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Case Study: Correlation between total phenol and anti-diabetic properties of black tea-stevia drink in a glass jar

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Abstract: This research was conducted with the aim to determine the correlation between antioxidant content and antidiabetic properties of stevia black tea mixtures with various proportions. The research design used was a completely randomized design with one factor. The factor is the proportion of black tea: stevia with 5 levels of proportion, namely the proportion of black tea: stevia (in % by weight) used respectively is 88:12, 76:24, 64:36, 52:48 and 40: 60. Tea is brewed by steeping every 2 g of stevia tea in 200 mL of hot water at 94 ± 5 °C for 4 ± 1 minute. And the tea was filled in a glass jar, then stored at ambient temperature (28 ± 2 °C) for 3 days. After 3 days, the tea drink was analyzed. The parameters analyzed were total phenols and inhibition of the alpha-amylase enzyme. The data obtained from testing these parameters were then analyzed using regression Minitab 17 with a significance level of 5%. The relationship between total phenol and amylase inhibitory ability is directly proportional, positive, and cubic equation type

Keywords: total phenol, 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. Antioxidant compounds can be in the form of vitamin E, vitamin C, flavonoids, polyphenols, and carotenoids. One type of tea that contains the highest antioxidant compounds is green tea [3]. Many of the benefits of tea have been researched and reported, including immunomodulatory, antigenotoxic, fighting heart disease (cardiovascular diseases), cancer prevention, chemoprevention of prostate cancer, hepatoprotective properties, fighting obesity, antibacterial and antiviral properties, and antidiabetic so that tea is classified as a product that has protective effects for human health [4]. In 2003, the antioxidative activity of extracts of green tea, oolong tea, and black tea was investigated [5] with the results that the antioxidative properties of various types of tea were not significantly different.

A study comparing the hypolipidemic and growth-suppressing effects of Oolong, Black, Pu-erh, and Green Tea Leaves in mice was also conducted [6], with the findings showing that partially or fully fermented tea was more effective than green tea in influencing these effects. Green tea has been shown to have anti-diabetic, anti-obesity, and anti-inflammatory activities in animal models, according to Cao et al [7], but the molecular mechanisms underlying these benefits are still unclear. There was no difference between the effects of green tea and black tea extracts on glucose management in persons with type 2 diabetes mellitus, according to research [8]. According to Nishiumi's research [9], green tea, and black tea can reduce insulin resistance and hyperglycemia. Black tea-grade broken orange pekoe fanning (BOPF) was discovered to have hypoglycemic, antihyperglycemic, and anti-diabetic properties by Sri Lankan researchers in 2011 [10]. 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 [11],[12]. According to Komissarenko et al. (1994) in [12], stevia leaf extract contains flavonoids, alkaloids, chlorophyll, water-soluble xanthophylls, hydroxycinnamic acid, oligosaccharides, free sugars, amino acids, lipids, oils, and minerals. Tadhani et al. (2007) in [12] concluded that extracts from stevia leaves exhibit high antioxidant activity and are a good source of antioxidants. In the beverage industry, glass bottle packaging is used as a carrier as well as a place to store packaged tea drinks, so that they are safe from physical and chemical damage. Storage or the process of distribution and marketing is often done using room temperature and refrigeration temperature.

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The use of this packaging material is a new trend in packaging systems [13]. The aim of the study is to be investigated the correlation between antioxidant activity and anti-diabetic properties of black tea-stevia drinks in a glass jar at various proportions of tea-stevia.

II. MATERIAL AND METHOD

Material. The material used was a black tea that has been packaged in aluminum foil, obtained from a plantation outlet in East Java. Meanwhile, dried stevia leaves were obtained from plantations in Central Java, which were packed in plastic packaging. The chemicals used for analysis are categorized as chemicals for analysis (p.a).

Methods. The research design used was a completely randomized design with one factor. The factor is the proportion of black tea: stevia with 4 levels of proportion, namely the proportion of black tea: stevia (in % by weight) used respectively is 88:12, 76:24, 64:36, and 52:48. Tea was brewed by steeping every 2 grams of stevia tea in 200 ml of warm water at 94 ± 6 °C for 4 ± 1 minute. And the tea was filled in a glass jar, then stored at ambient temperature (28 ± 2 °C) for 3 days. After 3 days, the tea drink was analyzed. The parameters analyzed were total phenols and inhibition of the alpha-amylase enzyme. The analysis was carried out with 5 replications with triple observations. The data obtained from testing these parameters were then analyzed using regression using Minitab 17 with a significance level of 5%.

The total phenol content of the tea-stevia drink was determined by the spectrophotometric method based on the method of Kumar et al. [14] and Jayasri et al. [15]. 1 mL of Folin-Ciocalteus Phenol reagent was diluted to 20 mL with distilled water, then 1 mL of sample (250 mg/mL) was added and shaken. Then 4 mL of sodium carbonate (75 g/L) and 10 mL of distilled water were added and shaken. The mixture was left at room temperature for 2 hours. Then centrifuged 2000 g for 5 minutes and the absorbance of the supernatant was measured at λ 760 nm. The curve is determined with the gallic acid solution. The results obtained are expressed by gallic acid equivalents (GAE).

The spectrophotometric approach was used to assess **the alpha-amylase enzyme's inhibitory activity** in the beverage sample. The main idea behind this test is to stop the alpha-amylase enzyme from hydrolyzing starch. Starch will be hydrolyzed into simple sugars by the enzyme amylase. DNS (dinitrosalicylic acid) is added to halt the enzymatic process and is detected spectrophotometrically at a wavelength of 540 nm. Dinitrosalicylic acid, sodium hydroxide, Rochelle salt, phenol, and sodium metabisulfite make up the DNS reagent [16][17]. This technique may be used to assess a sample's capacity to thwart the alpha-amylase enzyme, which has the power to convert starch into simple sugars. Alpha-amylase activity is inversely correlated with the rate at which starch is hydrolyzed into maltose**.** Data obtained from testing these parameters were then analyzed using regression Minitab 17 with a significance level of 5%.

III. RESULT AND DISCUSSION

From the experimental results, it is known that the total phenol value for each treatment varies as shown in Table 1. below:

TABLE I TOTAL PHENOLS OF BLACK TEA STEVIA DRINK

Black tea: Stevia proportion (w/w)	Average (mg GAE/100 mL)
0.88:0.12	97.28 ± 0.11
0.76:0.24	89.03 ± 0.44
0.64:0.36	93.75 ± 0.11
0.52:0.48	85.20 ± 0.85
0.40:0.60	87.00 ± 0.70

Table 1 shows the difference in the proportion of black tea stevia which can affect the total phenol of black tea stevia. The highest total phenol was in the proportion of 0.88:0.12 treatment with an amount of 97.2792 mg GAE/100mL which was stored at room temperature. The difference in total phenols can be due to the interaction between the phenolic compounds in black tea and stevia by bonding as glycosides or esters. The interaction resulted in the free hydroxyl groups being bound and unable to react with the Folin-Ciocalteau reagent. Phenolic compounds have many hydroxyl groups in free form which can serve as hydrogen atom donors when reacting with radical compounds through an electron transfer mechanism, so as to produce high total phenols. Fewer free -OH (hydroxyl) groups can result in lower total phenol, due to the less ability to donate hydrogen electrons. From the experimental results, it is known that the inhibition by alpha-amylase for each treatment varies as shown in Table II below:

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TABLE II DATA OF A-AMYLASE ENZYME INHIBITION ABILITY

Black tea: Stevia proportion (w/w)	%inhibition
0.88:0.12	73.50 ± 1.23
0.76:0.24	75.56 ± 1.05
0.64:0.36	76.08 ± 0.99
0.52:0.48	76.53 ± 1.09
0.40:0.60	76.33 ± 1.01

Comparing treatments at the same temperature, the 0.52:0.48 treatment had the strongest enzyme inhibitory effect. The inhibition of the alpha-amylase enzyme was affected differently depending on the ratio of black tea to stevia. The performance of enzymes was to blame for this. The active sites of enzymes are designed specifically to recognize their substrates. The formation of the product, in this case glucose, will also be inhibited if there is an inhibitor present. Tea also contains tannin compounds, which contribute to the bitter flavor of the beverage.

Tannins are divided into two groups, namely hydrolyzable tannins and condensed tannins, according to Westendarp [18] and Besharati [19]. The hydrolyzed tannins are polymers of gallic or ellagic acid compounds which are esters linked to a sugar molecule. Examples of hydrolyzed tannins are catechin (C), Epigallocatechin (EGC), Epicatechin (EC), Catechin gallate (CG), and Epigallocatechin gallate (EGCG) which are all antioxidants. Based on research by the USDA (United State Department of Agriculture) in 2002, it was shown that these gallic derivatives are useful in helping to accelerate the activity of the insulin hormone (in regulating blood sugar levels) so that they can act as anti-diabetic compounds. So that differences in proportions can have different effects, on the inhibitory ability of enzymes.

From the regression analysis, it is known that the relationship between total phenol and the ability to inhibit amylase is positively correlated, the higher the total phenol, the higher the inhibition of amylase, and vice versa. The value of the relationship between total phenol and amylase inhibition can be seen in the following table:

TABLE III REGRESSION EQUATIONS

Line Equations	Type	R dan R2 Value	Reference
FENOL = - 159.1 + 3.299 AMYLASE INHIBITION	**Liniear**	**R-Sq = 79.7%** **R-Sq(adj) = 78.8%**	**Appendix 1**
FENOL = 4268 - 114.3 AMYLASE INHIBITION + 0.7814 AMYLASE INHIBITION^2	**Quadratic**	**R-Sq = 88.5%** **R-Sq(adj) = 87.4%**	**Appendix 2**
FENOL = 149277 - 5886 AMYLASE INHIBITION + 77.35 AMYLASE INHIBITION^2 - 0.3385 AMYLASE INHIBITION^3	**Cubic**	**R-Sq = 90.2%** **R-Sq(adj) = 88.8%**	**Appendix 3**

By looking at Table III, and paying attention to the highest R-sq value of 90.2%, it means that the PHENOL equation = 149277 - 5886 AMYLASE INHIBITION + 77.35 AMYLASE INHIBITION^2 - 0.3385 AMYLASE INHIBITION^3, is the most suitable to represent the relationship between total phenol and amylase inhibitory ability.

IV. CONCLUSION

The results of the analysis showed that black tea stevia had an effect on total phenol and antidiabetic activity (the ability to inhibit alpha-amylase enzyme) in glass bottles. The relationship between total phenol and amylase inhibitory ability is directly proportional, positive, and cubic equation type.

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Appendix 1:

Regression Analysis: FENOL versus AMYLASE INHIBITION

The regression equation is
FENOL = - 159.1 + 3.299 AMYLASE INHIBITION
S = 2.09609 R-Sq = 79.7% R-Sq(adj) = 78.8%

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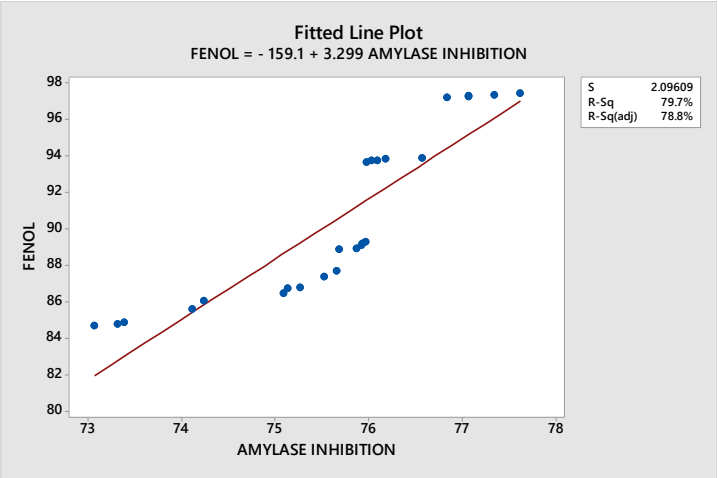
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	396.316	396.316	90.20	0.000
Error	23	101.053	4.394		
Total	24	497.369			

Fitted Line: FENOL versus AMYLASE INHIBITION



Appendix 2:

Polynomial Regression Analysis: FENOL versus AMYLASE INHIBITION

The regression equation is
FENOL = 4268 - 114.3 AMYLASE INHIBITION + 0.7814 AMYLASE INHIBITION^2
S = 1.61585 R-Sq = 88.5% R-Sq(adj) = 87.4%

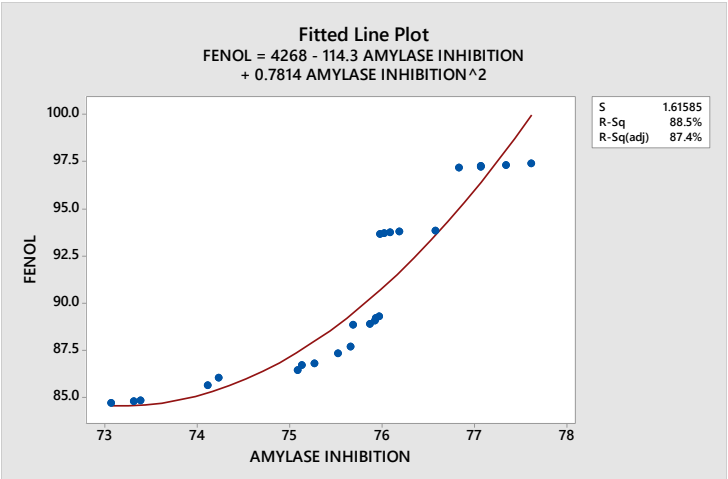
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	2	439.928	219.964	84.25	0.000
Error	22	57.441	2.611		
Total	24	497.369			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	396.316	90.20	0.000
Quadratic	1	43.612	16.70	0.000

Fitted Line: FENOL versus AMYLASE INHIBITION



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Appendix 3:

Polynomial Regression Analysis: FENOL versus AMYLASE INHIBITION

The regression equation is
FENOL = 149277 - 5886 AMYLASE INHIBITION + 77.35 AMYLASE INHIBITION^2
- 0.3385 AMYLASE INHIBITION^3
S = 1.52518 R-Sq = 90.2% R-Sq(adj) = 88.8%
Analysis of Variance

Source	DF	SS	MS	F	P
Regression	3	448.519	149.506	64.27	0.000
Error	21	48.850	2.326		
Total	24	497.369			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	396.316	90.20	0.000
Quadratic	1	43.612	16.70	0.000
Cubic	1	8.591	3.69	0.068

Fitted Line: FENOL versus AMYLASE INHIBITION