The Study of Tyrosinase Inhibition Efficiency by Glutathione and its Precursor Amino Acids and Food Supplement Formulas

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Abstract

This research aimed to study the inhibitory effect of L-Glutathione and its precursor amino acid, L-Glutamine, L-Cysteine and L-Glycine on the Tyrosinase activity in mushroom using Dopachrom method. The result indicated that L-Glutathione and its precursor amino acid, L-Cysteine markedly reduced tyrosinase activity approximately 97.64-99.30%, whereas L-Glutamine and L-Glycine had a slight effect only about 18% on such enzyme activity. Therefore, glutathione and its precursor amino acids, especially L-Cysteine could be good for development as a new tyrosinase inhibitor, supplementary product that can help to reduce the cost of raw materials in the production of dietary supplements.

Keywords**:** *L-Glutathione, Tyrosinase inhibition, supplementary product*

1. Introduction

Nowadays, food supplements or personal appliances related to the skin. Most of the substances are found "L-glutathione" (L-Glutathione) makes the present, L-glutathione has received attention from various sciences. (Gillbro & Olsson, 2011) many fields. Because of L-glutathione plays an important role as an antioxidant and can be found in many cells throughout the body especially in the liver. (Parvez et al., 2006). In which L-glutathione is involved in 4 different processes in the body, is the process of eliminating toxins from the body or various wastes in the body the process of controlling cell division. The process of balancing thiol levels antioxidant process. (Ramaiah, 1996; Davids, Van Wyk & Khumalo, 2016).

L-glutathione is a type of tripeptide, caused by the combination of the amino acids L-cysteine, L-glutamine and L-glycine, which the body can produce by itself and found in foods such as tomatoes, milk, eggs, etc. In medicine, it is used to treat diseases. Related to the nervous system and immune system. In addition, it is also an antioxidant, helps prevent cancer, heart disease and L-Glutathione. can inhibit the enzyme tyrosinase inhibit the synthesis of melanin, when melanin is not synthesized, brown skin pigment produced. (Sonthalia, et al., 2018), resulting in white skin. It is popular in beauty products related to skin care. With the many benefits of L-glutathione causing nowadays to be put into various products such as drinks, skin creams and dietary supplements. (Iwata et al., 1990)

Glutathione is an omnipresent compound that our bodies can produce. (Villarama, & Maibach, 2005). It is played the important role in human about antioxidant properties, antimelanogenic properties and skin-lightening agent. (Sonthalia, Daulatabad, & Sarkar, 2016). Sinee Weschawalit and coworker studies about a skin-lightening effected by glutathione (GSH). The results shown that female who gotten GSH have a convincing reduction in wrinkles and increased skin elasticity compared with placebo. (Sinee et al., 2017).

Amino acids known as the precursor of Glutathione (Li, Wei, & Chen., 2004), three amino acids glutamic acid, glycine and cysteine with ATP can significant increased. Moreover, a tripeptide of glutamate, cysteine and glycine are also amino that consisted in glutathione. (Pastore et al., 2003)

In this research, the objective was to study and test the tyrosinase inhibitory efficacy from food supplements containing L-glutathione and its precursor compounds in the formation of L-glutathione. Glutathione To set up formulas and develop dietary supplement formulas in the form of tablets, capsules, instant coffee, functional drinks, and gels, which can be used commercially, to continue making dietary supplements in order to obtain quality dietary supplements according to the laws of the Food and Drug Administration. (Dadzie, 2016) (Liu, 2022) (Sinha, 2017)

2. Objectives

1) To study the tyrosinase inhibition efficiency of L-glutathione precursor compounds (L-glycine, L-cysteine, L-glutamine)
2) To set up formulas and develop formulas for dietary supplements in various forms
3) To learn the process of quality inspection of food supplements

3. Materials and Methods

1.1 Auto pipette (Mettler Toledo, Switzerland)
1.2 Microplate 96 wells plate (Meark, Germany)
1.3 Microplate Spectrophotometer (BioTek EPOCH, US)
1.4 Electrical Balance 4 digit (Sartorius, Germany)
1.5 Sodium phosphate buffer solution (pH 6.8) (Merck)
1.6 Tyrosinase enzyme (Sigma)
1.7 L-DOPA solution (L-3,4-dihydroxyphenylalanine) (Sigma)
1.8 20% ethanol (Lab grade, RCI Lab-scan Ltd.)
1.9 L-Glutathione (Food grade, Changsha Huir Biological-tech Co., Ltd)
1.10 L-Cysteine (Food grade), Changsha Huir Biological-tech Co., Ltd)
1.11 L-Glutamine (Food grade), Changsha Huir Biological-tech Co.,Ltd)
1.12 L-Glycine (Food grade) Changsha Huir Biological-tech Co.,Ltd)

Methods

3.1 Preparation of enzyme buffer, stock solutions, and sample solutions.

1. Preparation of a concentrated sodium phosphate buffer solution 0.02 M (pH 6.8) by weighing 0.44 g of $Na_2HPO_4 \cdot 2H_2O$ and 0.39 g of $NaH_2PO_2 \cdot 2H_2O$, and adjusting the volume with distilled water to 250 mL.
2. Preparation of a 0.2 mg/mL solution of enzyme tyrosinase (314.8 units/mL) in 5 mL of 0.2 M sodium phosphate buffer (pH 6.8).
3. Preparation of a 0.34 mM solution of L-DOPA (L-3,4-dihydroxyphenylalanine) by weighing 0.32 mg of L-DOPA and dissolving it in 5 mL of 0.2 M sodium phosphate buffer (pH 6.8). (Nakajima, et. al. 2014)

2.2 Preparation of Sample Solution Set 1

Table 1 shows the preparation of Sample Solution Set 1

Amino acid	**Weight (mg)**					**0.2 M Na_2HPO_4 buffer (pH 6.8) (mL)**
L-Glutathione	50	100	150	200	250	10
L-Cysteine	50	100	150	200	250	10
L-Glutamine	50	100	150	200	250	10
L-glycine	50	100	150	200	250	10

*Concentration of sample solution Set 1 = 75mg/mL

2.3 Preparation of Sample Solution Set 2

Table 2 shows the preparation of Sample Solution Set 2

Set	**Amino acid (mg)**			**0.2 M Na_2HPO_4 buffer (pH 6.8) (mL)**
	L-Cysteine (500 mg/day)	**L-Glutamine** (2,000 mg/day)	**L-glycine** (600 mg/day)	
1	125	125	-	10
2	125	-	125	10
3	-	125	125	10
4	125	125	90	10
5	115	90	90	10

Set	Amino acid (mg)			0.2 M Na_2HPO_4 buffer (pH 6.8) (mL)
	L-Cysteine (500 mg/day)	L-Glutamine (2,000 mg/day)	L-glycine (600 mg/day)	
6	100	100	100	10
7	250	100	100	10

Note: The number of amino acids used follows the THAI Recommended Diary Intakes standard.

2.4 Tyrosinase activity inhibition assay

Sample solutions from section 2.1 (Preparation of buffer, enzyme, and sample solutions) were added to test sets A, B, C, and D, and separated into a 96-well plate, with three replicates per set, as shown in Table 3. The assay was repeated 3 times, replacing the sample solutions in section 2.2 with those in section 2.3 until all sample solutions were tested (Fan et al., 2021).

Table 3 shows the test sets for tyrosinase inhibition

Test Kid	0.2 M Na_2HPO_4 buffer (pH 6.8) (microliter)	Tyrosinase enzyme solution (microliter)	Sample Solution (microliter)	20% Ethanol (microliter)
Set A (control)	150	50	-	-
Set B (blank of A)	150	-	-	50
Set C (test sample)	150	50	50	-
Set D (blank of C)	150	-	50	50

Mix the reagents in each well thoroughly and incubate at 25 degrees Celsius for 10 minutes. Then add 50 microliters of L-DOPA solution to each well, shake, measure the light absorbance at 492 nanometers using a microplate reader, and incubate at 25 degrees Celsius for 2 minutes. Measure the light absorbance again at 492 nanometers.

Calculate the percentage inhibition of tyrosinase enzyme activity.

$$\%\text{Tyrosinase inhibition} = \left[\frac{(A-B)-(C-D)}{(A-B)}\right] x100$$

A, B, C, and D represent the differences in the absorbance values at a wavelength of 492 nanometers, where:

A is the absorbance value when there is no inhibitor but there is enzyme.
B is the absorbance value when there is neither inhibitor nor enzyme.
C is the absorbance value when there is both inhibitor and enzyme.
D is the absorbance value when there is inhibitor but no enzyme

4. Results and Discussion

The research results indicate the efficacy of inhibiting the function of the enzyme tyrosinase through experimental testing with L-Glutathione acid and other compounds as initial substances for producing L-Glutathione.

4.1. Testing the inhibition of the enzyme tyrosinase

4.1.1 Testing the inhibition of tyrosinase enzyme by L-glutathione, L-cysteine, L-glutamine, and L-glycine.

Inhibiting the enzyme tyrosinase with L-glutathione, L-cysteine, L-glutamine, and L-glycine at concentrations of 50, 100, 150, 200, and 250 milligrams, respectively, to determine the degree of inhibition of the tyrosinase enzyme (Table 4).

Table 4 Inhibiting the enzyme tyrosinase with L-glutathione, L-cysteine, L-glutamine, and L-glycine

Weight (mg)	Inhibiting the enzyme tyrosinase (%) (Average ± SD, n=3)			
	L-glutathione	L-cysteine	L-glutamine	L-glycine
50	7.76 ± 11.64	97.99 ± 0.19	ND	ND
100	17.41 ± 11.61	98.35 ± 0.29	ND	ND
150	33.69 ± 6.99	98.54 ± 0.05	3.87 ± 2.89	0.21 ± 6.91
200	65.19 ± 16.95	97.64 ± 0.45	2.05 ± 1.99	12.21 ± 7.74
250	95.35 ± 1.87	99.30 ± 0.15	10.37 ± 4.34	17.26 ± 6.72

Note: ND means not detected

Table 4 have shown when the percent inhibition of tyrosinase enzyme of L-glutathione, L-cysteine, L-glutamine, and L-glycine are compared, a graph showing the relationship between the percent inhibition of tyrosinase enzyme and the important substance level can be seen in Figure 1.

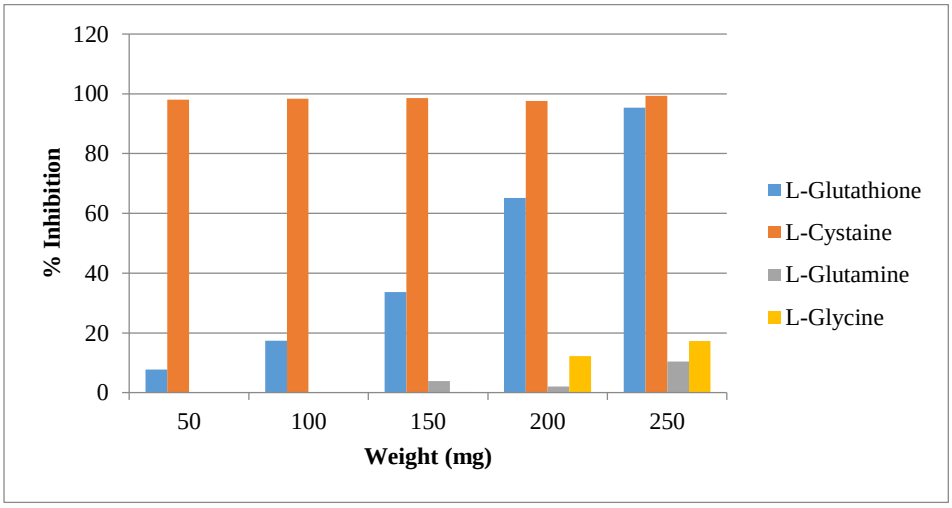

Figure 1 The graph shows the correlation between the percentage of inhibition of the enzyme tyrosinase and the amounts of L-glutathione, L-cysteine, L-glutamine, and L-glycine.

From Figure 1, it can be observed that as the amount of glutathione increases, the percentage of inhibition of tyrosinase enzyme also increases in order. When considering the initial substances in the production of glutathione, it was found that L-cysteine has a very high percentage of inhibition of tyrosinase enzyme, ranging from 97.64-99.30%, while L-glycine and L-glutamine have lower percentages of inhibition, less than 18%. This shows that they have a weak inhibitory effect on tyrosinase enzyme (Villarama & Maibach, 2005). Therefore, if a strong inhibitory effect on tyrosinase enzyme is desired, the use of L-cysteine will provide the highest effectiveness. This is due to the preliminary data on the function of L-cysteine, which plays a crucial role as a precursor in the production of glutathione, which affects the inhibition of the function of tyrosinase enzyme from converting into dopaquinone. (Chintamaneni, Nadimpalli, & Abburi, 1991). This leads to a decrease in the production of melanin, resulting in a high percentage of inhibition of tyrosinase enzyme, as shown in Figure 1. Similarly, L-cysteine is an amino acid that provides good results in the inhibition of tyrosinase enzyme, mainly due to the structure of L-cysteine that contains the R-group (-SH) that can form a disulfide bond with the oxidizing form (GHHG), as does L-glutathione. (Tsuji et. al., 2007)

1.2 Testing the inhibition of tyrosinase enzyme by a sample formula of dietary supplement product

Testing the inhibition of tyrosinase enzyme by a sample formula used in dietary supplement products to determine the inhibition values of tyrosinase enzyme (Table 5).

Table 5 Inhibition of Tyrosinase Enzyme by Sample Formula Used in Dietary Supplement Products.

Formula	L-cysteine (mg) (Thai RDI 500 mg/day)	L-glutamine (mg) (Thai RDI 2,000 mg/day)	L-glycine (mg) (Thai RDI 600 mg/day)	% Inhibition (AVG ± SD, n=3)
1	125	125	-	98.12 ± 0.42
2	125	-	125	98.84 ± 0.04
3	-	125	125	17.75 ± 15.69
4	125	125	90	97.33 ± 9.68
5	115	90	90	94.21 ± 1.29
6	100	100	100	91.22 ± 5.48
7	250	100	100	99.34 ± 5.16

Thai RDI stands for Thai Recommended Daily Intakes, which refers to the recommended daily intake of nutrients for Thai people aged 6 years and older, based on a daily energy requirement of 2,000 calories.

The information in Table 5 shows the comparison of the percentage inhibition of tyrosinase enzyme activity among different sample formulas used in dietary supplements. A graph illustrating the relationship between the percentage inhibition of tyrosinase enzyme activity and the sample formula is presented in Figure 2.

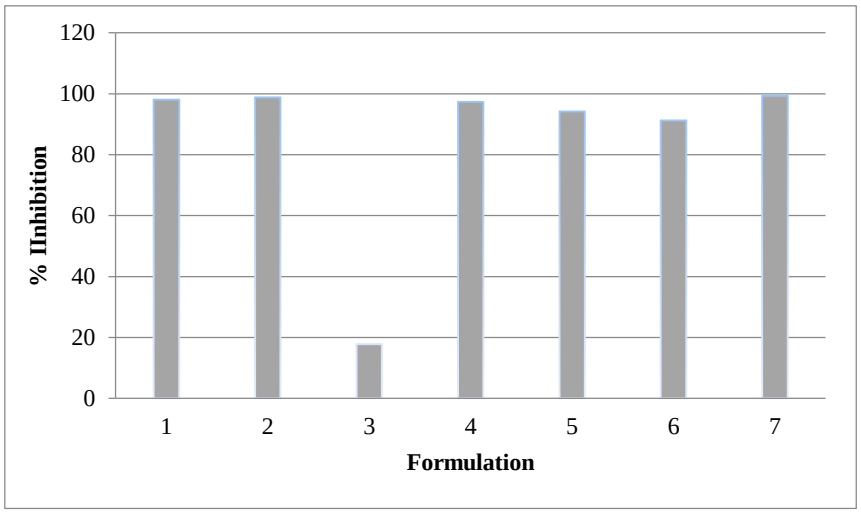

Figure 2 Shows the correlation between the percentage inhibition of tyrosinase enzyme activity and the sample formula used in the dietary supplement product

The relationship between the percentage of tyrosinase enzyme inhibition (Sonthalia et al., 2016) and the sample formulas used in dietary supplements can be observed from Figure 2. It is found that sample formulas containing 125 milligrams of L-cysteine in formulas 1 and 2 have a high percentage of tyrosinase enzyme inhibition, which are 98.12% and 98.84%, respectively. For formula 3, which does not contain L-cysteine, the percentage of tyrosinase enzyme inhibition is very low at 17.75%. When the formulations of the dietary supplements were adjusted in formulas 6 and 7 by keeping the amount of L-glutamine and L-glycine constant and increasing the amount of L-cysteine to 100 and 250 milligrams, respectively, it was found that increasing the amount of L-cysteine resulted in an increase in the percentage of tyrosinase enzyme inhibition. (Lim et al., 2009)

5. Conclusion

The efficacy of tyrosinase inhibition by L-glutathione and its precursors. When the amount of L-glutathione is increased, the percentage inhibition of the enzyme tyrosinase increases accordingly. (Riley, 1993) When considering the initial substance for the formation of L-glutathione, it was found that L-cysteine

has a very high percentage of inhibition of the enzyme tyrosinase, namely 97.64-99.30 percent. L-glycine and L-glutamine have less than 18 percent inhibition of the enzyme tyrosinase, indicating a very weak inhibitory effect on the enzyme. Therefore, if a strong inhibitory effect on the tyrosinase enzyme is desired, the use of acetylcholine would be the most effective.

Testing the inhibitory efficiency of the tyrosinase enzyme in a sample formula of dietary supplements (Dilokthornsakul, Dhippayom, & Dilokthornsakul 2019), in order to determine the level of inhibition of the tyrosinase enzyme. It was found that a sample formula of dietary supplements containing the amino acid L-cysteine has a high percentage of inhibition of the tyrosinase enzyme, with values ranging between 91.22 and 99.31 percent inhibition (Handog, Datuin, & Singzon, 2016), which are close to each other. And dietary supplement products that do not contain L-cysteine as an ingredient have a low level of inhibition of the tyrosinase enzyme, specifically only 17.75 percent inhibition. Amino acid L-cysteine has the same inhibitory effect on the enzyme tyrosinase as glutathione. This can be applied in the formulation and development of dietary supplement products, especially if L-cysteine is used instead of glutathione in production (Sinee et al., 2017), it can greatly reduce production costs since L-cysteine is cheaper than glutathione.

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