

OPTIMIZATION OF CONTINUOUS MICROWAVE INACTIVATOR FOR POLYPHENOL OXIDASE INACTIVATION ON GREEN TEA PROCESSING USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Microwave treatment is a promising technology for food processing such as drying, extraction, and enzyme inactivation because of its ionic heat transfer. This study develops and optimizes the fixation process in green tea using a microwave-based enzyme inactivator. A continuous microwave enzyme inactivator with a dimension of 3300 (L) × 550 (W) × 600 mm³ (H) was built to study the effect of temperature, microwave radiation time, and the number of microwaves on the catechin content of green tea. The optimum condition for the inactivation process was determined using response surface methodology and central composite design. The result shows that the model can predict the effect of temperature, microwave radiation time, and the number of microwaves on catechin content. Temperature, fixation time (conveyor velocity), and the number of microwaves, have a significant impact on enzyme inactivation when using a continuous microwave. The optimum microwave inactivation condition for polyphenol oxidase enzyme was four at 70°C temperature and 30 rpm conveyor speed.

Keywords: Catechin, Enzyme Inactivation, Green Tea, Microwave, Polyphenol

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INTRODUCTION

Tea is a beverage produced from *Camellia sinensis* leaves. Based on the processing method, tea can be classified into white and green tea (without fermentation process), oolong tea (semi-fermented), and black tea (fermented tea).¹ The health benefits of tea are due to polyphenol compounds. Polyphenols on green tea such as catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), and gallic acid, have been reported to have anti-cancer activity, anti-inflammation, prevent cardiovascular disease, obesity, and other degenerative diseases.²⁻⁵ During processing and storage, green tea catechins can be degraded, oxidized, fermented, epimerized, and polymerized.⁶ Generally, fermentation decreases the concentrations of EGCG, EGC, EC, and ECG by 74%, 91%, 51%, and 62%, respectively, and converts them to the aflavin and thearubigin.⁷ Oxidation or thermal degradation can reduce antioxidant capacities such as caffeine, ascorbic acid, saponin, and flavonol glycosides.⁸

Green tea processing starts with spreading fresh tea leaves to increase the hydrolysis of pectins and non-water-soluble carbohydrates, accumulation, and formation of non-gallate catechins, removal of the distinctive grassy aroma of fresh tea leaves, and dissipation of some moisture in fresh leaves.⁹ Then, the process is followed by fixation, rolling, shaping drying, sorting, grinding, and packing. To produce high catechin green tea, fresh tea leaves must be withered immediately to inactivate the polyphenol oxidase and hydroperoxide enzymes and prevent fermentation. In Indonesia, the fixation process is commonly accomplished by panning and steaming. The disadvantage of the pan-fired method is that conventional heating depends on the phenomenon of convection-conduction, and heat transfer occurs through a heat gradient, causing most of the heat to be lost to the environment. The degradation of chlorophyll to pheophytin results in the black color of the tea. Although steaming can improve the quality of green tea,

catechin epimerization still occurs. This is caused by individual catechins such as epicatechin, epicatechin gallate, and epigallocatechin, and epigallocatechin gallate is polymerized into theaflavin at temperatures above 80°C because of the complexity of the water vapor ion steamer.^{10,11}

Microwave treatment is a promising technology for food processing such as drying, extraction, and enzyme inactivation because of its ionic heat transfer. In microwave-assisted treatment, heat is generated inside the food being heated rather than being not dissipated from the surface. Electromagnetic waves transfer energy to water molecules, ions, and other food components. Research on microwave applications for drying processes has been conducted on red pepper powder, fish, chicken as well as beef, purple-fleshed sweet potato, red lentil seed, bitter melon, loquat, asparagus, tea, and other foods.^{12–19} Microwaves have the advantage over other means of food processing because they are faster, efficient, and provide better quality.^{16,17,20,21} Microwave energy reduces product deterioration due to prolonged heating and maintains temperature-sensitive content such as vitamins, as well as proteins, polyphenols, and carotenoids.^{17,22–24}

Microwave applications for enzyme inactivation have been tested in several products.²⁰ In the enzyme inactivation experiment of loquat, at an inactivation temperature of 83°C, an increase in microwave energy increases the amount of enzyme inactivated. In the microwave blanching process of asparagus, blanching time and microwave energy influenced the quality of the asparagus such as color as well as phenolic and antioxidant content. The best quality was achieved with a 4-min blanching time and 150 W energy, retaining 126.33% phenolic compound and 76.99% free radical scavenging ability retention.¹⁸ The energy value for enzyme inactivation is determined by several factors, such as the dielectric properties, chemical composition, pH, and other actual properties of the tissue.²⁰

Microwave thermochemical processes can also reduce energy consumption and increase the inactivation of polyphenol oxidase and hydroperoxides enzymes in the cytoplasm because of their ionic conduction heat transfer, which reduces oxygen diffusivity into the cytoplasm of tea leaves and reduces the catechin epimerization because some of the bound water inside the cell cavity of the tea leaves is released through capillary tubes by ionic conduction. Gulati et al.'s application of household microwave in polyphenol oxidase (PPO) enzyme inactivation of green tea showed the highest level of total phenols and catechins, brighter colors, sweet taste, and a subtle pleasant odor compared with steam and heating inactivation.¹⁹ Most of the research conducted using commercial microwaves with a batch process is difficult to be used in the industry. In this study, continuous microwave application for enzyme inactivation was developed to make it easy for industrial applications. When a product undergoes a thermochemical process in the microwave, several parameters, such as residence time, temperature, and energy, determine product quality such as color, phenol content, and antioxidants.¹⁸ The effect of these process parameters can be examined individually, but it will take time and be costly because the interactions between parameters are not considered and many experiments are required. Parameter optimization using response surface methodology can be used to solve these problems.^{25–27} This study determines the number of microwave use on the inactivation of polyphenol oxidase enzymes on continuous enzyme inactivators and the process parameters that have the most influence.

EXPERIMENTAL

Material and Methods

Fresh tea leaves (*Camellia sinensis* var. *assamica*) clone TRI 2025 were supplied by PT Rumpunsari Medini, Kendal, Indonesia.

The 3300 (L) × 550 (W) × 600 mm (H) continuous microwave enzyme inactivator was designed for laboratory use. As shown in Fig.-1, it consists of a hopper, microwave, fan, agitator, conveyor, electric motor, temperature sensor, thermo-controller, and control panel. Four commercial microwave ovens with a frequency of 2450 MHz were placed directly after the hopper. Each microwave has an agitator that stirs the tea leaves such that they are evenly exposed to the waves.

General Procedure

Plucked tea leaves are sorted until the third leaf is removed from the shoots, followed by a 2–4 min fixation process in the microwave. After the fixation process, the tea leaves are manually rolled and ready to be dried.

The microwave was set to the desired temperature (50°C–90°C), and 500 gr tea leaves were fed through the hopper once the temperature was reached. The conveyor transports tea leaves across the microwave to the cooling area. Furthermore, the tea leaves were microwaved at 70°C until their weight was constant. The process optimization design was determined using a central composite design and analyzed using Statistica 8. The experimental data were tabulated (Table-1). The variables of the fixation process were microwaves at temperature (50°C–90°C), conveyor velocity (20–40 rpm), which is equivalent to fixation time (2–5 min), and the number of microwaves (2–4), while the response was catechin content.

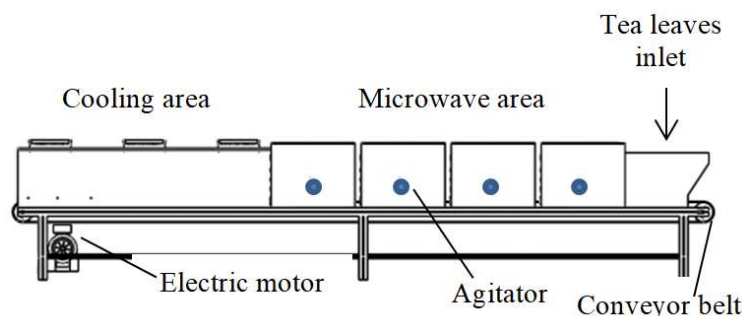


Fig.-1: Continuous Microwave Enzyme Inactivator

Table-1: Experimental Design of Fixation Optimization Process

Temperature (°C)	Conveyor Velocity (rpm)	Number of Microwaves	Catechin Content ($\mu\text{g/g}$)
50	20	2	41.53
50	20	4	44.68
50	40	2	56.67
50	40	4	21.41
90	20	2	52.4
90	20	4	76.15
90	40	2	30.37
90	40	4	64.83
42.9	30	3	39.69
97	30	3	96.83
70	16.5	3	57.81
70	43.5	3	48.72
70	30	2	76.93
70	30	4	112.47
70	30	3	91.85
70	30	3	45.91

Detection Method

Catechin content was analyzed using high-performance liquid chromatography (HPLC) Agilent Technologies 1260/DAD Detector. The detection of the active substance in tea using the HPLC method has the advantage of being simple, fast, and accurate.²⁸ The column was VDSpher PUR 100 C18-E, 150 x 4.6 mm, 3.5 μm , with a column temperature of 40°C and flowrate of 0.8 ml/min.

RESULTS AND DISCUSSION

The fixation process of tea leaves aims to inactivate the polyphenol oxidase and hydroperoxide enzymes located in the cytoplasm, resulting in high catechin green tea products. Microwave radiation, which spreads evenly because of the presence of agitators, can reduce the diffusivity of oxygen in the cytoplasm of tea leaves, reducing the enzymatic oxidation of catechin compounds to theaflavins and thearubigins as the tonoplast membrane walls are ripped. The dipole rotation on the tea leaves can keep the tea leaf temperature operating in a constant condition.

The R^2 correlation coefficient was used to assess the model's validity. This shows the number of combinations of independent variable combinations that affect the value of the variable simultaneously, and its values are between 0 and 1. When the value is closer to one, the regression model will be better. The R^2 value obtained was 0.67393 (Table 2). R^2 of 0.67393 states that 67.393% of the total variation in the fixation process using microwaves affects the process variables being studied. The p-value of the parameters studied was less than 0.01 (Table 2). This indicated that the model is efficient at predicting process variables. Table 2 shows that temperature (L), conveyor speed (Q), number of microwaves (L) had a significant effect on fixation using microwaves because their values are less than 0.05.

Table-2: Effect Estimation of the Fixation Process Parameters

Effect Estimates; Var.:Catechin Content (ppm); R-sqr=.67393; Adj.:18482 (Spreadsheet1) 3 factors, 1 Blocks, 16 Runs; MS Residual=513.5297 DV: Catechin Content (ppm)										
Factor	Effect	Std.Err.	t(6)	p	-95.% Cnf.Limt	+95.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	82.2976	12.47991	6.59441	0.000584	51.7604	112.8349	82.2976	12.47991	51.7604	112.8349
(1)Temperature (oC)(L)	23.4165	13.27373	1.76413	0.128161	-9.0631	55.8962	11.7083	6.63686	-4.5316	27.9481
Temperature (oC)(Q)	-22.6629	18.50687	-1.22457	0.266633	-67.9475	22.6218	-11.3314	9.25343	-33.9738	11.3109
(2)Number of Microwave(L)	12.3280	14.33220	0.86016	0.422733	-22.7416	47.3976	6.1640	7.16610	-11.3708	23.6988
Number of Microwave(Q)	0.3913	23.98987	0.01631	0.987514	-58.3098	59.0924	0.1957	11.99493	-29.1549	29.5462
(3)Velocity (rpm)(L)	-9.2317	13.28137	-0.69509	0.513007	-41.7300	23.2667	-4.6158	6.64069	-20.8650	11.6333
Velocity (rpm)(Q)	-39.2103	18.55740	-2.11292	0.079053	-84.6186	6.1980	-19.6052	9.27870	-42.3093	3.0990
1L by 2L	22.5800	16.02388	1.40915	0.208453	-16.6290	61.7890	11.2900	8.01194	-8.3145	30.8945
1L by 3L	-6.3050	16.02388	-0.39348	0.707570	-45.5140	32.9040	-3.1525	8.01194	-22.7570	16.4520
2L by 3L	-6.9250	16.02388	-0.43217	0.680719	-46.1340	32.2840	-3.4625	8.01194	-23.0670	16.1420

The response surface graph shows the individual and cumulative effects of the variables, as well as their subsequent effects on the response. Response Y is the catechin yield and temperature (X_1), conveyor speed (X_2), and the number of microwaves (X_3). The regression model for the microwave fixation process shows that temperature (X_1) and the interaction between temperature and conveyor speed (X_1X_2) have a positive effect on catechin levels. Meanwhile, the effect of conveyor speed (X_2) and the number of microwaves (X_3) has a negative effect. The squared term of the combination of temperature–number of microwaves (X_1X_3), and conveyor speed–number of microwaves (X_2X_3), also show a negative effect.

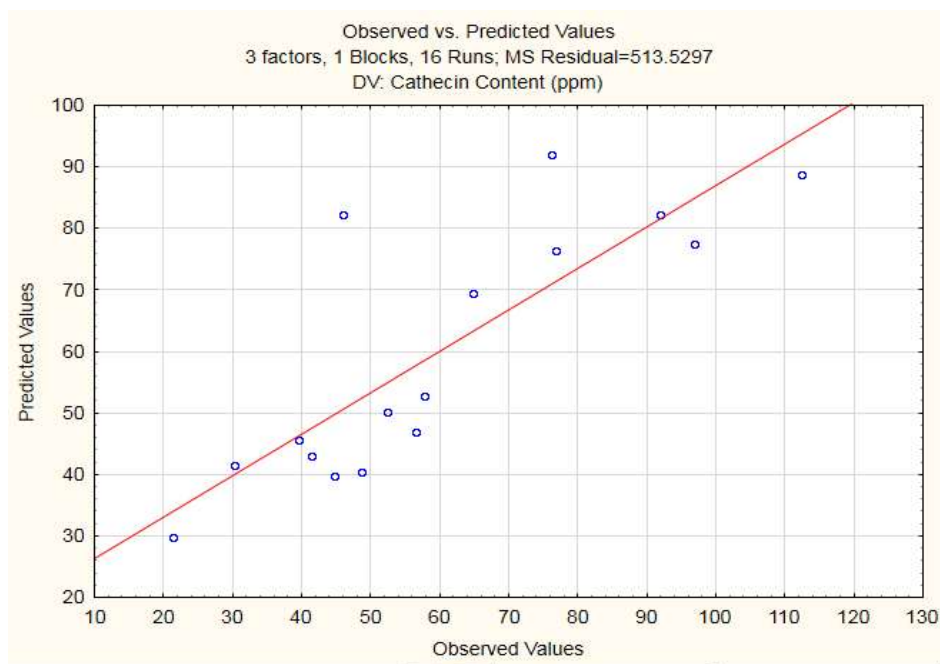


Fig.-2: Pareto Chart of the Standardized Effect

Figure 1 shows the closeness of the model based on the experimental data validation in the laboratory. Although the fitting of the model seems less precise, it still serves its purpose in representing the characterization of the fixation process. The Pareto chart illustration in Fig.-2 shows that the conveyor speed is the variable that most influences the microwave fixation process of green tea leaves. The conveyor speed shows the fixation time it takes for microwaves to penetrate green tea leaves. The lower the conveyor speed, the longer the fixation time, and thus the catechin content. This occurs because the ionic conduction penetrates deep enough into the cytoplasm of the tea leaves to inactivate most polyphenol oxidase and hydroperoxide enzymes, making them incapable of catalyzing the aerobic oxidation of catechins. Therefore, when there is cell damage, the catechins in the vacuole are not oxidized to theaflavins and thearubigins. However, increasing fixation time (lower conveyor speed) causes catechin to decrease. This is assumed to be due to the penetration of ionic conduction and the rotation of the dipole through the tonoplast membrane wall and diffusing into the vacuole, causing some catechins to thermally degrade. This result is consistent with previous research on loquat, the yield of total phenolic content in methanolic extract of loquat increased with treatment time²⁹. As well on pomace, the individual phenolic acid content of Kinnow and Feutrell's early pomace extract increased with increasing microwave irradiation time²³.

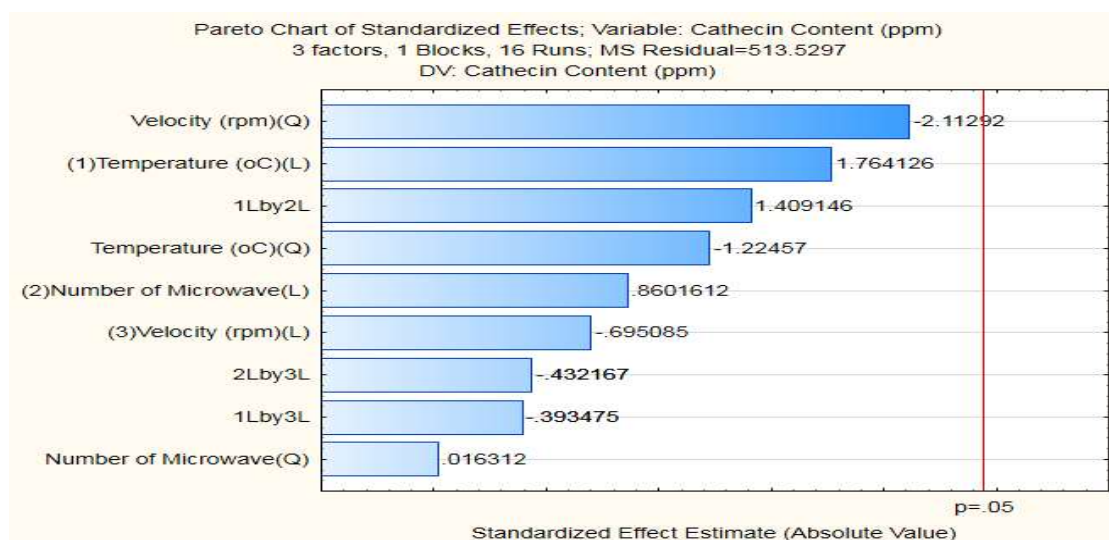


Fig.-3: Pareto Chart of the Standardized Effect

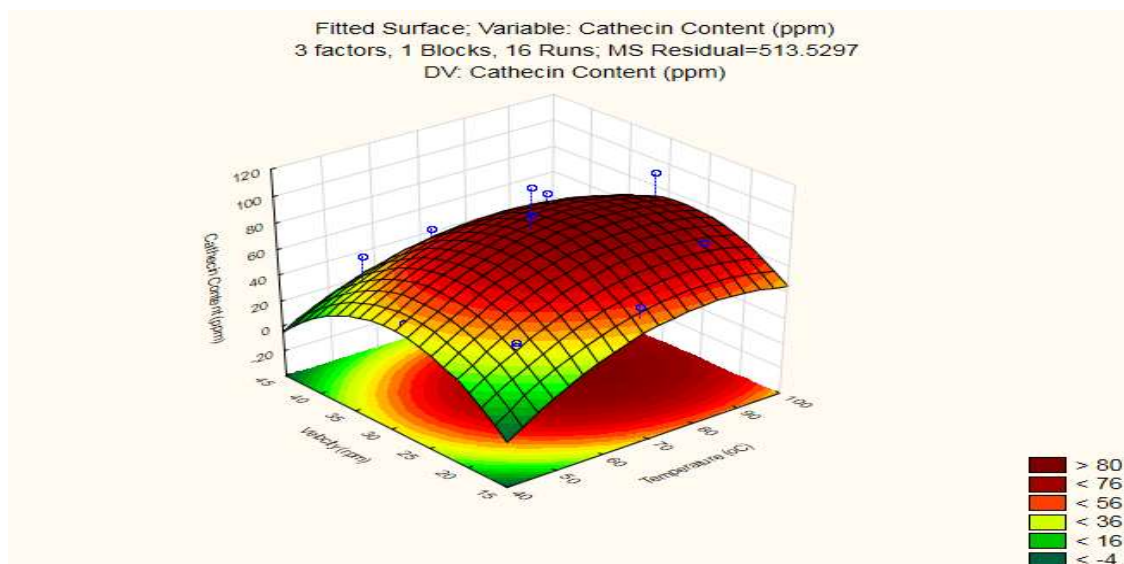


Fig.-4: Response Surface Contour Plot on the Effect of Temperature and the Conveyor Velocity

Figures-4 and 6 show that at 90°C, catechins are obtained in relatively high concentrations. This occurs because ionic conduction can penetrate effectively into the cytoplasm, rendering most polyphenol oxidase and hydroperoxide enzymes inactive and unable to catalyze the aerobic oxidation of catechins. Therefore, if there is cell damage in the following process, the catechins in the vacuole will not be oxidized to theaflavins and thearubigins.³⁰⁻³² The oxidation will transform catechins into theaflavins and thearubigins^{30,33} (Fig.-5) and change the color and taste of the tea. The oxidation reaction of polyphenol enzymes with self-oxidized catechins into strong oxidizing agents can cause the non-enzymatic oxidation of shoot substances. Quantitatively, the formation of theaflavins and thearubigins is the most important reaction that occurs during the enzymatic oxidation of tea polyphenols, but the oxidation of other substances by oxidized catechins is an important possibility in determining the quality of processed tea.^{32,34-36}

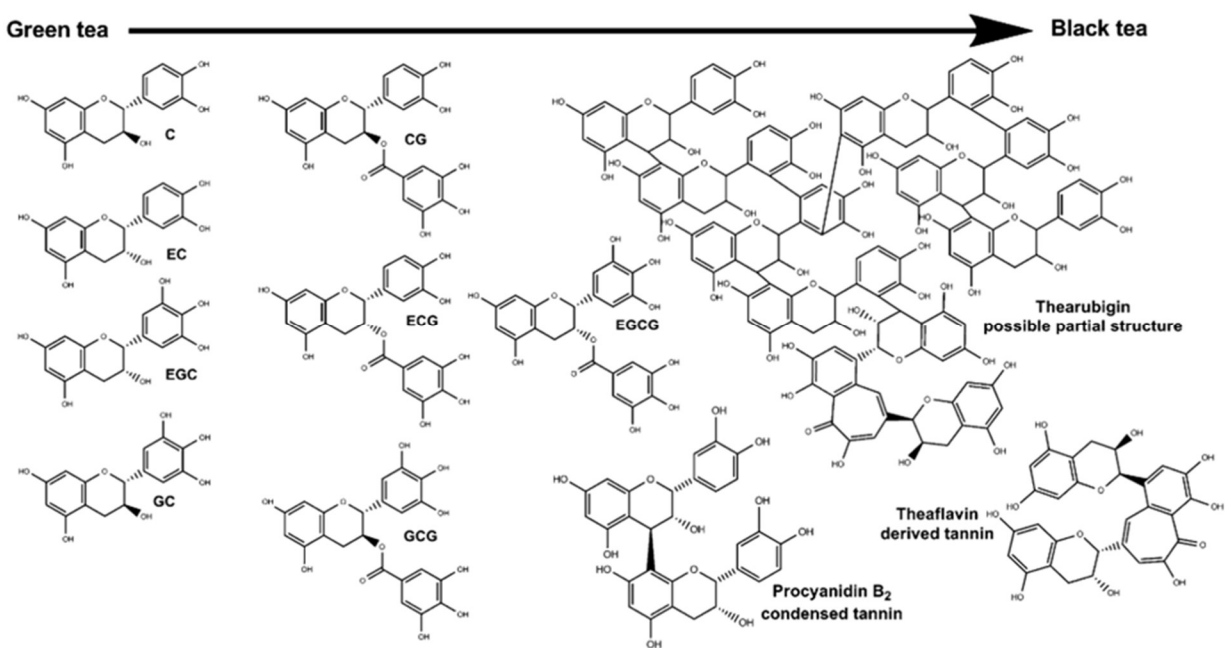


Fig.-5: Transformation of Catechin during Oxidation Process^{33,37,38}

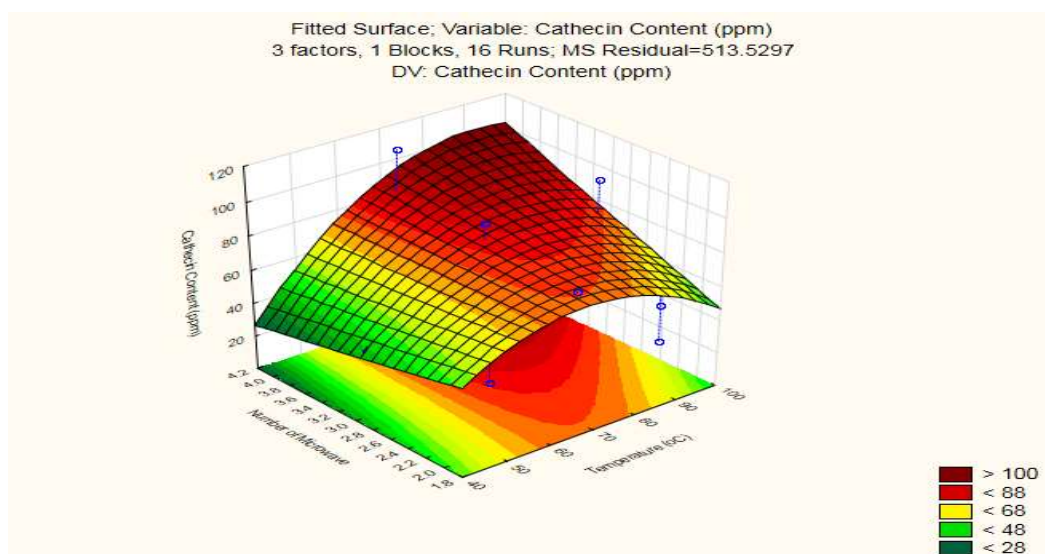


Fig.-6: Response Surface Contour Plot on the Effect of Temperature and the Number of Microwaves on Catechin Levels

The catalytic activity of the enzymes polyphenol oxidase and hydroperoxide decreases as the temperature increases. The enzyme begins losing activity at 80°C and decreases steeply at 90°C. This shows that there is a denaturation of the polyphenol oxidase and hydroperoxide enzymes. High temperatures can cause damage to the enzyme structure, rendering it inactive and inhibiting the catechin oxidation process. However, a further increase in temperature will cause ionic conduction to penetrate the tonoplast membrane wall, allowing the catechins in the vacuole to be penetrated, causing thermal degradation of the catechins to form theaflavins and thearubigins compounds. Thearubigin is an advanced oxidation product of catechins and theaflavins and can be formed because of the condensation of theaflavins and other oxidation products of catechin.³⁹

CONCLUSION

A study was conducted on the application of a continuous microwave enzyme activator in the fixation of fresh tea leaves during the processing of green tea. The optimization of the process was obtained using response surface methodology. According to the study, the model can predict the effect of temperature, conveyor velocity, and the number of microwaves on catechin content. Temperature, fixation time (conveyor velocity), and the number of microwaves have a significant effect on the enzyme inactivation using the continuous microwave. The optimum conditions for polyphenol oxidase enzyme inactivation using microwave were 70°C, 30 rpm conveyor speed, and four microwaves.

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