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Determination of Vitamin C Levels in Various Chilli (*Capsicum* sp) Varieties and In-Silico Activity Assessment

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ABSTRACT

Chili peppers are acknowledged as one of the fruits endowed with a notable vitamin C content, a crucial nutrient integral to various physiological processes. These include collagen synthesis, bone and tooth formation, wound healing, and bolstering the immune system's defense against infections. Vitamin C, as a water-soluble compound, is susceptible to degradation when exposed to atmospheric conditions. Hence, careful preservation of chili peppers is imperative to maintain their vitamin C levels. The primary objective of this study is to discern the concentrations of vitamin C within three distinct stages of chili pepper ripeness: young, semi-ripe, and fully ripe. The methodology employed in this investigation entails UV-Vis Spectrophotometry, with a maximal wavelength for vitamin C quantification established at 265 nm. This approach yielded absorbance values of 0.696, 0.564, and 0.478 for the respective stages. Quantitative analysis reveals that the vitamin C concentrations in young, semi-ripe, and fully ripe chili peppers are 8.1397 ppm, 5.9559 ppm, and 4.5313 ppm, respectively. These findings unequivocally demonstrate that vitamin C content is significantly higher in young chili peppers when compared to their semi-ripe and fully ripe counterparts. The docking results for capsaicin ligands yielded Gibbs energy (ΔG), Ki and IC_{50} value -6.14 kkal/mol, 31.34 μm and 19.143 ppm, respectively. The result indicate that capsaicin exhibited good interaction to catalytic site of glycogen phosphorylase which could serve as a potential antidiabetic agent.

Keywords: Vitamin C, Chili Pepper, glycogen phosphorylase, Spectrofotometry UV-Vis

INTRODUCTION

Indonesia's equatorial tropical climate supports the cultivation of diverse, nutrient-rich crops, especially fruits, which serve as significant sources of essential vitamins, including vitamin C. Vitamin C acts as a potent antioxidant, countering harmful free radicals in cells and tissues (Putri & Yunita, 2015).

Vitamins are crucial compounds that govern metabolic processes in the body. Essential vitamins for growth and reproduction include A, C, D, E, K, and B, sourced from foods such as fruits, vegetables, and dietary supplements (Ajis & Legowo, 2020). Chili peppers, among other fruits and vegetables, are rich in vitamin C, which serves as an antioxidant and improves immune function through enhanced calcium (Rosmainar et al., 2018). Fresh chili peppers have higher vitamin C and vitamin A content compared to dried chili peppers based on calorie and nutritional analysis (Tatengkeng et al., 2019).

Vitamin C is a highly effective antioxidant known for its immune-boosting properties and water solubility. It is abundant in vegetables and fruits such as oranges, guavas, custard apples, lemons, strawberries, broccoli, bell peppers, chili peppers, and tomatoes. Vitamin C is also referred to as ascorbic acid (Rahayuningsih et al., 2022).

Chili, like other fruits and vegetables, is rich in vitamin C, which serves as a beneficial antioxidant and enhances the body's immunity, aiding calcium absorption (Rosmainar et al., 2018). Fresh chili peppers have higher levels of vitamin C and vitamin A compared to dried chili peppers based on their calorie and nutritional content (Tatengkeng et al., 2019). The capsaicin level, responsible for the spiciness of chili peppers, influences fruit ripeness. Riper (red) fruits have higher capsaicin content.

Capsaicin content varies among different species and varieties and is affected by chili pepper ripeness (Ajis & Legowo, 2020).

Several methods have been developed to determine the vitamin C content, including UV-Vis spectrophotometry and iodometric methods. In UV-Vis spectrophotometry, a beam of light is directed at the sample (sample solution), and the intensity of the emitted light is measured (Shufyani & Sinurat, 2022).

Ascorbic acid in pharmaceutical formulations can be determined by iodometric titration or UV spectroscopy at a wavelength of 265 nm. Absorption at 260 nm exhibits the maximum absorbance for ascorbic acid. Absorbance is directly proportional to particle quantity, indicating that the majority of particles absorb at 260 nm. The vitamin C content in chili peppers can be obtained by converting absorbance data into concentration (ppm). The determination of the maximum wavelength is achieved by measuring the concentration of ascorbic acid in ppm with the pre-established wavelength (Fajriyani et al., 2023).

Glycogen phosphorylase, a pivotal catalyst in the modulation of glycogen metabolism, facilitates the disassembling phosphorolysis of glycogen into glucose-1-phosphate. This enzyme has been utilized as a precise target for inhibitors aimed at preventing glycogenolysis during elevated glucose concentrations in type II diabetes. Certain antidiabetic medications exhibit one or more deleterious side effects, rendering the management of diabetes mellitus a persistent quandary in the medical realm. Lately, the quest for suitable antihyperglycemic agents has shifted towards traditional medicinal products, attributed to their reduced adverse effects (Herowati & Widodo, 2014).

This research employs UV-Vis spectrophotometry, a method suitable for both qualitative and quantitative analysis. The study aims to investigate the influence of chili ripeness on the vitamin C content in chili peppers, using samples of ripe, semi-ripe, and young chili fruits. Also, this *in silico* study aimed to screen the active compound of Chilli such as *capsaicin*, to see whether it has role in hypoglycemic activity as glycogen phosphorylase inhibitor by molecular docking using AutoDock Vina software. By molecular docking, the bound conformations and the binding affinity between chemical constituent of *Capsaicin* and glycogen phosphorylase as target, could be predicted.

METHODOLOGY

This research employs a descriptive experimental approach using UV-Vis Spectrophotometry. The study sample consists of different chili pepper ripeness levels, including ripe, semi-ripe, and raw, selected through random sampling (Dewi, 2018). The in silico (computational) method is carried out using a computer system.

Tools

The utilized equipment consisted of an analytical balance (Radwag AS 220.R1 PLUS), glass beaker, volumetric flask, volumetric pipettes, volumetric glassware (Pyrex), filter paper (Whatman 41), micropipettes (DragonLab), blender, and UV-Vis spectrophotometer (Genesys).

Materials

The materials consisted of young chili samples (green skin), semi-ripe chili (yellow skin), and ripe chili (red skin), distilled water (aquadest), and L-ascorbic acid Pro analysis (Merck).

The in-silico analysis was performed using a computer equipped with an Intel Inside CORE i7 CPU 2.00 GHz, 16 GB of RAM, and Windows 10. Docking analysis was carried out using AutoDock Vina, AutoDock Tools, Discovery Studio, Chemdraw. Receptor 1GPA, with PDB code 1GPA, was downloaded from www.rcsb.org/pdb, and capsaicin ligands were sourced from www.chemspider.com.

Research Design

The study employed an experimental and descriptive design to assess vitamin C levels in various chili peppers (ripe, semi-ripe, and young). The analysis was conducted in triplicate using UV-Vis spectrophotometry.

Qualitative Analysis Vitamin C

Mix 2 ml of the sample solution with 2 drops of 0.1% $KMnO_4$ and observe color changes. A positive reaction is characterized by fading color (Shufyani & Sinurat, 2022).

Preparation of 100 ppm ascorbic acid stock solution

0.01 g of Vitamin C was weighed and introduced into a 100 ml volumetric flask wrapped in aluminum foil. It was dissolved in distilled water to the mark and homogenized, resulting in a concentration of 100 ppm.

Preparation of standard curve

Vitamin C solution at 100 ppm was pipetted into five 100 ml volumetric flasks wrapped in aluminum foil, with volumes of 4 ml, 6 ml, 8 ml, 10 ml, and 12 ml, respectively. Distilled water was then added to the mark, homogenized, resulting in concentrations of 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm.

Determination of maximum wavelength

A 4 ppm solution was withdrawn and placed in a cuvette, followed by measurement in the 200-400 nm wavelength range using distilled water as the blank.

Calibration Curve Determination

The absorbance of solutions at concentrations of 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm were measured at the maximum wavelength. Subsequently, a calibration curve was generated, and a linear regression equation was calculated from the obtained data.

Determination of sample content using UV-Vis spectrophotometry method

Chilli fruits, categorized as young, semi-ripe, and ripe, were finely chopped and homogenized. Sample solutions were prepared by weighing 25 grams, dissolving them in distilled water in a glass beaker, transferring to a 50 ml volumetric flask, and homogenizing. The sample solution was then filtered using filter paper. A 2 ml aliquot of the sample solution was pipetted into a 50 ml volumetric flask. Vitamin C content in the sample was determined by measuring absorbance at the maximum wavelength, and the vitamin C concentration was calculated using the absorbance value in a linear regression equation (Dewi, 2018)

Analisis In-Silico

1. Receptor and Ligand Structure Preparation

Protein structures were downloaded from the www.rcsb.org/pdb website. The target receptor with code 1GPA was selected, and protein preparation was performed using Discovery Studio and AutoDock Tools software. Test ligands were obtained from www.chemspider.com and prepared using Discovery Studio and AutoDock Tools. Autodock Tools were employed to convert ligand and receptor into .pdbqt file formats, adjust the number of torsions, and configure the grid box on the receptor.

2. Moleculer docking

Binding mode and interaction of glycogen phosphorylase (1GPA) with individual chemical constituent of *Capsaicin* was performed using AutoDock Vina software. Optimization of the geometrical isomeric structure of *Capsaicin* was done by minimizing energy (MM2) using Hyperchem 7.0 and then saved as .mol. Docking was performed to obtain a population of possible conformations and orientations for the ligand at the binding site.

The protein was loaded in PyRx software, creating a PDBQT file that contains a protein structure with hydrogens in all polar residues. In this condition, all bonds of ligands were set to be rotatable. All calculations for protein-fixed ligand-flexible docking were done using the Lamarckian Genetic Algorithm (LGA) method. The docking site on protein target was defined by establishing a grid box with the dimensions of x (46.52), y (-0.265), and z (27.283).

The best conformation was chosen with the lowest docked energy, after the docking search was completed. Ten runs with AutoDock Vina were performed. Docking results are analyzed by examining Gibbs energ values and Ki, followed by visual assessment using Discovery Studio.

RESULT AND DISCUSSION

Vitamin C Levels Determination

Table 1 revealed positive outcomes, indicating the presence of vitamin C compounds in each chili sample, confirmed by the $KMnO_4$ reagent. This is denoted by a color change observed upon $KMnO_4$ addition, where the color disappears. $KMnO_4$ served as an oxidizing agent and an indicator for the presence of vitamin C in the sample. Permanganate ions oxidized vitamin C into dehydroascorbic acid by accepting electrons released by vitamin C, leading to the disappearance of $KMnO_4$ color (Guntarti & Hutami, 2019).

Table 1. Qualitative Analysis of Vitamin C Results

No.	Sample of Chili	Reagent	Result	Description
1	Young	$KMnO_4$	Fading Purple	+
2	Semi-ripe	$KMnO_4$	Fading Purple	+
3	Ripe	$KMnO_4$	Fading Purple	+

The linear regression calculation yielded the equation: y = 0.0606x + 0.2034 with a correlation coefficient (r) of 0.989. The correlation coefficient (r) approaching 1 indicates strong linearity, demonstrating that the curve of absorbance versus concentration exhibits a clear and consistent increase (Lestari et al., 2011). Using 5 standard concentration variations, the linearity test for method validation confirms linear correlation, meeting acceptance criteria and affirming the method's excellent performance across the measurable concentration range (Kantasubrata, 2008).

Figure 1 depicted the calibration curve generated for vitamin C to determine the linear range of standard vitamin C solutions. The calibration curve for vitamin C is established using absorbance values obtained from concentration variations (Shufyani & Sinurat, 2022).

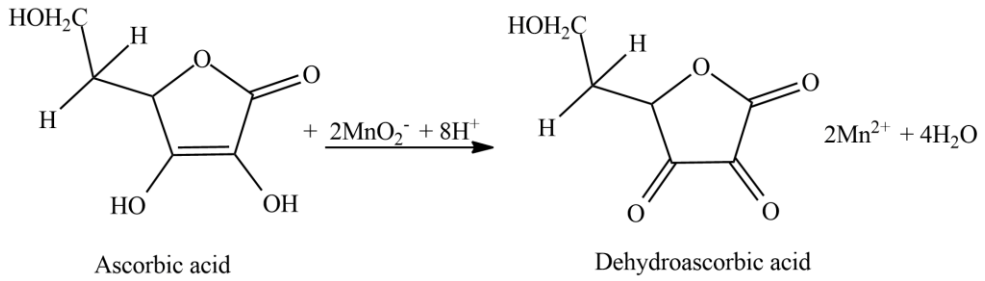


Figure 1. The reaction of Vitamin C with $KMnO_4$
Source: (Guntarti & Hutami, 2019)

The average absorbance values for young, semi-ripe, and ripe chili peppers were 0.696, 0.564, and 0.478, respectively. The vitamin C content in these chili peppers was 8.1397 ppm, 5.9559 ppm, and 4.5313 ppm, respectively. The findings align with the investigation conducted by (Badriyah & Manggara, 2015), which quantified the concentration of vitamin C in red chili peppers through UV-Vis spectrophotometry, yielding a vitamin C content of 4.463 ppm. Another investigation by Ajis & Legowo, (2020), reported a vitamin C level in Cayenne chilli (cabe rawit) of approximately 3.444 ppm (Ajis & Legowo, 2020).

The results demonstrate higher vitamin C levels in young chili peppers than in semi-ripe and ripe ones, with decreasing content associated with fruit maturity (Winarno, 2004).

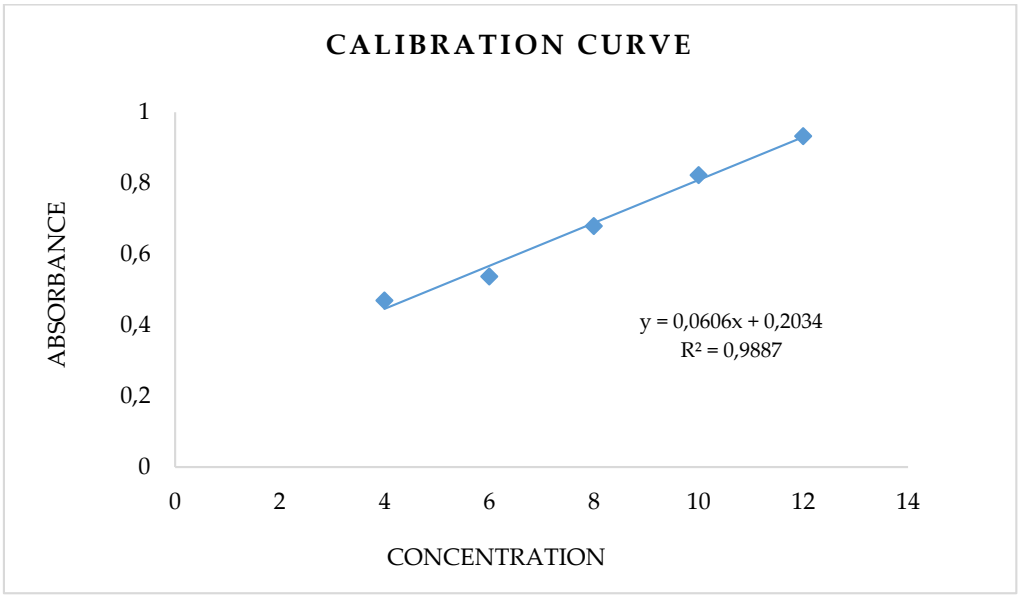


Figure 2. Vitamin C calibration curve

Table 2. Absorbance Values for Different Varieties of Chili Peppers

No.	Standard solution concentration (ppm)	Absorbance
1	4	0.469
2	6	0.537
3	8	0.679
4	10	0.822
5	12	0.932

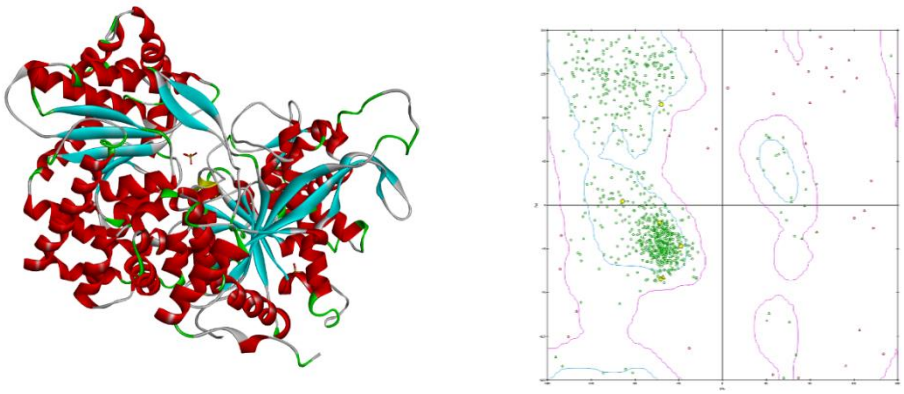
Table 3. Vitamin C Levels in Different Varieties of Chili Peppers

No.	Sample of Chili	Absorbance	Average Absorbance	Vitamin C Level (ppm)
1	Young	0.692 0.689 0.709	0.696	8.1397
2	Semi-ripe	0.578 0.598 0.517	0.564	5.9559
3	Ripe	0.486 0.447 0.501	0.478	4.5313

***In-Silico* Analysis**

Molecular docking studies were employed to explore the potential interaction/binding between ligands and proteins involved in 1GPA. Capsaicin ligands were selected according to its majority compounds found in Chili pepper. The structure of the target 1GPA was downloaded from a PDB data bank. 1GPA served as the receptor in this study. The receptor's stability analysis, using a Ramachandran plot, consists of four regions. The blue-lined region is permissible and favored, while the areas outside the pink lines are undesirable (Andrio Suhadi; Rizarullah, 2021).

The Ramachandran diagram is a visual representation displaying dihedral angle conformations to determine amino acid positions and assess the receptor structure's quality obtained from crystallization. Receptor stability analysis is conducted within the Ramachandran plot, divided into disallowed, allowed, and favored regions (Andrio Suhadi et al., 2019).



(a) **(b)**

Figure 3. Visualization: (a)1GPA receptor, (b) Rahmachandran Plot of 1GPA

The receptor analysis results indicated that almost all the amino acids are in favored regions. According to these results, it can be concluded that the 1GPA receptor exhibits excellent quality and is suitable for further use.

Docking methods are a primary computational approach in drug discovery and development. They involved binding ligands to a receptor to form a molecular complex and assess the stability between the ligand and receptor (Noviardi & Fachrurrazie, 2015).

The docking results for capsaicin ligands yielded Gibbs energy (ΔG), Ki and IC_{50} value -6.14 kkal/mol, 31.34 μm and 19.143 ppm, respectively. Gibbs energy is a parameter representing the stability of the ligand-receptor complex. The Gibbs energy principle states that lower or more negative Gibbs values indicate a stronger binding interaction between the ligand and the receptor (Andrio Suhadi et al., 2019).

Binding energy is indicative of the affinity in ligand-receptor interactions, quantifying the requisite energy for ligand attachment to a receptor. More negative binding energy values denote a more stable ligand-receptor complex, reflecting stronger binding affinity (Fajrin *et al*., 2018).

Table 4. Binding Energy

Ligand	Target	Gibbs Energy (ΔG)	Ki (μM)	IC_{50} value (ppm)	Log P	X, Y, Z value	Binding Site Residues
Capsaicin	1GPA	-6.14	31.34	19.143	1.29	Center_x = 46.52 Center_y = -0.265 Center_z = 27.283	ARG16, ARG69, VAL15, ILE13

The physicochemical parameters evaluated include lipophilicity, denoted by partition coefficient (Log P) values. Lipophilicity indicates the compound's ability to permeate cell membranes, while the structural friction of the test compounds increases the likelihood of beneficial interactions with the target site.

The findings reveal that Capsaicin exhibits a log P value of 1.29, reflecting a favorable partition coefficient. According to Lipinski's rule, an optimal partition coefficient is characterized by a log P value below 5, as higher log P values can diminish activity (Puspaningtyas *et al*., 2012).

The 1GPA receptor consists of 828 amino acid residues available for binding, with active site amino acids including ARG16, ARG69, VAL15, and ILE13. The active site of this receptor serves as the location for inhibitor binding to inhibit the receptor's reaction (Anitha et al., 2013).

The capsaicin molecule was segmented into three regions: aromatic (A), an amide linkage (B), and a hydrophobic side-chain (C). Modifying the structural configuration at regions B and C altered the pharmacodynamic properties as previous study. The replacement of the amide group in capsaicin augmented its hydrophobic character and efficacy. The amide moiety in capsaicin engages in hydrogen bonding at the Ile13 locus.

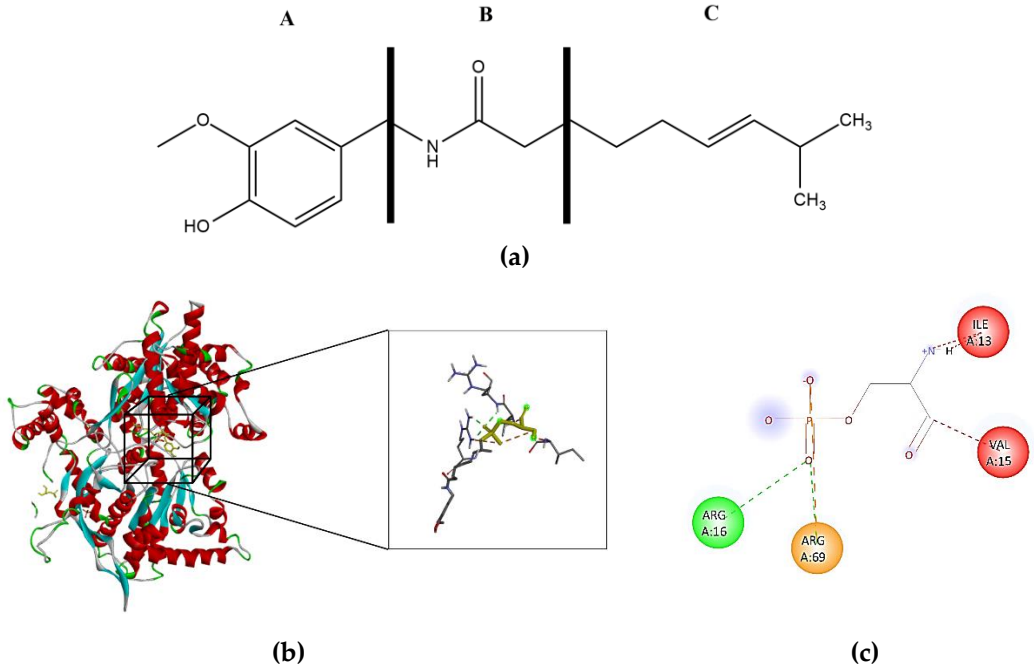


Figure 4. Visualization: (a) Structure of capsaicin; (b) Ligand Capsaicin-1GPA Receptor; (c) 2D Ligand Capsaicin-1GPA Receptor

Meanwhile, glycogen phosphorylase contains at least six distinct ligand-binding sites: (1) the Ser14-phosphate recognition site, (2) the catalytic site that accommodates glycogen, glucose-1-phosphate, glucose, and glucose analogues, (3) the AMP allosteric site, (4) the purine nucleoside site, (5) the glycogen storage site, and (6) the dimer interface site, which interacts with indole derivatives. The catalytic site, AMP allosteric site, inhibition site, and

dimer interface site are known for their affinity to inhibitors (Fajrin et al., 2018).

Glycogen phosphorylase (GP) serves as a pivotal enzyme in the glycogenolysis pathway. Inhibitors of GP are presently being explored as a novel hepatocentric strategy for the treatment of type 2 diabetes mellitus (DM). Consequently, the pharmacological inhibition of glycogen phosphorylase could attenuate plasma glucose concentrations by promoting glucose sequestration as glycogen, thereby mitigating the deleterious effects associated with hyperglycemia (Rocha et al., 2022).

The docking results for capsaicin ligands produced Gibbs energy (ΔG), Ki, and IC50 values of -6.14 kcal/mol, 31.34 μM, and 19.143 ppm, respectively. These findings suggest that capsaicin ligands have the potential to act as inhibitors of the 1GPA receptor. Amino acids ARG16, ARG69, VAL15, and ILE13 play a crucial role in the active site. The result indicated that the capsaicin exhibited good binding interaction to catalytic site of glycogen phosphorylase which could serve as a potential antidiabetic agent.

CONCLUSION

The average absorbance values for young, semi-ripe, and ripe chili peppers were 0.696, 0.564, and 0.478, respectively. The vitamin C content in these chili peppers was 8.1397 ppm, 5.9559 ppm, and 4.5313 ppm, respectively. The results demonstrate higher vitamin C levels in young chili peppers than in semi-ripe and ripe ones, with decreasing content associated with fruit maturity.

The docking results for capsaicin ligands produced Gibbs energy (ΔG), Ki, and IC50 values of -6.14 kcal/mol, 31.34 μM, and 19.143 ppm, respectively. These findings suggest that capsaicin ligands have the potential to act as inhibitors of the 1GPA receptor which could serve as a potential antidiabetic agent.

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