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Product-Driven Process Synthesis for the Extraction of Polyphenols from Fresh Tea Leaves

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Polyphenolic compounds present in fresh tea leaves are related to health benefits, and they can be used as food items and vitamin supplements. However, during conventional tea processing that includes cutting, rolling, and drying, the extraction step is missing. Moreover, process conditions are harsh for example, to destroy the remaining enzymes and to reduce the moisture content in a leaf, a high temperature has to be applied (above 80 °C). Therefore, there is a need for an alternative process for the extraction of polyphenols under mild conditions. The Product-driven process synthesis methodology a well-defined structured approach for the conceptual design of an extraction process for polyphenols from fresh tea leaves was highlighted in this study. Starting with specification of the input (fresh tea leaves) and output (polyphenols), we subsequently defined fundamental tasks to convert our raw material into the desired product. Among the different mechanisms that could be used to perform the tasks, pulsed electric field is selected as non-invasive and non-thermal mechanism for opening the cell structure in the plant raw material. Next we need to define the operating window for the pulsed electric field unit. An experimental design is setup and executed (varying several setting of the Pulsed Electric Field unit such as electric field strength, number of pulses and pulse duration) and from the collected data, the analysis of variance is used to determine which variables are significant. Furthermore, the outcomes of the statistical analysis help in defining the best operating conditions that maximizes the extraction yield of the polyphenols. We found that the optimal pulsed electric field settings are: 1.1 kV/cm field strength, 0.0001 s pulse duration and number of pulses 50. With obtained optimum settings maximum value of 32.5 % of extraction yield was achieved. The next step in the product-driven process synthesis methodology is to translate all gathered information into suitable process design.

1. Introduction

Polyphenolic compounds present in fresh tea leaves may reduce the risk of a variety of illnesses, including cancer and coronary heart diseases. The usefulness of these polyphenols (PPs) may be extended by combining them with other consumer products such as food items and vitamin supplements. Industrial tea extraction is mainly based on the maceration method combined with stirring, circulation, ultrasonic, microwave or enzymatic treatment (Perva-Uzunalić et al., 2006; Li et al., 2010). These methods require large volumes of solvent, have high temperature and energy costs, and obtain relatively low extraction efficiencies. However, during conventional tea processing that includes cutting, rolling, and drying, the extraction step is missing. Moreover, process conditions are harsh for example, to destroy the remaining enzymes and to reduce the moisture content in a leaf, a high temperature has to be applied (above 80 °C). Therefore, there is a need for an alternative process for the extraction of polyphenols under mild conditions. New alternative process has to be an effective method that will allow economical and comprehensive utilisation of tea resources to extract tea polyphenols from fresh tea leaves.

This work describes the Product-Driven Process Synthesis (PDPS) methodology proposed by Bongers and Almeida-Rivera (2012) for the conceptual design of an economic and sustainable process for the extraction of polyphenols from fresh tea leaves. This methodology delivers flow sheets that allow economical and environmentally responsible conversion of specific feed stream(s) into desired and specific product(s).

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2. Product-driven process synthesis methodology (PDPS)

The PDPS methodology has several hierarchical levels and focuses on finding fundamental tasks that could be performed by different mechanisms. These mechanisms are then linked with unit operations that are running under defined optimum operating conditions. A case of extraction of polyphenols (product stream) from fresh tea leaves (feed stream) is taken here as an example to demonstrate some of the key steps of the PDPS methodology.

2.1 Framing level

At the framing level, the background of the project, the business context and the potential of polyphenols (PPs) as food additives (mostly known for their antioxidant effects) are identified. The presence of PPs in fresh tea leaves is responsible for health benefits associated with green tea consumption. PPs are now gaining significant attention from both technical and consumer point of view due to potential health benefit. It has been reported that PPs may reduce risk of cancers, cardiovascular diseases, dental decay, obesity, diabetes, and improvement in the immune system (Wang, et al., 2003).

2.2 Input-output level

At this level a complete specification of all exchange streams to the process (inputs/raw material(s) and target output/product(s) are identified. Fresh tea leaf (input stream) contains approximately 75 % moisture and 25 % of total solids. PPs account approximately 30 % of dry weigh of fresh tea leaves (Zhu et al., 2012). Fresh tea leaves contain caffeine, tea polyphenols, tea polysaccharides, and necessary nutrients, such as protein, amino acids, lipids, and vitamins. Generally, some chemical components – free amino acids, total tea polyphenols, and soluble sugars – are considered important indicators of tea quality (Ruan, et al., 2010).
The output of the process is fraction of polyphenols. The special flavor and astringency of a tea brew is affected by the total tea polyphenols (including catechin, epigallocatechin-3-gallate (EGCG), epigallocatechin epicatechin-3-gallate, epicatechin and their epimers), which amount to 18-36 % of the weight of the total dry matter of a tea leaf (Graham, 1992).

Table 1: Fundamental tasks and mechanisms

Steps	Fundamental task*	Mechanisms*
Particle size reduction	C2	C21: attrition C22: impact C23: ultrasound C24: cutting C25: enzymes
Cell wall disruption	C3	C31: internal cell phase change C32: electro-magnetic fields (PEF, ultrasound) C33: shear C34: enzymes C35: chemical
Extraction of PPs	G1	G11: molecular size G12: particle size G13: electrical charge G14: solubility G15: chemical affinity G16: chemical reaction G17: (vapour) pressure G18: gravity G19: molecular size and electrical charge G20: shear
Physical/biological stabilization	J1	J11: freezing J12: cooling

* “Mechanisms” is the nomenclature used in the paper of Almeida and Bongers (2010)

2.3 Task network level

The next step in the methodology is to identify the fundamental tasks that are needed to convert the input into the desired output *i.e*. determination of the task network. The aim is to isolate PPs from fresh tea leaves originally present in small compartment (organelle) inside the cells. To make the polyphenols accessible the following tasks need to be executed (reference code is included for the sake of simplicity), see also Table 1:
All possible combinations result in more than 100 routes that could be followed. As this number of alternatives is far from manageable a further simplification is proposed for the network based on the following engineering-driven heuristics (H) and project constraints:
H1: Mechanisms C21, C22, C23 and C25 are not considered because fresh tea leaves and components inside during processing would be damaged.
H2: C33, C34, and G20 have been excluded due to the project constraints (*i.e*. no harsh conditions).
H3: C35, G15 and G16 have been excluded because of environmental reasons. This project aims at isolating PPs from fresh tae laves under mild conditions, hence without the use of chemicals. Furthermore, PPs will be used in the food industry and these compounds have to be approved by obeying strict law regulations.
H4: G12 has not been considered because PPs are molecules, not particles.
H5: J12 is rejected because PPs (as a final product) have to be stored at -18 °C to avoid contamination of the product. Therefore, cooling is not sufficient.
After applying these heuristics, number of possible and feasible mechanisms is reduced. Selected mechanisms are identified and presented in Table 2.

Table 2: Selected tasks and mechanisms

Steps	Fundamental task*	Mechanisms*
Particle size reduction	C2	C24: cutting
Cell wall disruption	C3	C32: electro-magnetic fields (pulsed electric field)
Extraction of PPs	G1	G11: molecular size G13: electric charge G14: solubility G19: molecular size and electric charge
Physical/biological stabilization	J1	J11: freezing

All listed mechanisms in Table 2 are experimentally tested. In the step cell wall disruption, pulsed electric field (PEF) is selected mechanisms due to the fact that PEF is non-thermal and non-invasive method. PEF processing of foods involves the application of short pulses (duration of micro- to milliseconds) of high electric field intensity. PEF applied on fresh tea leaves (depending on the settings) caused opening the cell structure and transport of cellular material occurred. By measuring the amount of PPs in the aqueous media after PEF treatment, it was possible to monitor the effect of pulsed electric field on opening the cell structure. PEF experiments have been carried out and results as well as extensive discussion have been presented earlier (see Zderic, et al, 2013)

2.4 Mechanism and operating window

For each mechanism the operating window has to be defined. Since all experiments were performed on the lab scale, for the first step C24: cutting, leaves were cut manually (1 cm width). The most critical step in the task network is cell wall disruption (C3: pulsed electric field) and for this step operating window has to be defined. Electric field strength is an important factor that controls the efficiency of electroporation of the cellular tissue. (Bazhal, et al., 2003) presented classification of the PEF modes as low ($E \leq 100$-200 V/cm), moderate ($E=300$-1500 V/cm) and high ($E>1500$ V/cm). With low electric field strength, the treatment time should be longer for electroporation of the cellular membranes. It has been found experimentally that the time needed for electroporation of cellular membrane of the different biological tissues is inversely proportional to the electric field strength by factor dependency (Lebovka, et al., 2000). Cell wall disruption depends not only on electric field strength, but on other process parameters including pulse duration or pulse width (PD), and number of pulses (N). In the present study, statistical analysis was employed to find optimal PEF settings to maximize the amount of extracted PPs (extraction yield). Box-Behnken design was used to optimize significant process factors: electric field strength (E), pulse duration (PD) and number of pulses (N). This design is suitable for exploration of quadratic response surfaces and constructs second order polynomial model, thus helping in optimizing a process using small number of experimental runs. The design consists of replicated center points and set of points lying at the midpoints of each edge of the multidimensional cube that defines operational region (minimum and maximum value of each factor). The experimental data were processed using the StatGraphics Centurion XVI Software.

Three selected factors for the Box-Behnken design E, PD and N were designed as A, B, and C respectively. Analysis and model fitting have been performed using codded design variables (A, B and C) and not design factors with their original units. When the original units are used, obtained numerical results in comparison to the codded unit analysis are different and not easy to interpret (Montgomery, 2013). Therefore, the low, middle and high levels of each design variable are -1, 0 and +1 and presented in the Table 3.

Table 3: Selected factors and levels of each design variable selected for the Box-Behnken design

Design variable	A: electric field strength, kV/cm	B: pulse duration, s	C: number of pulses, #
	-1 (0.1)	-1 (0.0001)	-1 (10)
	0 (0.6)	0 (0.05)	0 (30)
	1 (1.1)	1 (0.1)	1 (50)

Box-Behnken design requires experiment minimum number of experiments where experiments were run in randomized way (Souza, et al., 2005). Hence, for three-factor design, a total 15 experimental runs (including three center points) were executed and their observation were fitted to following second order polynomial model Eq(1):

$$Y = C_0 + C_1 \cdot A + C_2 \cdot B + C_3 \cdot C + C_{12} \cdot A \cdot B + C_{13} \cdot A \cdot C + C_{23} \cdot B \cdot C + C_{11} \cdot A^2 + C_{22} \cdot B^2 + C_{33} \cdot C^2 \quad (1)$$

where, Y = predicted extraction yield of PPs, C_0 = constant; C_1, C_2, and C_3 = linear coefficients; C_{12}, C_{13}, and C_{23} = cross product coefficients; C_{11}, C_{22}, and C33 = quadratic coefficients.

Independent and depended factors were related using mathematical relationship. Therefore, for predicting the optimal values of extraction yield (EY) of PPs within experimental constraints, a second order polynomial model was fitted to the experimental results using Analysis of Experiments software. The regression model for predicting extraction yield was expressed as following equation Eq(2):

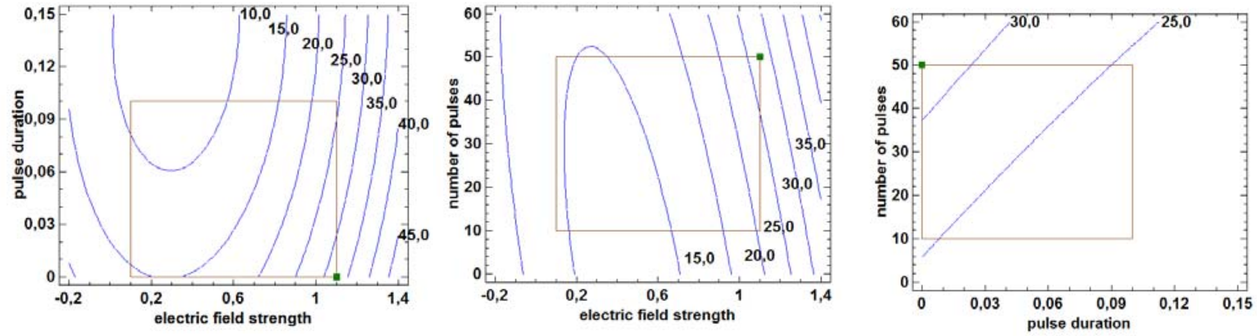
$$EY = 18.46 - 23.27 \cdot E - 44.59 \cdot PD - 0.075 \cdot N + 25.93 \cdot E^2 - 16.62 \cdot E \cdot PD + 0.18 \cdot E \cdot N + 358.05 \cdot PD^2 - 1.05 \cdot PD \cdot N + 0.001 \cdot N^2 \quad (2)$$

where codded variables A, B and C represent E, PD and N. Positive sign represents a positive effect while a negative sign indicates an antagonistic effect. The developed model was evaluated using statistical analysis of variance (ANOVA) and Fisher's test, and the quality of the model was expressed by the coefficient of determination R^2 . After generating polynomial equation relating dependent and independent factors, optimization step was performed in order to maximize extraction yield (EY). The optimal value of extraction yield 32.5 % could be achieved for $E_{optima\ l}$ = 1.1 kV/cm. $PD_{optima\ l}$ = 0.0001 s and $N_{optimal}$ =50 pulses (Table 4).

Table 4: Optimal PEF settings for maximized value of extraction yield

Variables	Optimal settings
A: electric field strength, kV/cm	1.1
B: pulse duration, s	0.0001
C: number of pulses, #	50

The contour plot was used as the graphical representation to show interactions among three variables (E, PD and N). Figure 1 presents the contour plot for each extraction yield value obtained from the regression model. Because the model contains interactions between factors, the contour lines of constant extraction yield are curved. It is desirable to operate in the region where extraction yield is between 30 and 35 %. The contour plots show that several combinations of E, PD and N could satisfy this objective. In the Figure 1a (E versus PD and for fixed N=50) the extraction yield increases with increase of electric field strength and decrease with increases of number of pulses reaching maximum of 32.5 % when E is 1.1 kV/cm and is 50 pulses. Therefore, contour plot n Figure 1b represents maximum amount of extracted PPs (extraction yield) versus number of pulses (N) and pulse duration (PD) for the fixed E = 1.1 kV/cm. with increasing number of pulses, extraction yield increases. On the other hand, at lower pulse duration, extraction yield is higher which means that increasing pulse duration, extraction yield decreases (Figure 1c).



(a) *(b)* *(c)*

Figure 1: Contour plot representing extraction yield of PPs versus: electric field strength (E) and pulse duration (PD) for fixed number of pulses N = 50 (a); electric field strength (E) and number of pulses for fixed PD = 0.0001s (b) and pulse duration (PD) and number of pulses (N) for fixed electric field strength 1.1 kV/cm (c)

3. Conclusions

In this work we have demonstrated the use of the product-driven process synthesis methodology for the conceptual design of PPs extraction from fresh tea leaves. First we started with specification of the input stream (fresh tea leaves) and output stream (polyphenols). Next, we identified the fundamental tasks to convert input into a final product which led to new cell wall opening method: pulsed electric field. In order to define operating window for PEF statistical analysis was employed. From Box-Behnken design we found that three variables such as electric field strength (E), pulse duration (PD), and number of pulses (N) have significant effect on extraction yield. The levels of these variables were predicted to obtain maximized response, extraction yield (EY). Observed responses were close to the predicted values. Optimization of E, PD and N followed by Box-Behnken design showed that electric field strength (linear and quadratic effects) and pulse duration are significant model terms. Moreover, optimized PEF settings for maximized extraction yield of 32.5 % are E = 1.1 kV/cm, PD = 0.0001 s and N = 50.

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