IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

ISO 3297:2007 Certified ⁘ Impact Factor 8.066 ⁘ Peer-reviewed / Refereed journal ⁘ Vol. 10, Issue 6, June 2023

DOI: 10.17148/IARJSET.2023.106103

Case Study: Optimization of antidiabetic properties of stevia black tea brewing

Tarsisius Dwi Wibawa Budianta[1], Adrianus Rulianto Utomo[2]

Laboratory of Food Processing Technology, Food Technology Department, Faculty of Agricultural Technology,

Widya Mandala Catholic University Surabaya, Indonesia[1,2]

Abstract: This research was conducted to determine the effect of adding stevia leaf powder on the anti-diabetic properties of stevia-black tea drinks. A design is needed to find the best proportion of stevia black tea to get the best anti-diabetic properties. The research design used was a response surface method completely, with a central composite, 2 factors, and 1 replication. The factor was stevia and black tea, with varying weights according to the design proposed by the RSM program. The combination of stevia and black tea was put into a tea bag, weighing 2 g. Tea was brewed by steeping every 2 g of stevia black tea in 200 mL of hot water at 94 ± 5 °C for 4 ± 1 min. And then cool the infusion to room temperature for 15 min, and the tea was filled in a glass of dark brown color, to be analyzed one hour later. The parameter analyzed for anti-diabetic properties was the inhibition of the alpha-amylase enzyme. The data obtained from testing this parameter was then analyzed using RSM design Minitab 17. The results of the optimization show that stevia has a direct and positive effect on amylase inhibitory ability, while black tea has an inverse and negative effect.

Keywords: optimization, antidiabetic, amylase, black tea, stevia

I. INTRODUCTION

Tea (Camellia sinensis) is one of the plants known by the world community. Tea can be consumed by brewing the leaves. This tea drink is very liked by the world community because it has a distinctive taste and aroma and has a good effect on the health of the body [1]. Several studies have reported that consuming tea regularly can reduce and prevent degenerative diseases such as heart disease, hypertension, diabetes, obesity, cancer, and so on [2].

Compounds that play a role in preventing degenerative diseases are antioxidant compounds by counteracting free radicals and inhibiting the oxidation process [3]. Antioxidant compounds can be in the form of vitamin E, vitamin C, flavonoids, polyphenols, and carotenoids. Many of the benefits of tea include immunomodulatory, antigenotoxic effects, heart disease (circulatory disease) control, cancer prevention, prostate cancer chemoprevention, hepatoprotection, anti-obesity regulation, antibacterial and antiviral effects, and antidiabetic effects. are studied and described. Products classified as having a protective effect on human health [4]. In 2003, the antioxidant activity of green, oolong, and black tea extracts was tested [5].

As a result, there was no significant difference in the antioxidant properties of different types of tea. A study was also conducted on the differences in lipid-lowering properties and growth-inhibitory effects of oolong, black, pu-erh, and green tea leaves in mice [6], and the results showed that partially or fully fermented tea had a growth-inhibitory effect. effectively influenced. - Lipid suppression and hypolipidemia compared to green tea. A study of the effects of green and black tea extracts on glucose control in adults with type 2 diabetes [7] found no difference in the effects of the two on glucose control. A study by Saikai et al. [8] found the ability of green tea and black tea to suppress hyperglycemia and insulin resistance. Researchers from Sri Lanka have found the hypoglycemic, antihyperglycemic, and antidiabetic effects of black tea on the quality level of Broken Orange Pekoe Fanning (BOPF) [9].

Stevia rebaudiana Bertoni M is a source of natural sweeteners and low in calories. The main component contained in stevia is steviol glycosides with a content of 4–20% dry weight and gives a sweet taste sensation 200-450 times compared to sucrose [10],[11]. According to Komissarenko et al. in [11], stevia leaf extract contains flavonoids, alkaloids, chlorophylls, water-soluble xanthophylls, hydroxycinnamic acids, oligosaccharides, free sugars, amino acids, oils, lipids, and minerals. Tadani et al. (2007) [11] concluded that stevia leaf extract has high antioxidant activity and was an excellent source of antioxidants.

 This work is licensed under a Creative Commons Attribution 4.0 International License

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

ISO 3297:2007 Certified ⁜ Impact Factor 8.066 ⁜ Peer-reviewed / Refereed journal ⁜ Vol. 10, Issue 6, June 2023

DOI: 10.17148/IARJSET.2023.106103

The phytochemical composition and antioxidant activity of a black tea stevia tea mixture was studied using RSM [12]. From this study, it can be seen that the optimal concentration of a mixture of stevia and black tea that produces total phenol and a high DPPH value has been determined. To complete the study, optimization of the use of a blend of stevia and black tea was performed in terms of the anti-diabetic properties of this brew. The aim of the study was to be investigated the optimal mixing between stevia and black tea which results in the highest inhibition of the enzyme amylase.

II. MATERIAL AND METHOD

Materials used in this study were divided into 2, namely the material of manufacture stevia-black tea drinks and chemicals. Material manufacture of black stevia-tea drink: stevia dry powder is procured from suppliers in Java Central and offered online, while black tea is obtained from an outlet tea plantation in Surabaya. Chemicals for analysis consist of dinitrosalicylic acids (DNS), acetate buffer pH 5 solution, distilled water, 2% starch, and amylase saliva. These materials were used for testing the sugar reduction activity which was carried out simultaneously with the % inhibition test, the data obtained as supporting data for the % inhibition test.

Method. To obtain the combination treatment between stevia and black tea, a reference was used from the results of the initial absolute threshold test and the difference threshold [13], and by using new stevia and black tea that was different from the reference, the stevia data used per dose was a maximum of 0.3% (w/v) and maximum black tea data was 5% (w/v) of the steeping volume**.** The data was then entered into the Minitab 17 program on the Design of the Experiment using the RSM, then obtained a central composite design with two factors and one replication, with a total run of 13, with an axial point of 4, and an alpha of 1.414421 and the treatment (run order) as in the following table:

TABLE I DESIGN RSM

StdOrder	RunOrder	PtType	Blocks	Concentration (w/v)	
				Stevia	Black Tea
9	1	0	1	0.22	3
2	2	1	1	0.33	1
13	3	0	1	0.22	3
5	4	-1	1	0.09	3
1	5	1	1	0.13	1
7	6	-1	1	0.22	0.18
3	7	1	1	0.13	5
8	8	-1	1	0.22	5.8
10	9	0	1	0.22	3
11	10	0	1	0.22	3
4	11	1	1	0.3	5
6	12	-1	1	0.33	3
12	13	0	1	0.22	3

The combination of stevia and black tea was put into a tea bag, weighing 2 g. Tea was brewed by steeping every 2 g of stevia black tea in 200 mL of hot water at 94 ± 5 °C for 4 ± 1 min. The brew was allowed to cool at room temperature for 15 min, and the tea was filled in a glass of dark brown color, to be analyzed one hour later. The parameter analyzed for anti-diabetic properties was the inhibition of the α-amylase enzyme.

Analysis of sugar-reducing activity. The ability of mixed black tea stevia extract to hydrolyze starch or sucrose was based on Bernfeld's [16] method using the dinitrosalicylic acid test. Various concentrations of stevia black tea mixed extract were added to 0.5 mL of substrate (2% starch dissolved in buffer pH 5), then incubated at 40 °C for 10 min. Add 2 mL of 3.5 dinitrosalicylic acids (DNS) [14][15]. and heat at 100 °C for 5 minutes, cool with running water, and add 20 mL of distilled water, then measure the absorbance at λ = 540 nm. The reaction was indicated by a change in the color of the solution from yellow to red-brown. Judgment based on qualitative changes in color.

Analysis of the activity of inhibiting the α-amylase enzyme was determined based on the method of Bernfeld [16]. 100 µl of 100 mM acetate buffer solution pH 5, 100 µl of starch, various concentrations of stevia black tea mixed extract, and 50 µl of α-amylase [saliva] enzyme. A control was similarly prepared but without the addition of the stevia black tea blend extract. The extract was incubated at 37°C for 10 minutes and the absorbance measured was at **λ** = 540 nm. Black

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

ISO 3297:2007 Certified ⁝ Impact Factor 8.066 ⁝ Peer-reviewed / Refereed journal ⁝ Vol. 10, Issue 6, June 2023

DOI: 10.17148/IARJSET.2023.106103

tea stevia mixed extract which has the potential to inhibit the α-amylase enzyme was shown to have lower enzyme activity than the control. Rate inhibition (%) = (1 - AS/Ab) x 100, where As = absorbance of sample and Ab = absorbance of control. The data obtained from testing this parameter were then analyzed using RSM from Minitab 17. The analysis was carried out in 3 replications, with each being observed 3 times (triple), and the mean was entered as a single data in RSM as one replication.

III. RESULT AND DISCUSSION

The test for the ability to reduce sugar by the stevia black tea mixture showed a positive result, this was indicated by a change in the color of the solution from yellow to red-brown. From the analysis of the inhibitory activity of the alpha-amylase enzyme experimental results, it was known that the inhibitory activity of the alpha-amylase enzyme varies from 12% to 14%. The data was then entered into the Minitab RSM program according to Table 1., above and the surface plot results are obtained as follows:

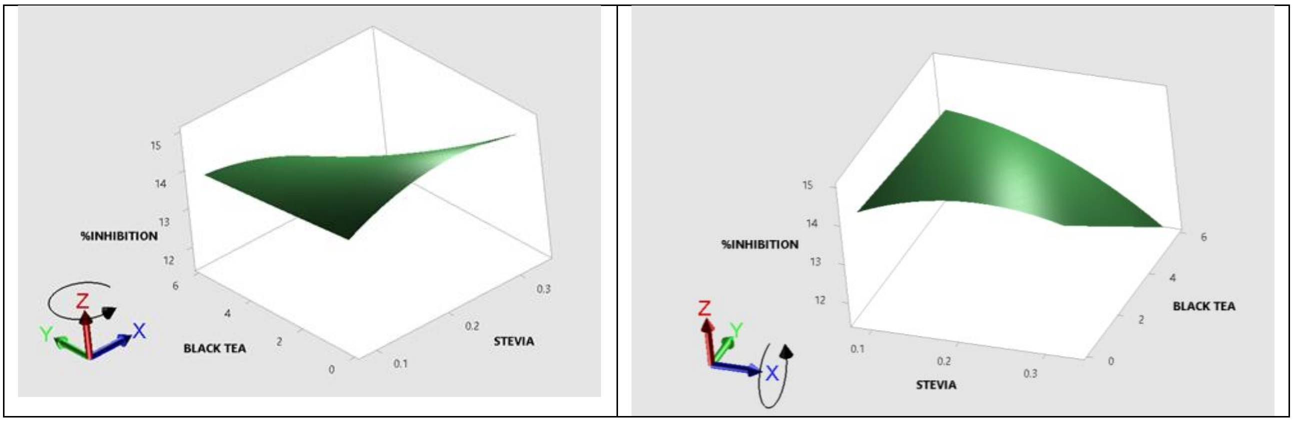


Fig. 1. Surface Plot RSM Stevia-Black tea vs %inhibition

From Figure 1 above it appears that there is an effect between stevia and black tea on inhibition, but it needs to be investigated further considering there was a hypothesis that stevia and black tea individually or together each have an effect on inhibition. For this purpose, Anova was needed which has been processed by the RSM program, with the following results [Appendix 1], Calculations proved that stevia and black tea had an effect individually and in combination, thus proving the hypothesis correct. Different ratios of black tea and stevia have different effects on inhibiting the enzyme alpha-amylase. Therefore, varying amounts may have different effects on the % inhibition of the α-amylase enzyme. This is due to the performance principle of enzymes. In addition, enzymes have specific active sites to recognize their substrates. The presence of inhibitors also inhibits product formation. In this case, the desired product is glucose. Additionally, tea contains tannin compounds that give it a bitter taste. According to Westendarp [17] and Besharati [18], tannins are classified into two groups, namely hydrolyzable and condensed tannins. Hydrolyzed tannins are polymers of gallate compounds, which are esters attached to sugar molecules. Hydrolyzed tannins include catechin (C), epicatechin (EC), catechin gallate (CG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), all of which are antioxidants. Based on USDA (United States Department of Agriculture) research in 2002, it was shown that these gallic derivatives can promote insulin hormone activity (regulation of blood sugar levels) and act as anti-diabetic. However, Singla et al [19] conducted a study on the effects of stevia on amylase. The results showed that the aqueous extract, compared to other extracts, had the highest amylase inhibitory activity. Furthermore, rebaudioside A (compound 20) isolated from aqueous extracts showed an excellent binding profile with α-amylase.

From the RSM regression analysis, it is known that the relationship between stevia and the ability to inhibit amylase is positively correlated, the higher stevia, the higher the inhibition of amylase, and vice versa. On the other hand, black tea has a negatively correlated effect with inhibition, the greater the concentration of black tea, the lower the inhibitory ability, and vice versa. The regression results are as follows:

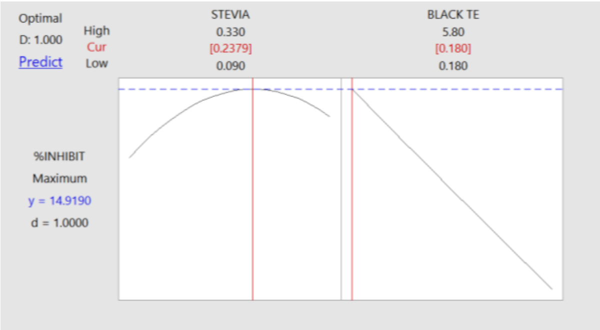
Regression Equation in Uncoded Units

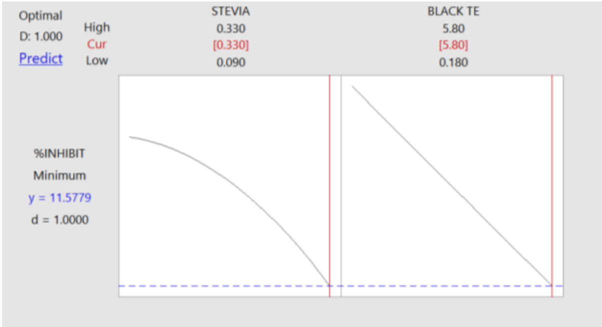
%INHIBITION = 14.098 + 4.56 STEVIA - 0.037 BLACK TEA - 6.54 STEVIA*STEVIA
- 0.0017 BLACK TEA*BLACK TEA - 0.804 STEVIA*BLACK TEA

Fig. 2. RSM Regression Analysis of Stevia Black tea vs %inhibition.

DOI: 10.17148/IARJSET.2023.106103

To further see the effect of each on the inhibition of the amylase enzyme can be seen in Fig. 3 and Appendix 2. The figure shows a graph showing the role of stevia and black tea respectively if there is a target to get maximum and minimum % inhibition. In this figure (Fig 3. (a)), the target for achieving maximum % inhibition can be seen that the effect of stevia is in the form of a quadratic curved equation, while the effect of black tea is a straight line. The maximum target %inhibition was reached at 14.919%, with 0.2379% stevia and 0.18% black tea. Meanwhile, the predicted minimum target (Fig 3. (b)) was reached at the %inhibition level of 11.5779%, with 0.330% stevia and 5.8% black tea.





(a) Maximum Target % Inhibition (b) Maximum Target % Inhibition

Fig. 3. Target prediction Plot RSM Stevia-Black tea vs %inhibition

From the figure in Fig. 3, it is clear that stevia and black tea do not work synergistically or can even be said to be opposite antagonists and inhibit them to a certain concentration. This can be presumably caused by the comparison of the amount of amylase enzyme used with the amount of stevia black tea steeping mixture. The amount of stevia black tea steeped is far more than the amount of the amylase enzyme used, so when the inhibition occurs, the enzyme is used up. Therefore, more study is required to pinpoint the precise degree of inhibition provided by a stevia black tea mix.

IV. CONCLUSION

The results of the analysis showed that black tea stevia had an effect on antidiabetic activity (the ability to inhibit alpha-amylase enzyme) in glass bottles. The effect between stevia and amylase inhibitory ability is positive and directly proportional, while the effect of black tea on the ability to inhibit amylase enzymes is negative and inversely proportional.

ACKNOWLEDGMENT

The authors would like to thank the Rector cq Chairperson of the LPPM Widya Mandala Catholic University Surabaya who has funded this research.

REFERENCES

[1]. P. Ljubuncic et al. Antioxidant activity and cytotoxicity of eight plants used in traditional Arab medicine in Israel, *Journal of Ethnopharmacology* 99: 43–47. 2005.
[2]. Kris-Etherton PM et al. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine 113: 71S-88S. 2002.
[3]. N. Kamalakkannan, P.S.M. Prince. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic Wistar rats.Basic dan Clinical Pharmacology dan Toxicology 98: 97–103. 2006.
[4]. K. Jain, M. Siddiqi, J.H. Weisburger. Protective effect of tea on human health. 2006.
[5]. K. Yanagimoto, H. Ochi, Kwang-Geun Lee, T.Shibamoto. Antioxidative activities of volatile extracts from green tea, oolong tea, and black tea. 2003. J Agric Food Chem 2003 Dec 3;51(25):7396-401. doi:10.1021/jf030127i.
[6]. K-L. Kuo, K-L, M-S Weng, C-T Chiag et al. Comparative Studies on the Hypolipidemic and Growth Suppressive Effects of Oolong, Black, Pu-erh, and Green Tea Leaves in Rats. *J. Agric. Food Chem.* 2005, 53, 2, 480–489. https://doi.org/10.1021/jf049375k.
[7]. T. Mackenzie, L. Leary, W B. Brooks, The effect of an extract of green and black tea on glucose control in adults with type 2 diabetes mellitus: double-blind randomized study. Metabolism. 2007 Oct;56(10):1340-4. doi: 10.1016/j.metabol.2007.05.018.

ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

ISO 3297:2007 Certified ⁙ Impact Factor 8.066 ⁙ Peer-reviewed / Refereed journal ⁙ Vol. 10, Issue 6, June 2023

DOI: 10.17148/IARJSET.2023.106103

[8]. S. Nishiumi, H. Bessyo, M. Kubo, et al. Green and black tea suppress hyperglycemia and insulin resistance by retaining the expression of glucose transporter 4 in muscle of high-fat diet-fed C57BL/6J mice. J Agric Food Chem. 2010 Dec 22;58(24):12916-23. doi: 10.1021/jf102840w.
[9]. K. R. W. Abeywickrama , W. D. Ratnasooriya, A M T Amarakoon. Oral hypoglycaemic, antihyperglycaemic and antidiabetic activities of Sri Lankan Broken Orange Pekoe Fannings (BOPF) grade black tea (Camellia sinensis L.) in rats. J Ethnopharmacol. 2011 May 17;135(2):278-86. doi: 10.1016/j.jep.2011.02.035.
[10]. A. N. Starratt, C.W. Kirby, R. Pocs. J.E. Brandle. Rebaudioside F, a diterpene glycoside from Stevia rebaudiana. Phytochemistry. 2002; 59:367–70.
[11]. M. Kobus-Moryson, A. G. Michalowska. Directions on the use of stevia leaves (Stevia Rebauidana) as an additive in food products. Acta Scientiarum Polonorum, Technologia Alimentaria 14(1):5-13. DOI: 10.17306/J.AFS.2015.1.1
[12]. T. D. W. Budianta, A. R. Utomo, S.J. Raharjo. Optimization of concentration of stevia leaves (Stevia rebaudiana Bert.) and black tea: study on phytochemical composition and antioxidant activity of mixed stevia-black tea solution. IOP Conf. Series: Earth and Environmental Science 472 (2020) 012010 IOP Publishing doi:10.1088/1755-1315/472/1/012010
[13]. T. D. W. Budianta, A. R. Utomo, S. J. Raharjo, Sensory Evaluation of Brewed Black Tea (Camellia sinensis sp) and Stevia (Stevia rebaudiana Bert) Leaves. Case Study: Threshold of Phenolic Bitterness Consumer Preference Test. International Advanced Research Journal in Science, Engineering and Technology. Vol. 7, Issue 1, January 2020. DOI 10.17148/IARJSET.2020.7113.
[14]. A. A. N. Saqib, P. J. Whitney. Differential behaviour of the dinitrosalicylic acid (DNS) reagent towards mono- and di-saccharide sugars. Biomass and Bioenergy, Volume 35, Issue 11, November 2011, Pages 4748-4750, https://doi.org/10.1016/j.biombioe.2011.09.013
[15]. P. Bernfeld 1955. Amylases, a and b. In S. P. Colowick dan N. O. Kalpan (Eds.). Methods in enzymology (Vol. 1, pp. 149–158). New York: Academic Press
[16]. N. S. Wang, 2009, Biochemical Engineering Laboratory: Glucose Assay by Dinitro salicylic Colorimetric Method, University of Maryland, Maryland.
[17]. H. Westendarp. Effects of tannins in animal nutrition. Dtsch Tierarztl Wochenschr, 2006 Jul;113(7):264-8.
[18]. M. Besharati, A. Maggiolino, V. Palangi, et al. Tannin in Ruminant Nutrition: Review. Molecules. 2022 Nov 27;27(23):8273. doi: 10.3390/molecules27238273
[19]. R. Singla, N. Singla, V. Jaitak, *Stevia rebaudiana* targeting α-amylase: An *in-vitro* and *in-silico* mechanistic study. Formerly Natural Product Letters, Volume 33, 2019 - Issue 4. https://doi.org/10.1080/14786419.2017.1395433

Appendix 1:
Response Surface Regression: %Inhibition versus Stevia Black tea

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	4.67251	0.93450	5.40	0.024
Linear	2	3.96632	1.98316	11.46	0.006
STEVIA	1	1.06503	1.06503	6.15	0.042
BLACK TEA	1	2.90129	2.90129	16.76	0.005
Square	2	0.35217	0.17608	1.02	0.409
STEVIA*STEVIA	1	0.34864	0.34864	2.01	0.199
BLACK TEA*BLACK TEA	1	0.00033	0.00033	0.00	0.966
2-Way Interaction	1	0.35403	0.35403	2.05	0.196
STEVIA*BLACK TEA	1	0.35403	0.35403	2.05	0.196
Error	7	1.21163	0.17309		
Lack-of-Fit	3	0.34395	0.11465	0.53	0.686
Pure Error	4	0.86768	0.21692		
Total	12	5.88413			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.416040	79.41%	64.70%	35.39%

Coded Coefficients

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant		13.998	0.186	75.23	0.000	

International Advanced Research Journal in Science, Engineering and Technology
ISO 3297:2007 Certified ⁜ Impact Factor 8.066 ⁜ Peer-reviewed / Refereed journal ⁜ Vol. 10, Issue 6, June 2023
DOI: 10.17148/IARJSET.2023.106103

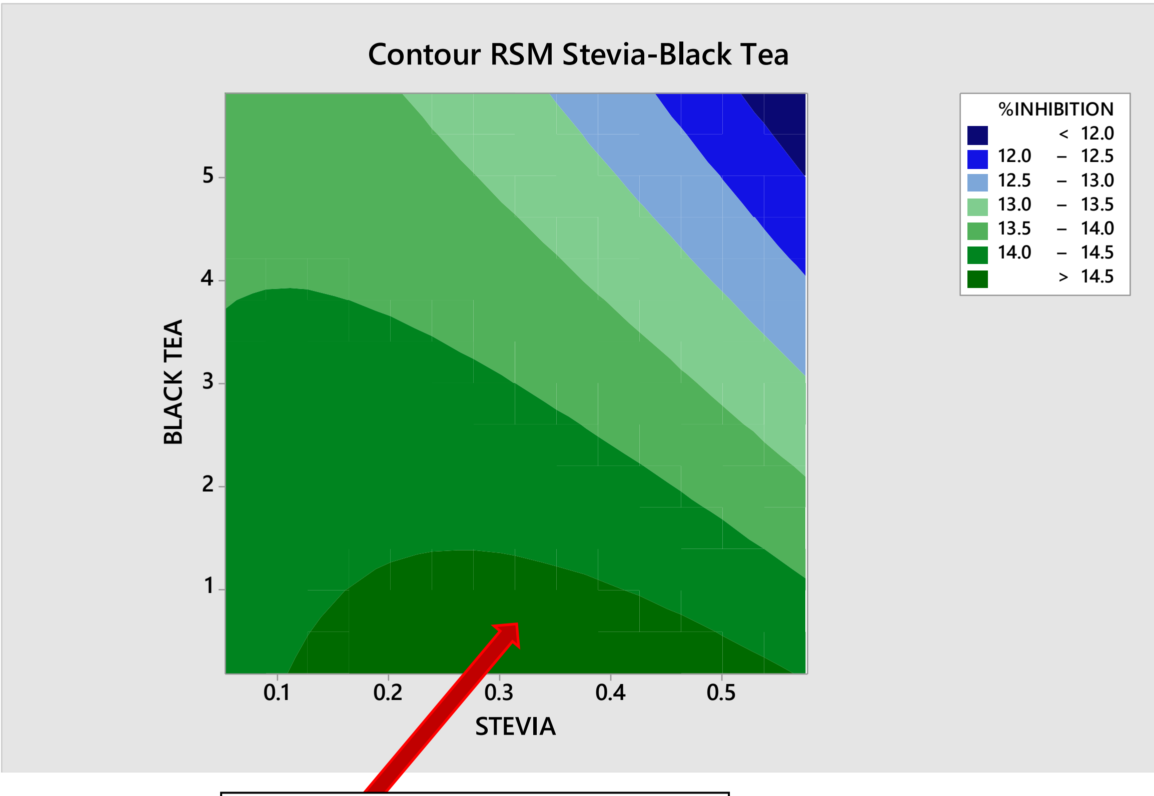
```
STEVIA              -1.032  -0.516   0.208   -2.48   0.042  1.00
BLACK TEA           -1.703  -0.852   0.208   -4.09   0.005  1.00
STEVIA*STEVIA       -0.895  -0.448   0.315   -1.42   0.199  1.02
BLACK TEA*BLACK TEA -0.028  -0.014   0.315   -0.04   0.966  1.02
STEVIA*BLACK TEA    -1.190  -0.595   0.416   -1.43   0.196  1.00
```

Regression Equation in Uncoded Units
%INHIBITION = 14.098 + 4.56 STEVIA - 0.037 BLACK TEA - 6.54 STEVIA*STEVIA
- 0.0017 BLACK TEA*BLACK TEA - 0.804 STEVIA*BLACK TEA

Fits and Diagnostics for Unusual Observations

```
                          Std
Obs  %INHIBITION    Fit  Resid  Resid
 13       14.800  13.998  0.802   2.16  R
R  Large residual
```

Appendix 2:
Contour Plot of % Inhibition vs Stevia-Black Tea



The highest % inhibition area >14.5