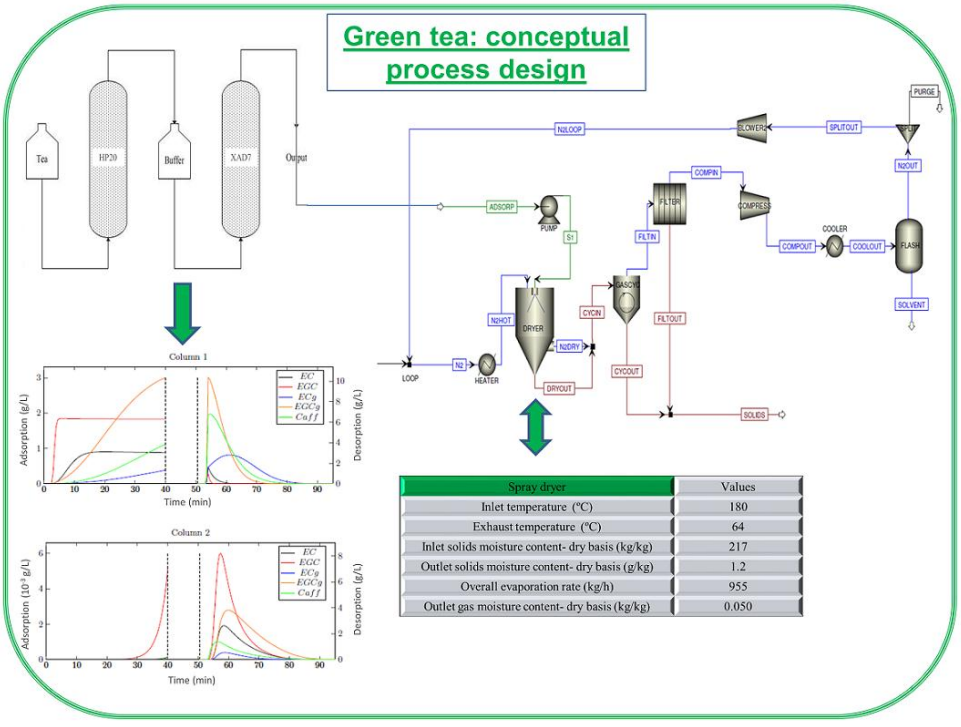
The values of the regressed parameters for the XAD7HP and HP20 resins demonstrate the different affinities towards the macroporous resins. The XAD7HP resin has a higher affinity for all the components, when comparing to the HP20 resin. On the other hand, the HP20 resin has a much higher affinity for the caffeine than for the catechins, resulting in a good option to preferentially adsorb caffeine, e.g. in a pretreatment step.

6.6. List of symbols

τ	Tortuosity factor
ε_b	Bed porosity
ρ_p	Density of the adsorbent
b	Langmuir constant, dm^3/g
c_i	Concentration of the component *i*, g/dm^3
$D_{p,i}^e$	Effective pore diffusivity, m^2/s
$D_{az,i}$	Axial dispersion coefficient, m^2/s
D_{LP}	Liquid-phase diffusivity coefficient, m^2/s
d_p	Particle diameter, m
$k_{ext,i}$	External mass transfer resistance, s^{-1}
$k_{int,i}$	Internal mass transfer resistance, s^{-1}
$k_{ov,i}$	Overall mass transfer resistance, s^{-1}
Q_m	Maximum adsorption capacity, g/g
p	Probability of axial displacement
Q_i	Concentration of component *i* on adsorbent, g/g
v	Superficial fluid velocity, m/s
v	Interstitial fluid velocity, m/s
Re	Reynolds number
Sc	Schmidt number
Sh	Sherwood number

7.

Conceptual process design



Spray dryer	Values
Inlet temperature (°C)	180
Exhaust temperature (°C)	64
Inlet solids moisture content- dry basis (kg/kg)	217
Outlet solids moisture content- dry basis (g/kg)	1.2
Overall evaporation rate (kg/h)	955
Outlet gas moisture content- dry basis (kg/kg)	0.050

ABSTRACT: In this chapter, a conceptual process design for the production of a purified catechins powder from green tea is presented. In the first step two different operational designs for the adsorption of catechins in a packed bed column are presented and evaluated. For the second step a spray drying process is modeled to determine the equipment and the operational conditions, as well as the economic potential. The conceptual study shows that it is possible to obtain a powder of catechins with 83.5 % purity and a particle sauter mean diameter of 7.5 micron.

7.1. Introduction

In this chapter a process design for the recovery of catechins from green tea is presented. Level 5 (Mechanism and operational window) and Level 7 (Equipment selection and design) of the PDPS methodology are applied to achieve the final conceptual design. Level 6 (Multi product integration) does not apply in this case. The starting material is a green tea aqueous extract and the output is a catechins rich dry powder.

7.2. Process adsorption schemes (green tea)

The model that describes the column dynamics (see chapter 6) can be used to estimate operational parameters of the adsorption process in a packed bed process, for the recovery of catechins from green tea. The Matlab adsorption model was expanded to include two packed bed operating designs: in Design 1 a two columns process is used with the objective of maximizing the amount of catechins and minimizing the contamination of the catechins with caffeine. In Design 2 a single column is used with the purpose of maximizing the amount of catechins recovered. All the columns have the same dimensions: a length of 2.2 m and a diameter of 0.2 m. For the desorption a 70 % (wt %) ethanol-water mixture was used.

Although it is not included in the model, after the elution, the last step should be a washing with water to prepare the column for the next cycle. In addition to the washing procedures, after a certain number of operating cycles, a resin regeneration procedure should be performed, e.g. elution with sodium hydroxide and afterwards rinsing until neutral pH. In this study the regeneration step is not considered.

The objective is to achieve an efficient process design for the separation of green tea catechins with a high yield and purity. In a preliminary process design, the regressed model is optimized for the operational time of the columns, in one adsorption and desorption cycle.

7.2.1. Design 1

Design 1 (Figure 7.1) is based on two adsorption columns connected by a buffer tank and operated in a batch mode. The green tea extract is fed to the first column at the bottom with a flow rate of 0.84 m^3/h. The outlet stream at the top of the column is afterwards collected in the buffer tank. The flow from the buffer tank is the feed of the second column. The output of the second column is the final stream of the process.

Based on the resin screening results reported by Sevillano et al. (2014), the first column will be packed with HP20 resin (favorable for caffeine adsorption) and the second column will be packed with the resin XAD7HP (preferred for the adsorption of catechins).

In total four operating modes are considered, based on the possibility of using the output from the adsorption or from the desorption in each column (Figure 7.1). The feed for the buffer tank, can be taken from the output of either the adsorption or desorption of the HP20 column. The output of the whole process can either be the adsorption or the desorption of the second column (XAD7 column).

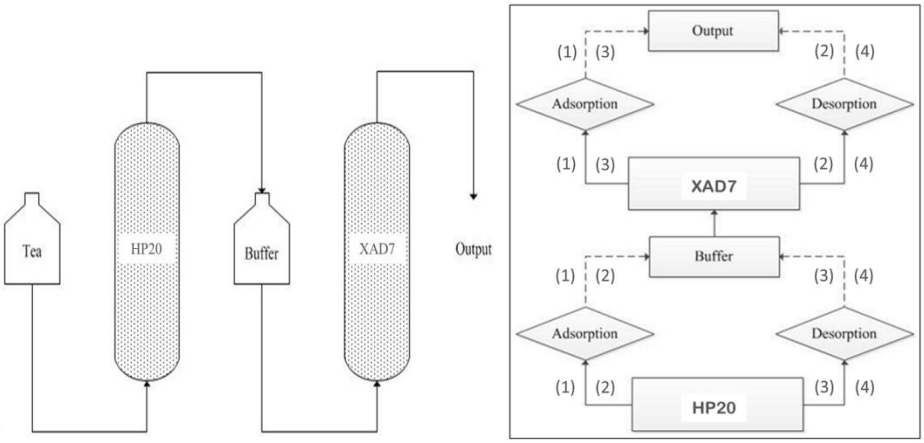


Figure 7.1. Two columns process design diagram (left) and the four possible operational modes (right).

The objective of Design 1 is to maximize the yield of catechins (Y_{cat}) and to minimize the yield of caffeine (Y_{caff}), see Equations 7.1 and 7.2.

$$Y_{cat}(\%) = \frac{mass\ of\ catechins\ in\ output(g)}{mass\ of\ catechins\ in\ feed(g)} * 100 \quad (7.1)$$

$$Y_{caff}(\%) = \frac{mass\ of\ caffeine\ in\ output(g)}{mass\ of\ caffeine\ in\ feed(g)} * 100 \quad (7.2)$$

The operational mode which achieves the best result is the one that uses the adsorption output of column 1 (HP20 resin), as the input for the buffer, followed by the desorption

(elution) output of column 2 (XAD7HP resin), represented in Figure 7.1 by operational mode (2). In operational mode (2), column 1 is used to preferably adsorb the caffeine, while column 2 (XAD7HP resin) mostly adsorbs the catechins.

In Figure 7.2. the concentration profiles for a complete operational cycle are presented. For the two columns cycle, the model optimized operating time is 40 minutes for the adsorption step and 45 minutes for the desorption step. A washing step of 2 bed volumes (BV) is performed between the adsorption and desorption steps. One complete operational cycle achieves a 52 % yield of catechins, while reducing the yield of caffeine to 19 %.

To better evaluate the relative amounts of catechins and caffeine after one adsorption/desorption cycle, a relative purity of catechins ($P_{cat,caff}$) is defined (Equation 7.3):

$$P_{cat,caff}\,(\%) = \frac{concentration\ of\ catechins\ \ (\mathrm{g/\,dm^3})}{concentration\ of\ catechins + caffeine\ \ (\mathrm{g/\,dm^3})} * 100 \qquad (7.3)$$

In the green tea extract feed for the first column the relative purity of catechins was 78 %. After one cycle the catechins relative purity increased to 91 %.

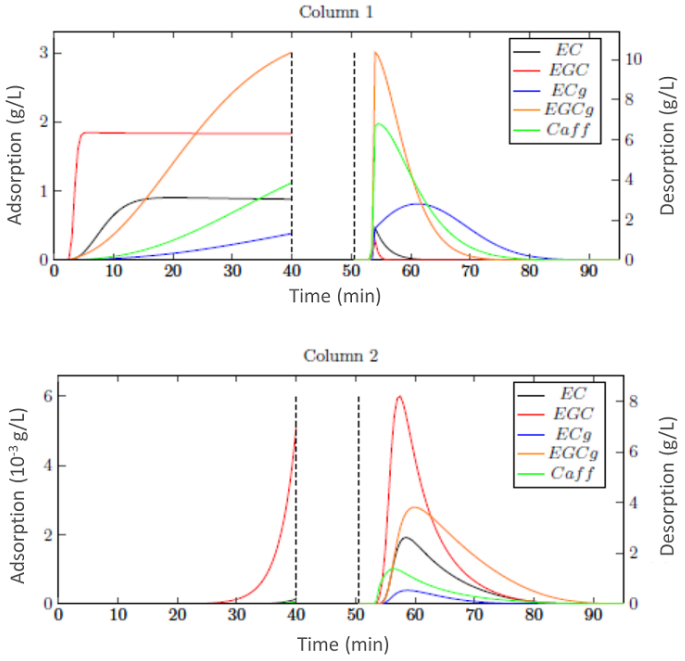


Figure 7.2. Column operation cycle (adsorption + washing + desorption) for Design 1.

7.2.2. Design 2

In Design 2 a single column packed with the XAD7HP resin was used and the operational time is optimized with the single objective of maximizing the yield of catechins (Y_{cat}).

The green tea extract flow rate is set at 0.56 m^3/h and a washing step of 2 bed volumes (BV) is performed between the adsorption and desorption steps. The concentration profiles of the components (four catechins and caffeine) in one operational cycle are presented in Figure 7.3. For design 2, the optimal operational time is 20 minutes for the adsorption step and 65 minutes for the desorption step (Figure 7.3), achieving a yield of catechins of 89 % and a yield of caffeine of 88 %.

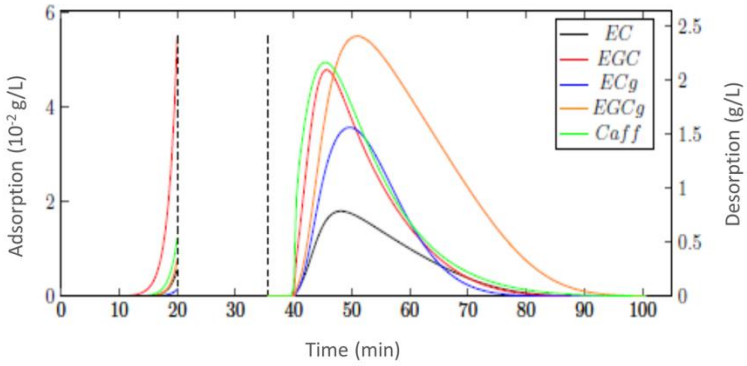


Figure 7.3. Column operation cycle (adsorption + washing + desorption) for Design 2.

Design 1 is capable of separating most of the caffeine and still recover more than half of the catechins, while Design 2 is very efficient for the recovery of catechins together with caffeine.

7.3. Spray Drying

The catechins solution resulting from the sorption process, is proposed to be subjected to a spray dry process to achieve a high purity catechins dry powder. Drying can be expensive, especially when large amounts of water, with its high heat of vaporization, must be evaporated. For drying high feed rate solutions with more than 50 % of moisture, spray drying is recommended when compared to other methods of drying, like freeze drying or drum drying (Seader and Henley, 2005).

7.3.1. Introduction

Drying is the removal of moisture from solids, solutions, slurries and pastes to produce solid products. Drying is widely used in several products, e.g. crystalline particles of inorganic salts and organic compounds to produce a free-flowing product, biological

materials (including food) to prevent spoilage and decay by microorganisms, as well as pharmaceuticals, and detergents. Spray drying is widely used in industry for particle formation by drying. In many cases the final product needs to comply with specific quality standards. The feed to the spray dryer can be a liquid or slurry that is rapidly dried with hot gas. The dry particles obtained from spray drying are typically very fine (Re, 2006; Tang et al., 2011). The most common applications are in the food and pharma industries, as a way to ensure microbiological stability, to avoid degradation or to obtain very specific properties e.g., high bulk density, instantaneous solubility, uniform particle distribution (Chen and Patel, 2008; Kerkhof, 1979).

A spray drying unit normally includes a heat exchanger, a drying chamber, centrifugal disks, an atomizer (or spray nozzle) and a cyclone. The first process step is the atomization of the liquid into a spray of droplets, which come in contact with the hot gas in the dryer. Spray drying can achieve high evaporation rates (up to 160 $kg.h^{-1}.m^{-2}$ of particle area) making it appropriate to dry thermally sensitive materials. Spray drying is very fast, with a specific time constant of a few seconds (Kerkhof, 2001; Mujumdar, 2014).

The atomization step is the critical step in the process, to obtain a low moisture content and a specific droplet size. There are three types of atomizers: pneumatic nozzles, pressure nozzles and centrifugal disks (Seader and Henley, 2005). The pneumatic (also called two-fluid) nozzles use the gas in the feed at low pressures, but are not efficient at high capacities. The pressure or single-fluid nozzles require high pressures to achieve breakup of the liquid feed stream. Pressure nozzles can achieve a very narrow range of droplet sizes, but the droplets are the largest obtained by the three types of atomizers. When using large spray dryers multiple nozzles must be used, as well as tall drying chambers (height 4 or 5 times the diameter). Centrifugal disks (also called spray wheels) can be used for solutions or slurries. The feed forms thin films that break up into small droplets in a nearly radial direction. The disks have the largest-diameter spray pattern and the largest drying chambers. For centrifugal disks the particle-size distribution is not very sensitive to the selected operational parameters.

When organic solvents that need to be recovered are used, closed cycle dryers are used, together with an inert gas as heat carrier medium, to avoid possible ignition as well as oxidation of the product. In this type of dryers the solvent vapors, after filtering, can be condensed in a single stage or multistage condenser. The hot and humid effluent gas can be cooled in a scrubber-condenser, where the solvent vapors partially condense. The drying cycle is closed by returning the spent gas into the dryer in the following steps: separation from the dry material, dehumidification and heating. As inert gas usually nitrogen is used. Nitrogen is supplied continuously in a fresh make-up stream (Seader and Henley, 2005).

The majority of the dry particles are usually recovered in the dryer, with the remaining particles being recovered in highly efficient cyclones, bag filters or other gas-solid

separators. The hot gas can be moved by a fan (Araujo et al., 2010; Mujumdar, 2014). The operation of a spray dryer is similar to that of a pneumatic-conveyor dryer because particles are small, gas temperature is high and mainly surface moisture is removed during a short residence time (Seader and Henley, 2005).

7.3.2. Process description

For the drying of tea polyphenols after the adsorption step a closed cycle spray dry process model is used. The design of the process is done in Aspen Plus Version 8.6. The spray drying unit is used to evaporate the solvent and to dry the solids. Spray drying is commonly used in the food industry because it can handle temperature sensitive materials and produces solid rounded particles with a uniform size. Another advantage is the potential combination of several steps (evaporation, crystallization, filtration, size reduction, classification and drying) in a single operation.

After the packed bed adsorption process, to achieve the product in the form of a dry powder, a spray drying process is used. The spray dryer is modeled in Aspen Plus based on the atomization and particle motion. The input data and assumptions of the model as well as the selected equipment description are reported in Appendix D.

The feed stream for the adsorption process is an aqueous green tea stream with a flow rate of 1000 kg/h. The feed stream for the spray dyer is based on the calculated stream leaving the two column adsorption process (Design 1, see section 7.2.1). Since the solvent is an ethanol-water mixture, nitrogen (inert gas) is used as heat carrier medium, to prevent ignition. Using nitrogen also has the advantage of avoiding the oxidation of the polyphenols. In addition, a closed cycle drying process is used to recirculate the nitrogen to the spray dryer (Figure 7.4).

The heated gas is introduced through a roof disperser around the atomizer, creating a co-current flow of product and gas. The contact between the gas and the droplets as soon as they are formed causes rapid surface evaporation, and keeps the solids at relatively low temperatures. The particle formation in the dryer starts after the critical moisture content in the droplet is reached. When evaporation becomes limited by the liquid diffusion from the center of the droplet to the surface, the particles are in a less hot zone of the dryer. This is the reason why heat-sensitive products can be spray-dried even at elevated temperatures. The final product from the drying model should be a solid product with less than 0.2 % (wt %) moisture content, at a maximum temperature of 70 °C. The drying temperature is set at 180 ºC based on the optimized spray drying conditions reported by (Tang et al., 2011) and on the desired output settings. The nitrogen flow rate is set at 18500 kg/h.

The most important part of the spray dyer is the atomizer. In the atomizer the droplets that are formed close to the atomizing device have a direction and velocity well defined by the

atomizer. The evaporation begins when the hot gas is mixed with the droplets. The gas flow determines the droplets motion inside the dryer chamber, to allow solvent evaporation and to avoid contact with the dryer walls. In a closed cycle preferably rotary or liquid nozzle atomizers are used, with the advantage that no gas is needed for the spraying (Mujumdar, 2014). The selected liquid atomizer for the dryer is a hollow cone nozzle. The spray dryer is operated in a co-current mode from the top to the bottom. The diameter of the dryer is proportional to the particle size desired in the final powder. The main characteristics of the modeled spray dryer are collected in Table 7.1.

Table 7.1. Spray dryer description.

Spray dryer	Values
Tower height (m)	6
Tower diameter (m)	2
Spray angle (deg)	45
Number of atomizers	9
Nozzle orifice diameter (mm)	1
Number of droplet size classes	4

The spray drying process flowsheet is depicted in Figure 7.4. In the dryer, the solvent evaporates into the stream of nitrogen, resulting in a dry solid material. The evaporation rate in the dryer is directly proportional to the product of the temperature difference along the dryer and the mass flow rate of the gas. Part of the particles are formed and collected in the dryer. The remaining particles entrained in the spent drying gas are recovered in a high efficiency cyclone or in the fabric filter with a baghouse design. All the particles formed are recovered into the solids stream. Since the formed powder is very fine, it is collected in the cyclone, with a sauter mean diameter (SMD) of 22.5 micron, as well as in the bag collector (SMD of 5.24 micron), which is the main collection point.

The humid gas afterwards passes through the filter and goes to a condenser-scrubber, where the solvent vapors are condensed. The gas without the solvent is then recirculated to the dryer. The recovered solvent is recirculated to the column adsorption process, to be used in the desorption step.

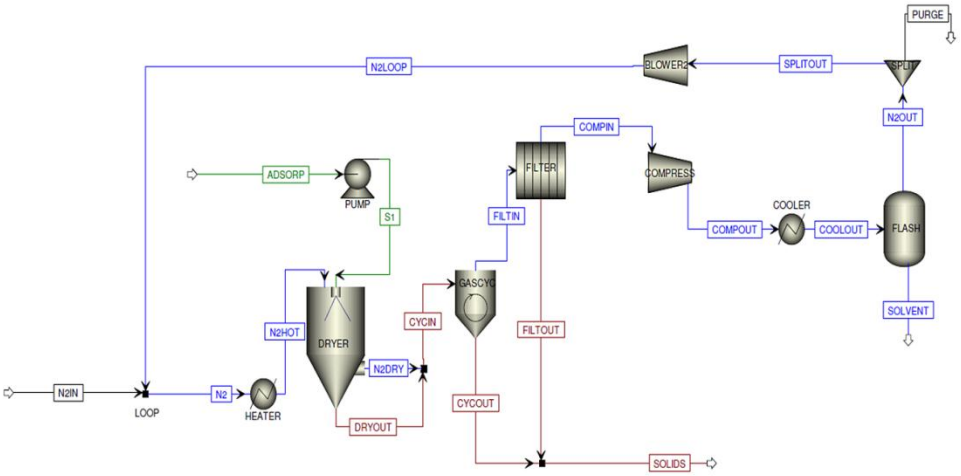


Figure 7.4. Spray drying process flowsheet. The gas streams are shown in blue, the solids streams in red and the input stream from the adsorption process in green.

7.3.3. Results

Spray dryer

For the droplet size distribution in the atomizers, the formation of four droplet size classes is assumed (one for each droplet interval). The calculated results from the droplet classes and gas temperature variation along the axial position in the dryer are shown in Figure 7.5. At the beginning of the drying chamber classes 1 and 2 are already heated to the gas temperature. Between 1 and 2 meters height the class 3 particles are dried. The last class of particles only reaches the gas temperature near the bottom of the chamber.

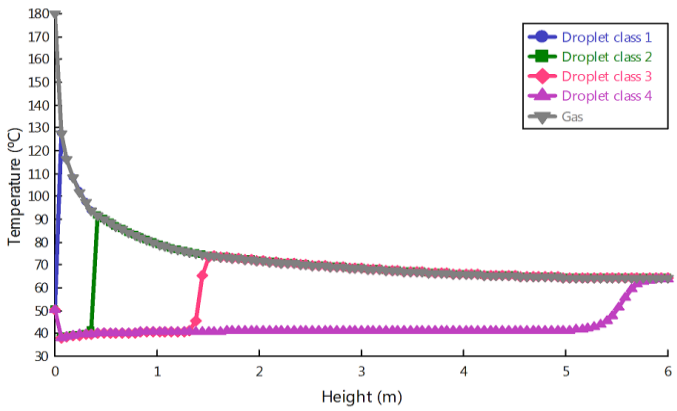


Figure 7.5. Temperature curve along the axial position (m) of the dryer (0 = top).

In Figure 7.6 the results of the calculations for the moisture content in the droplets are depicted for each droplet size class. Again very high drying rates are calculated for classes 1 and 2. Class 4 has a lower drying rate and only reaches the a low moisture content at the bottom of the drying chamber. At the end of the drying process the solid particles have a final moisture content of 1.2 g/kg.

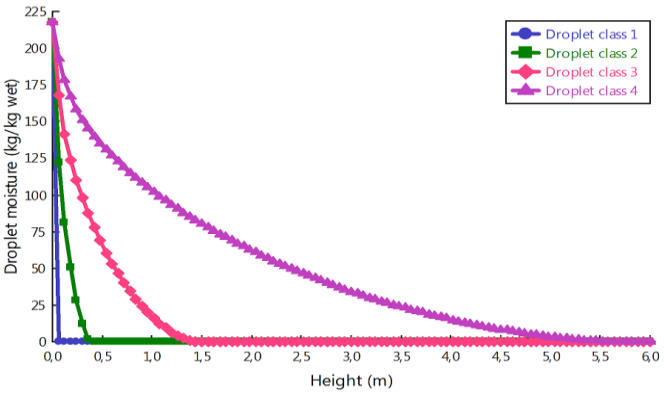


Figure 7.6. Droplet moisture curve along the height of the dryer.

The dryer model calculated results are collected in Table 7.2.

Table 7.2. Spray drying results for the feed from the adsorption process.

Spray dryer	Values
Inlet temperature (ºC)	180
Exhaust temperature (ºC)	64
Inlet solids moisture content- dry basis (kg/kg)	217
Outlet solids moisture content- dry basis (g/kg)	1.2
Overall evaporation rate (kg/h)	955
Outlet gas moisture content- dry basis (kg/kg)	0.05

Solids stream

The model results for the solids stream demonstrate that from 1000 kg/h of tea extract, 4.47 kg/h of catechins are produced with 83.5 % purity, at a temperature of 64 ºC. The SMD of the dry particles is 7.5 micron. The advantage of obtaining such a fine powder is that it can be easily dissolved when added to other products. This specification tends to increase the commercial value of the catechins powder.

7.4. Economic evaluation

Although cost data is scarce, it is expected that the costs of the spray drying process (including the solvent recovery) will be much higher than the adsorption costs. Both the

spray dryer and the condenser for the solvent recovery are high energy consumption operations. Nevertheless, the make-up ethanol solution costs for the adsorption process are included in the overall costs in the economic analysis. The total heating duty is 3467 KW.

The summary with all the considered process operating costs is included in Table 7.3. The price for the green tea extract is assumed to be 50 €/ton and the price for the purified catechins is assumed to be 95 €/kg. The yearly economic calculations performed by Aspen Plus are based on a processing time of 4800 hours per year (DACE Price Booklet, 2011).

Table 7.3. Summary of the annual operating costs.

Utility	Values
Total heating cost flow:	0,75 M€/year
Total cooling cost flow:	0,048 M€/year
Net cost (Total heating cost + Total cooling cost):	0,80 M€/year
Stream cost	**Values**
Net cost flow of feeds:	0,49 M€/year
Net cost flow of products:	3,00 M€/year
Overall net cost flow:	2,50 M€/year

The net cost flow of feeds is the cost of the raw materials in M€/year and the net cost flow of products is the value of the product stream, also in M€/year.

The process operating profit is calculated according to Equation 7.4 and it is a preliminary evaluation of the process potential profitability.

[Operating profit] = [Products sales] - [Raw material costs] - [Utility costs] (7.4)

For this process the operating profit is 1.7 M€/year. For this simplified evaluation mass and energy balances are considered, but no real equipment design constraints.

To estimate the full capital and operating costs, the Aspen Process Economic Analyzer® tool has been used to perform the economic evaluation within the Aspen Plus model simulation. The capital expenditures (CAPEX) and the operational expenditures (OPEX) were calculated, together with the total annual costs (TAC). The total OPEX are estimated to be 2.1 M€/year, for which the main contribution is from the cooler and the compressor. The CAPEX value is estimated to be 7.1 M€, with the major contribution coming from the compressor. The calculation of the TAC is done by summing up the OPEX with 20 % of the CAPEX (Seider et al., 2004). The TAC is estimated to be 3.5 M€/year.

7.5. Conclusions

In this chapter a detailed conceptual design for the separation and purification of catechins from a green tea extract has been presented. In the first part of the process a packed bed adsorption is used, followed by a spray drying process. For the green tea adsorption, two operating designs are simulated and optimized for the operational time of the packed bed columns.

Design 1 uses a two column process with the multi-objective of maximizing the amount of catechins and minimizing the amount of caffeine. After one operational cycle the yield of catechins was 52 % and the yield of caffeine was 19 %, for an operation time per column of 95 minutes. The relative purity of catechins to caffeine increased from 78 % to 91 %, when compared to the feed green tea solution. If instead of the multi-objective optimization, the only objective is to maximize the amount of catechins, a single column with an operation time of 100 minutes can be used (Design 2). Design 2 achieves a yield for the catechins of 89 %. Overall, Design 1 proves to be effective for separating the caffeine and still allows the recovery of more than half of the catechins. Design 2, however, is very efficient for the recovery of catechins.

Following the green tea column adsorption process (Design 1), a spray dry conceptual design is proposed to produce a high purity catechins dry powder. The spray drying process is able to produce a fine catechins powder with 83.5 % purity. The fact that the powder has a small particle sauter mean diameter means that it can easily be dissolved for future applications.

Based on the estimated economic evaluation it can be concluded that the process has the potential to be profitable.

7.6. Nomenclature

BV	Bed volumes
CAPEX	Capital expenditures
OPEX	Operational expenditures
($P_{cat,caff}$)	Relative purity of catechins
SMD	Sauter mean diameter
TAC	Total annual costs
Y_{caff}	Yield of caffeine (%)
Y_{cat}	Yield of catechins (%)

8.

Conclusions and outlook

8.1. Conclusions

With time, as consumers become more demanding, it is clear that the market for natural products will keep growing. The demand for polyphenols follows this trend due to their potential use as bioactive ingredients in several industries.

This thesis deals with the need to achieve a food grade and environmentally friendly process for the separation of polyphenols from liquid tea streams, which allows keeping the "natural" character of the products. This brings competitive marketing advantages and premium prices, as natural products are perceived as superior by the consumers.

The PDPS methodology has been applied for the recovery of tea polyphenols, by combining product and process synthesis principles with an engineering overview, in a structured and systematic approach. The polyphenols separation and purification proved to be very challenging, due to instability and degradation. Furthermore, the presence of several components in tea that interact with the polyphenols or have similar behavior and/or properties, increases the degree of complexity. Nonetheless, different properties and separation methods are presented and evaluated in this thesis for the recovery of polyphenols.

However, the modeling of multicomponent solute systems is very complex and the process optimization should not be underestimated. The use of process optimization for the separation and purification of polyphenols, allows the creation of desired functional properties and applications on the final food products. By developing dynamic models it is possible to incorporate changes to the system and/or to the selected process operational conditions.

An increasing knowledge of the functional properties of the components in the mixture allows the expansion of application areas and, together with the development of separation technologies, provides the possibility of having an adequate supply to the growing market of bioactive products and healthy food additives. In addition, it should also be considered that the tea stream after certain separation units still has commercial value, as only some components are partially removed, like for example during the adsorption process. If quantifiable, the value of this stream should be added to the value of the purified polyphenols to improve the economic potential.

As referred before, the PDPS is a multidisciplinary methodology. A continuous collaboration and communication between the R&D department and the marketing department is very important in the critical decision-making path. Sometimes, it is possible to produce similar products or products with different characteristics but with a similar market value at lower production costs. A striking example in this thesis is the possibility of producing a purified powder with both polyphenols and caffeine. These powders could be used in energy products with a health component, e.g. energy drinks, milkshakes and

energy bars. Although the range of application is narrower, the economic potential of a powder containing caffeine and polyphenols would be higher than of a powder containing only purified polyphenols. In addition, since the separation of the caffeine from the polyphenols is not easy, the production costs for the caffeine/polyphenols powder would also be lower.

8.1.1. Black tea

One interesting process alternative for the black tea would be a process at low temperatures, to prevent the thermal degradation of the polyphenols. The problem would be the tea cream formation. However the results obtained show that, with the correct combination of factors (temperature, amount of tea solids, pH and amount of EDTA) it is possible to keep 80-90 % of the black tea polyphenols in the non-cream phase. Adsorption could be afterwards used to separate and purify the polyphenols in the non-cream phase.

Solvent swing adsorption was tested for the separation of catechins and theaflavins from black tea. In a systematic resin screening study four food grade commercial macroporous resins were tested. The results of these tests show that the resin Amberlite XAD7HP has the best performance for the sorption of catechins (recovery rate of 60 %). The resin Amberlite FPX66 is the best for the theaflavins sorption, with a recovery rate of 59 %. The Langmuir multicomponent isotherm model shows a good fit to experimental results for both catechins and caffeine, and a reasonable fit for theaflavins. The eluent with the best results for the desorption is a solution with 70 % of ethanol (wt %) in water.

8.1.2. Green tea

The statistical analysis applied to the results from the Design of Experiments demonstrates that the green tea cream precipitation can be intensified to maximize the amount of catechins recovered, while minimizing the amount of caffeine (considered a contaminant), in a relatively simple process. Polynomial models were used to determine the optimal conditions. In addition it was found that it might be beneficial to use a tea with a relatively large amount of gallated catechins. The optimal results allow the recovery of 69 % of the catechins, while increasing the ratio catechins/caffeine in the cream phase by 60 %.

For the green tea it was possible to develop a multicomponent sorption model for the separation of catechins. The adsorption and desorption has been described with a one dimensional plug flow model including axial dispersion, to simulate the dynamics of the solvent swing sorption columns. The four main catechins and caffeine were included in the model for competitive sorption. The results demonstrate that the Matlab model based on the regressed parameters correspond well with experimental data. The XAD7HP resin has a higher affinity for all the modeled components and the HP20 resin has a higher affinity for

the caffeine than for the catechins, resulting in a good option for preferential adsorption of caffeine.

For the green tea a conceptual design study of the adsorption and spry drying process show that it is possible to produce a fine catechins powder with 83.5 % purity, which can easily be dissolved in the final food products. In addition, the process yield is 52 % and the operating profit is positive. The use of spray drying in the last step of the process allows to achieve particles with a uniform particle distribution, ensures microbiological stability and avoids thermal degradation.

Therefore, the conclusion can be drawn that the combination of packed bed adsorption with spray drying is promising for the separation and purification of catechins from a green tea extract.

8.2. Outlook

8.2.1. Product application

The last level of the PDPS is the multi product-equipment integration. In the case of tea there can be an overlap in the unit operations used for the separation, both for green and black tea. The packed bed adsorption and the spray drying can be used in both cases. In addition, other products, e.g. juice, beer, also commonly use packed bed adsorption in the production process. There is, therefore, room for integration between these processes. There is also a possibility to optimize the selected unit operations and operational window. Since spray drying is a very energy intensive operation there is room to optimize the energy requirements e.g., pump pressure, gas flow rate and gas temperature.

8.2.2. Batch process vs continuous process

It would be more advantageous to use a continuous separation process, e.g. simulated moving beds (SMB), true moving beds, instead of the selected cyclic batch packed bed process, where the adsorbent bed is alternately saturated and regenerated. When affinity differences between molecules are very small (as in the case of polyphenols), it is sometimes not possible to improve resolution via mobile- or stationary-phase changes. In these cases, SMB can separate mixtures of those compounds by allowing their small retention time differences to accumulate (Cong and Lin, 2007; Wang et al., 2012).

The use of moving beds (continuous counter current operation modes) would maximize the overall sorption rate and optimize the use of the adsorbent, when compared to the traditional batch mode. The disadvantages of the moving beds are high investment and maintenance costs, as well as higher complexity, than for the batch wise operation.

Nevertheless, these disadvantages are usually compensated by higher product yields and a lower solvent consumption.

8.2.3. Alternative feeds

Besides the use of aqueous tea extracts it would also be interesting to use tea byproducts as an alternative source of polyphenols. If recovered these polyphenols might have a high economic potential, since the byproducts have negative net value (due to disposal costs) or very low economic value. After extraction of this byproducts, it would also be possible to recover the polyphenols using the procedures described in this thesis. The discovery and the characterization of alternative sources of plant-based phenolic compounds can also potentially provide an increasing source of plant materials for the recovery of polyphenols.

Another potential alternative is the use of decaffeinated tea leaves to produce decaffeinated tea. As described throughout this thesis, caffeine is the biggest contaminant when the target products are purified polyphenols. If caffeine is not present, or it is only present in very small amounts, it would simplify the separation process tremendously. According to the model applied in section 7.2.2. potentially almost 90 % of the catechins present in the green tea can be recovered. This means that a very high yield and high purity product can potentially be obtained, although the cost of the starting material is also higher. Nevertheless, the option of using an alternative material seems to be a very promising.

8.2.4. Packed bed adsorption columns

A model for adsorption in a packed bed has been developed to optimize the operational time for the separation of caffeine and catechins from an aqueous green tea extract. A more complete model should also include other parameters e.g., column dimensions, operating flow rates, estimations of operating and investment costs. For example, bigger adsorption columns or extra adsorption cycles would potentially allow higher recoveries for the target components. The current model can also be extended to include cleaning and regeneration steps, and to determine the optimum number of cycles until regeneration is needed.

There is also the option of using gradient elution to separate the tea polyphenols and caffeine. Gradient elution may allow an effective separation of the target components. Furthermore, gradient elution may also allow fractionation of the individual polyphenols, with the corresponding increase in commercial value.

Another option would be to use a two-steps packed bed column elution. In the first step the column is eluted with a diluted aqueous ethanol solution (20-40 % ethanol) for recovering the caffeine. In the second step a more concentrated ethanol solution (70-80 % ethanol) is used for recovering the polyphenols (Lu et al., 2010).

Appendix A:

Economic potential calculation: data, assumptions and results for Level 3 and 4 of the PDPS methodology.

Input-output (Level 3)

The economic potential gives a first indication of the process profitability. The product prices were obtained from Unilever. Several assumptions are made for this calculation:

- 1 ton/h of BMF green tea (GT) extract with 4% solids
- 1 ton/h of BMF black tea (BT) extract with 4% solids
- GT extract has 17,62 % dry weight catechins (8,31 % EGCG)
- BT extract has 4,75 % dry weight catechins (0,56 % EGCG) and 0,67 % dry weight theaflavins
- Continuous process: 7200 working hours per year
- Raw materials costs:

1. Green tea extract: 50 €/ton
2. Black tea extract: 75 €/ton

- Product sales price:

1. Catechins (90% purity): 100 €/kg
2. Theaflavins (60% purity): 200 €/kg
3. EGCG (95% purity): 515 €/kg

- 80 % of process yield

4. Consider a 40 % profit margin for the company: catechins (90 % purity): 71 €/kg, theaflavins (60% purity): 143 €/kg, EGCG (95% purity): 368 €/kg

Task Network (Level 4)

Scenario 1. The preliminary economic evaluation is calculated in the same way as in the Input-Output level of the PDPS and takes into consideration the polyphenols degradation for a three hours period, at a temperature of 50 °C.

Table A.1. Economic potential evaluation for green tea: scenario 1.

Green tea: produce catechins (90 % purity)	3 hours degradation
Maximum product costs(M€/year) =	3.04
Raw material costs(M€/year) =	0.36
Economic potential (M€/year) =	2.68

Table A.2. Economic potential evaluation for black tea (catechins and theaflavins): scenario 1.

Black tea: produce cathechins (90% purity) and theaflavins (60% purity)	Tea cream loss + 3 hours degradation
Maximum product costs(M€/year) =	0.91
Raw material costs(M€/year) =	0.54
Economic potential (M€/year) =	0.37

The total economic potential for this scenario is 3.05 M€/year.

Scenario 2. In the second scenario the process temperature is 70 °C. To calculate the preliminary economic potential evaluation we will assume that the full process time will be 2 hours or 3 hours.

Table A.3. Economic potential evaluation for green tea: scenario 2.

Green tea: produce catechins (90 % purity)	2 hours degradation	3 hours degradation
Maximum product costs(M€/year) =	2.56	2.27
Raw material costs(M€/year) =	0.36	0.36
Economic potential (M€/year) =	2.20	1.91

Table A.4. Economic potential evaluation for black tea (catechins and theaflavins): scenario 2.

Black tea: produce cathechins (90% purity) and theaflavins (60% purity)	2 hours degradation	3 hours degradation
Maximum product costs(M€/year) =	0.90	0.77
Raw material costs(M€/year) =	0.54	0.54
Economic potential (M€/year) =	0.36	0.23

For the process time of 2 hours the total economic potential is 2.56 M€/year. For the process time of 3 hours the total economic potential is 2.15 M€/year.

Appendix B:

Design of experiments

Green tea. The factors (A: amount of solids, B: pH, C: temperature and D: EDTA) are varied in 3 levels (-1, 0, 1), representing the low, intermediate and high values of the range respectively.

Table B.1. Design of experiments table for the 27 experiments generated with the Box-Behnken design for green tea.

Experiment	A	B	C	D
	HPMCL (g/dm^3)	PVP (g/dm^3)	T (°C)	pH
1	0	0	1	1
2	-1	0	-1	0
3	0	0	-1	1
4	0	-1	0	-1
5	0	0	-1	-1
6	1	1	0	0
7	0	0	0	0
8	-1	0	0	1
9	-1	0	1	0
10	0	1	0	1
11	1	-1	0	0
12	-1	0	0	-1
13	1	0	0	1
14	0	0	0	0
15	0	-1	0	1
16	0	0	1	-1
17	1	0	0	-1
18	-1	1	0	0
19	0	-1	1	0
20	0	1	1	0
21	1	0	1	0
22	0	0	0	0
23	0	-1	-1	0
24	1	0	-1	0
25	0	1	0	-1
26	0	1	-1	0
27	-1	-1	0	0

Black tea. The factors (A: amount of solids, B: pH, C: temperature and D: EDTA) are varied in 3 levels (-1, 0, 1), representing the low, intermediate and high values of the range respectively.

Table B.2. Design of experiments table for the 27 experiments generated with the Box-Behnken design for black tea.

Experiment	A	B	C	D
	Solids (%)	T (°C)	pH	EDTA (g/dm^3)
1	1	0	-1	0
2	0	0	-1	-1
3	-1	1	0	0
4	0	-1	0	1
5	1	-1	0	0
6	-1	0	-1	0
7	1	1	0	0
8	1	0	0	1
9	0	1	-1	0
10	0	-1	-1	0
11	-1	0	0	1
12	0	0	0	0
13	-1	0	1	0
14	1	0	0	-1
15	-1	0	0	-1
16	0	0	1	-1
17	0	1	0	-1
18	0	0	1	1
19	0	1	0	1
20	0	0	0	0
21	0	-1	1	0
22	0	0	0	0
23	0	1	1	0
24	-1	-1	0	0
25	1	0	1	0
26	0	0	-1	1
27	0	-1	0	-1

Appendix C:

Model residual plots for the 3 adsorption experiments

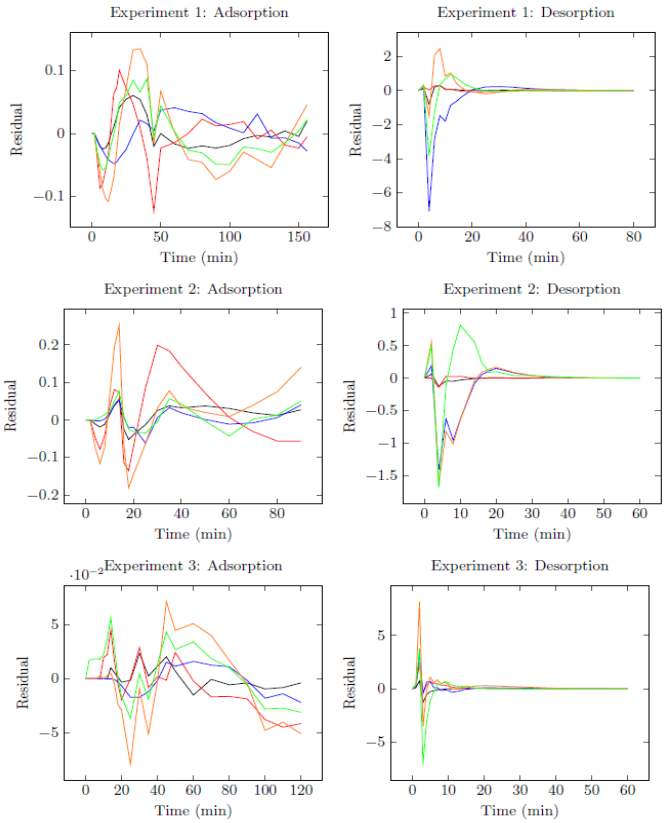


Figure C.1. Plot of the residuals (EC, EGC, ECG, EGCG and Caff)

Appendix D:

Spray drying model input and assumptions. Selected equipment description.

- **Dryer**

The spray dryer is operated in co-current flow and is based in an axial flow. The model neglects the agglomeration of particles and droplets coalescence, as well as lift. Both gravity and drag force are considered. For the mass and heat transfer calculations the model uses the Ranz-Marschall Correlation. The falling drying period is described by a normalized drying curve model.

The heat loss for the environment is neglected and no reduced heat and mass transfer in the spray zone is considered

- **Atomizer**

For the atomization a pressure nozzle is used (hollow cone nozzle) with 9 atomizers, 4 droplets intervals and 1mm nozzle orifice diameter. Only solid particles are considered to be formed.

- **Gas cyclone**

The model calculations for the gas cyclone are based on the Muschelknautz cyclone model and the cyclone is considered to be highly efficient. The cyclone design is based in the Stairmand geometry concept with 1.5 meters diameter.

- **Filter**

The filter is based on a fabric filter with baghouse design (5 cells and 80 bags per cell). The maximum pressure drop is set at 10 mbar and it is assumed an ideal separation efficiency.

- **Compressor**

An isentropic compressor is used with a discharge pressure of 1100 mbar. For the heat capacity calculations the model uses a rigorous calculation method.

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List of Publications

Journal Publications

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Monsanto, M., Hooshyar. N, Meuldijk, J., Zondervan, E., Modeling of packed bed adsorption columns for the separation of green tea catechins, *submitted to Chemical Engineering Science*.

Peer Reviewed Conference Proceedings

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Monsanto, M., Thota Radhakrishnan, A., Méndez Sevillano, D., Hooshyar, N., Meuldijk, J., Zondervan, E., Modeling and Optimization of an Adsorption Process for the Recovery of Catechins from Green Tea, *Proceedings of the 24th European Symposium on Computer Aided Process Engineering (ESCAPE 24)*, 15-18 June 2014, Budapest, Hungary, 33, pp. 1441-1446.

Oral Presentations

Monsanto, M., Hooshyar, N., Meuldijk, J., Zondervan, E., Precipitation behaviour of catechins and caffeine in green tea, *9th European Conference of Chemical Engineering,* 21-25 April 2013, The Hague, the Netherlands.

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Monsanto, M., Zondervan, E., Bongers, P., Product-driven process synthesis methodology application in the food industry, *Netherlands Process Technology Symposium 2010 (NPS10),* 25-27 October 2010, Veldhoven, Netherlands.

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Curriculum Vitae

Miguel Monsanto was born on 25 of May 1977 in Lisbon, Portugal. After finishing the secondary school, he started his study of Chemical Engineering (5 year degree) at the Instituto Superior Técnico in 1995 in Lisbon. In 2002 he started a 1 year internship at Globalcor, Lda as a Chemical Engineer, for technical sales assistance and customer support in Portugal and Spain (paint and coating industries). In 2004 he joined INETI (Department of Energy Engineering and Environmental Control) in Lisbon, for the position of Junior Researcher, where he worked in fluidized bed combustion and gasification. In 2007 he accepted a position of Environmental/ Chemical Engineer at DPM - Water treatment and air monitoring, in Lisbon. His work included technical support for water treatment and air quality control, as well as clients and suppliers management. In 2009 he started his Master degree in Chemical Engineering at the Instituto Superior Técnico. During his Master's program, he did his internship at Merck KGaA, Darmstadt, Germany and he wrote his thesis on "Investigation of secondary treatment steps of the effluent of a membrane bioreactor". In 2010 he received his MSc degree in Chemical Engineering from Instituto Superior Técnico and started the PhD work in the field of separation technology on the project "Separation of vitality ingredients" at the Eindhoven University of Technology, in the group of prof.dr.ir. André de Haan (Process Systems Engineering Group), under the supervision of prof.dr.ir. Peter Bongers and dr.ir. Edwin Zondervan. Since 2011 he moved to the Polymer Reaction Engineering Group at the Eindhoven University of Technology under the supervision of prof.dr. Jan Meuldijk and dr.ir. Edwin Zondervan. His PhD project was sponsored by ISPT in cooperation with Unilever and his work during the PhD led to this thesis.