The 3rd International Seminar of Science and Technology
ISST 2023 Vol 3 (2024) 02 003
Trends in Science and Technology for Sustainable Living
Faculty of Science and Technology
Universitas Terbuka

The effect of processing temperature on vitamin C content of *Citrus aurantifolia* drink using high performance liquid chromatography (HPLC)

Agung Supriatna and Rina Rismaya*

Universitas Terbuka, Food Technology Department, South Tangerang, Banten, Indonesia, 15437

Abstract - Lime is rich in vitamin C, however, it is easily degraded by high processing temperatures. The aim of this study was to determine the effect of processing temperature variations on the vitamin C content in lime drinks using the HPLC method. This study used a completely randomized design with one factor, namely different processing temperatures (no processing, 5°C, 28°C, 38°C, and 90°C) with 5 repetitions. The results showed that the processing temperature significantly affected in decreasing vitamin C content of lime drinks. The higher the processing temperature, the lower the measured vitamin C content. Lime drinks without processing have a higher vitamin C content (0.0330%) compared to 5ºC temperature (0.0328%), 28°C temperature (0.0318%), 38°C temperature (0.0317%), and 90°C temperature (0.0309%).

Keywords: *Citrus aurantifolia*, HPLC, lime drink, vitamin C degradation

1 Introduction

Lime is a fruit that is quite popular in society because it is affordable, easy to obtain, comes from nature, and has no side effects for consumers [1]. The fruit known by the scientific name *Citrus aurantifolia* has quite good nutritional content. Every 100 g of lime contains around 27 mg of vitamin C, 40 mg of calcium, 22 mg of phosphorus, 0.04 mg of vitamin B, 0.6 mg of iron, 12.4 g of charcoal hydrate, 37 calories, 0.1 g of fat, 0.08 g protein, and 86 g water [2].

Ascorbic acid or vitamin C is a nutrient that is really needed by the body because it has functions as an antioxidant and medicine [3], [4]. Vitamin C also plays a role in many biological processes, one of which is collagen formation [5], [6]. Ascorbic acid functions as a cofactor for the prolyl-hydroxylase enzyme which catalyses the hydroxylation reaction in the synthesis of hydroxyproline, the main component of collagen [7]. Without ascorbic acid, the collagen found in various body tissues will experience damage and weakness. Therefore, vitamin C is very important for the growth and health of subcutaneous tissue, cartilage, bones, teeth, and to prevent fiber deficiency [4], [8].

Lime is a source of vitamin C which can be consumed directly or processed into a warm drink. Most people prefer to enjoy lime in the form of a warm drink rather than fresh lime because the taste is too sour. Hot lime drinks can be made in various ways, for example by adding plain, cold, warm, or hot water [2]. One of the properties of ascorbic acid is that it is unstable to temperature, where increasing temperature can accelerate the degradation process [9]. The rate of degradation of

*Corresponding author: rinarismaya@ecampus.ut.ac.id

unprotected ascorbic acid usually doubles every time the temperature rises by 10°C [10]. According to Yulianto et al. [2], there is a significant influence of processing temperature on reducing vitamin C levels in lime drink. However, in previous research, analysis of vitamin C levels in lime drink still used the UV-VIS spectrophotometric method.

Several methods such as the titration method, spectrophotometric method, DPPH method and the HPLC method can be used to analyze vitamin C levels [11]. However, the HPLC method is the most selective method in separating the compounds contained therein, including vitamin C [12]. Apart from that, other advantages of the HPLC method are faster analysis times, and the sample volume required is small, high sensitivity, the column can be reused, and can be used to process organic and inorganic samples [13]. Based on this background, this research aims to determine the effect of variations in processing temperature on vitamin C levels in lime drink.

2 Materials and methods

2.1 Material

To conduct this research, researchers used several tools and materials. The tools required include HPLC Waters e2695, measuring cup, pipette, ordinary glass, orange juicer, spoon, thermometer, scale, and measuring flask with volumes of 10 mL, 50 mL, and 100 mL. The ingredients used to make lime drink are mineral water, lime juice and ice. The materials used to analyze the vitamin C content are distilled water, KH_2PO_4 and a standard solution of ascorbic acid.

2.2 Stages of the research process

2.2.1 Stages of making lime drink

Fresh and ripe limes are selected from the Cianjur main market to be used as the main ingredient. Lime skin is green or yellow, while the flesh is greenish yellow. The lime is squeezed with a citrus squeezer to get the juice. Then, four lime drink formulas were made, each containing 10 mL of lime juice and 100 mL of water. The four lime drink formulas (Table 1) have different temperatures, namely 5°C, 28°C, 38°C and 90°C. Temperature is measured with a water thermometer [2].

Table 1. Lime drink formula.

Ingredients	**Formula (mL)**			
	F1	**F2**	**F3**	**F4**
Lime juice	10	10	10	10
Ice water	90	-	-	-
Room temperature mineral water	-	90	-	-
Warm temperature mineral water	-	-	90	-
Hot temperature mineral water	-	-	-	90

2.2.2 HPLC chromatographic conditions

The specifications used are as follows: a UV/Vis detector with a wavelength of 245 nm, a flow rate of 1 mL/minute, a C18 column with a length of 250 mm and a diameter of 4.6 mm, with an injection loop of 20 µL. With a mobile phase of phosphate buffer pH 2.5. The HPLC method used is based on United State Pharmacopeia 42, NF 37 of 2019.

2.2.3 Determination of Vitamin C levels

a) Creation of calibration curve

In this research, the HPLC method uses phosphate buffer pH 2.5 as the mobile phase and a wavelength of 245 nm. A standard solution of 100 ppm ascorbic acid is made by dissolving 10 mg of ascorbic acid in a 100 mL volumetric flask containing distilled water up to the mark [14]. Then, the standard stock solution was diluted to obtain a standard series of ascorbic acid with a concentration of 0.4; 0.6; 0.8; 1.0; and 1.2 ppm. Each solution was put into a 10 mL measuring flask and distilled water was added up to the mark to obtain concentrations of 4 ppm, 6 ppm, 8 ppm, 10 ppm and 12 ppm. Standard curves and linear equations are used for quantitative analysis of samples containing vitamin C [2]. After the standard series is ready, each solution is filtered with a 0.45 µm sieve and put into a vial before being injected and analyzed for area. The linear regression equation y = a + bx and the correlation value are determined from the analysis results [14], [15].

b) Determination of vitamin C content of sampel

In determining the vitamin C content, lime drink is prepared first. The sample is filtered to facilitate embalming of the filtrate, then the sample is taken at the temperature that has been determined and diluted by taking 1 mL of the sample filtrate, then diluted into a 10 mL measuring flask and homogenized [2]. The sample flow is filtered using a 0.45 µm filter then injected into HPLC [14]. Sample measurements were carried out 5 times with repetition. The concentration obtained is then calculated into Equation 1.

$$\text{Vitamin C\%} = \frac{\text{x}\left(\frac{\text{mg}}{\text{L}}\right)\text{ x Volume (L)x Fp x 100\%}}{\text{Berat sampel (mg)}} \quad (1)$$

To measure vitamin C levels in each lime drink formula, this study used the HPLC method with reverse phase. The stationary phase chosen was C18 with phosphate buffer pH 2.5 as the mobile phase. HPLC operating conditions applied to sample analysis were a Lichosphere column 250 x 4 mm, injection volume 20 µL, flow rate 1 mL/minute. The chosen wavelength is 245 nm. The retention time of each injection is the time required by the sample from entering the sample until leaving the column and is detected by the detector as a maximum peak and displayed in the form of a chromatogram [15], [16]. Analysis of vitamin C in samples begins by injecting a standard solution of vitamin C into HPLC, so that the area and retention time of the standard solution of vitamin C and the sample are obtained.

2.3 Experimental Design and Data Analysis

This research used a Completely Randomized Design (CRD) with difference processing temperature in making lime drink, namely no processing, 5°C, 28°C, 38°C, and 90°C. The research was repeated 5 times. The measurement results were then analyzed using one-way analysis of variance (ONE-WAY ANOVA) at a 95% confidence level. If the ANOVA results show a real effect, then Duncan's further test is carried out to see the differences between treatments using SPSS Statistics version 21.0 for windows (IBM Corp., Armonk, NY, USA).

3 Results and discussion

3.1 Vitamin C content of lime drink

Real peak measurement results (Fig 1). The following standard solution of ascorbic acid is shown in peak form in the appearance of retention time in the vital vitamin C series.

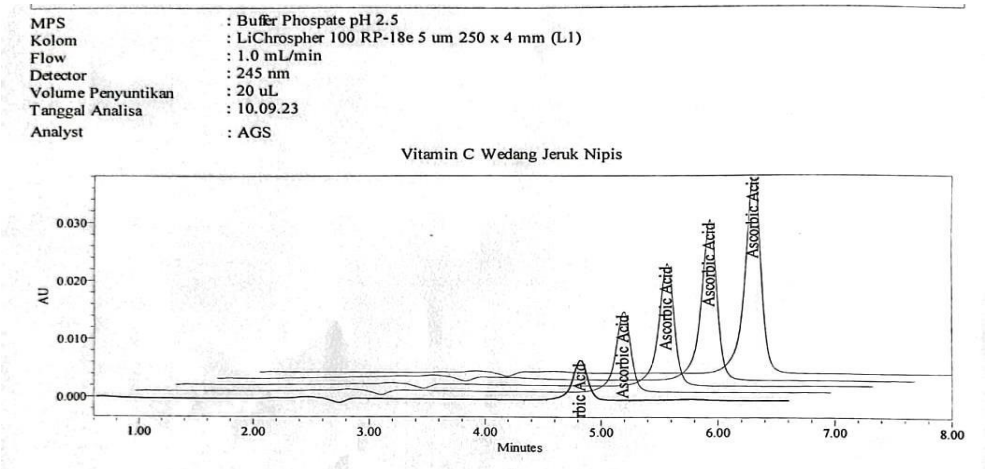

Fig 1. Standard chromatogram results.

Measurements were carried out on the results of a standard series of Vitamin C with concentration ranges of 4, 6, 8, 10 and 12 ppm. The results obtained are recorded as follows:

Table 2. Peak area calibration curve.

Volume of standard solution (mL)	Final volume of standard solution (mL)	Concentration (ppm)	Peak Areas	Retention time
0.4	10	4 ppm	255701	4.966
0.6	10	6 ppm	359694	4.972
0.8	10	8 ppm	512866	4.976
1.0	10	10 ppm	632079	4.975
1.2	10	12 ppm	766170	4.978

The measurement results of the Vitamin C standard series can be seen in Table 2. The Vitamin C standard calibration curve shows a linear relationship between the standard concentration of Vitamin C (expressed in ppm) along the x-axis and the peak value recorded along the y-axis through the instrument response. Based on this data, it can be concluded that the higher the concentration, the higher the peak value recorded. This is in line with what was stated by Putri & Rismaya [17] that there are indications of a positive relationship between concentration and peak. Then a curvilinear calculation was carried out to obtain a linear regression equation, namely Y = 64666x - 12027 with a correlation coefficient (r) of 0.9975 as in Fig 2.

Based on previous research, orange drink has been analysed using the heating method over a certain period to evaluate changes in vitamin C levels in it. The vitamin C content in natural lime juice is around 0.07359%. However, if lime juice is mixed with ice at a low temperature (3°C), the content drops to around 0.07096%. When a mixture of lime and water is prepared at room temperature

(28°C), the vitamin C content is lower, namely around 0.06328%. Meanwhile, when the mixture was prepared with warm water at a temperature (38°C), the vitamin C content fell even further to around 0.05116%. When mixed with hot water at a temperature (90°C), the vitamin C content reaches the lowest level, namely around 0.02740% [2].

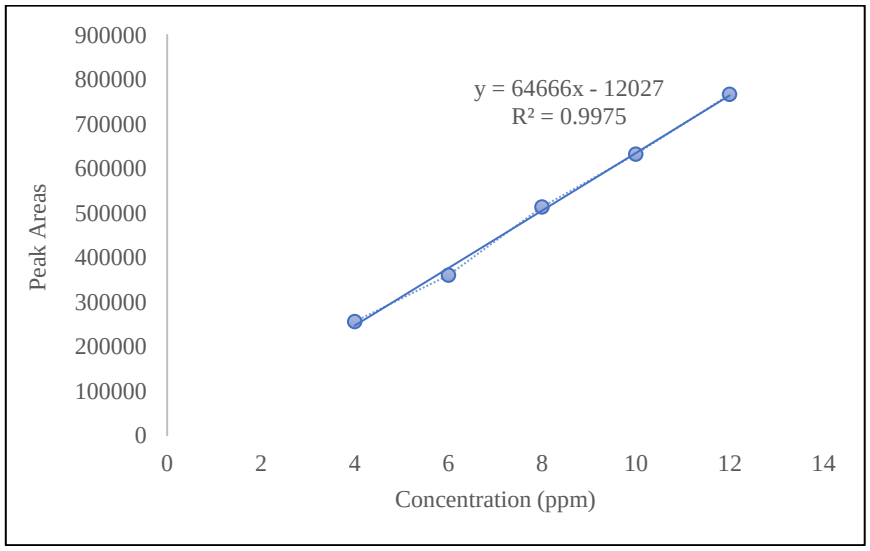

Fig 2. Vitamin C standard series curve.

Table 3. Vitamin C levels in lime drink with various temperature processing.

Sample	Vitamin C content (%)
Unprocessed Lime	0.03299
Lime (5°C)	0.03276
Lime (28°C)	0.03180
Lime (38°C)	0.03174
Lime (90°C)	0.03092

Based on Table 3, in samples without processing, the vitamin C content was 0.03299%, whereas after treatment at 5°C, the vitamin C levels decreased the smallest among the others, with an average content of 0.03299%. In previous research, a temperature treatment of 3°C was used with a concentration of 0.07096% [2]. This can happen due to differences in samples, temperature treatment and methods used. In previous research, the UV-Vis spectrophotometric method was used, and this research uses HPLC with a high level of accuracy and selectivity [12]. Vitamin C levels at 5°C have the highest levels after treatment, this can occur because at low temperatures the oxidation reaction process takes longer, while with increasing temperature the oxidation process becomes greater which causes the reaction to run faster [18].

Then, at a room temperature of 28°C and a warm temperature of 38°C, vitamin C levels decreased as the treatment temperature increased. At a temperature of 28°C the average content was 0.03180%, while at 38°C the average content was 0.03174%. The comparison of the effects of room and warm temperatures is not much different, with room temperature having a higher level than warm temperatures. This is in accordance with previous research where at the same temperature treatment, the results obtained were not much different, with vitamin C levels at room temperature being higher than warm temperatures, at room temperature 28°C the results were 0.06328% and at warm temperature 38° C is 0.05116% [2]. And in other research, it was explained that the vitamin C content decreases with increasing temperature [19].

At a hot temperature of 90°C the concentration was the lowest with an average content of 0.03092%. In general, the higher the reaction rate due to the influence of temperature, the higher the rate of degradation of vitamin C. So, there is an influence between temperature and the degradation of vitamin C. In research by Tambunan et al. [20], temperature affects the speed of chemical reactions catalysed by an enzyme, at low temperatures the activity of the enzyme is inhibited and will maintain vitamin C. A decrease in vitamin C content has been observed in previous research, and the lowest vitamin concentration was found at the highest temperature treatment (90 °C), namely 0.02740% [2]. Meanwhile, in other studies, the highest concentration of vitamin C was at a temperature of 40°C and the lowest was at a temperature of 80°C. This shows that as the temperature increases, the degradation of vitamin C due to heat also increases when heated at the same time. This is because at high temperatures, vitamin C molecules break their bonds, causing vitamin C to decompose or be damaged [21].

Vitamin C is a type of vitamin that is very sensitive to changes in temperature and easily dissolves in water. Vitamin C is quickly destroyed by heat. The results of heating cause degradation of vitamin C [22], [23]. Meanwhile, Ameliya et al. [24], longer heating times make the structure of vitamin C more susceptible to damage (degradation), especially if exposed to high temperatures. Therefore, cooking vegetables, for example, can easily reduce the vitamin C content. Degradation of vitamin C occurs when L-ascorbic acid, a very strong reducing agent and very easily oxidized, is converted into dehydro-L-ascorbic acid through an intermediate stage called dehydro-L-ascorbic acid. hemi hydro radical -L-ascorbic acid (sometimes called mono hydro ascorbate). These three forms of L-ascorbic acid form a redox system that can occur reversibly. When in the form of L-ascorbic acid, its activity reaches 100%. However, when it is converted into dehydro-L-ascorbic acid, its activity decreases to around 80% [24].

4 Conclusion

Based on results analysis, the highest concentration of vitamin C found in lime drink which is not processing (0.03299%), while in lime drink stored at 5 °C (0.03276%), lime drink processed at 28 °C (0.03180%) and lime drink processed 38 °C (0.03174%). These results showed that temperature processing significantly influenced to decline vitamin C content in lime drink. The higher temperature processing, the lower concentration of vitamin C in lime drink.

References

[1] S. W. Lauma, D. H. C. Pangemanan, and B. S. P. Hutagalung, “Uji efektifitas perasan air jeruk nipis (Citrus aurantifolia S) terhadap pertumbuhan bakteri Staphylococcus aureus secara in vitro [Test the effectiveness of lime juice (Citrus aurantifolia S) on the growth of Staphylococcus aureus bacteria in vitro],” *Ilm. Farm.*, vol. 4, no. 4, pp. 9–15, 2015.

[2] S. Yulianto, M. Jamilatun, and F. A. Pangesti, “Analisis kadar vitamin C wedang jeruk nipis (Citrus Aurantifolia) berdasarkan variasi suhu menggunakan metode spektrofotometri UV – VIS [Analysis of vitamin C levels of lime wedang (Citrus Aurantifolia) based on temperature variations using the UV – VIS],” *J. Formil (Forum Ilmiah) Kesmas Respati*, vol. 7, no. 2, pp. 208–218, 2022, doi: 10.35842/formil.v7i2.441.

[3] S. Goodman, *Vitamin C, The Master Nutrient*. New Canaan: CT: Keats Publishing, 1991.

[4] Y. A. N. Fitriana and A. S. Fitri, "Analisis kadar vitamin C pada buah jeruk menggunakan metode titrasi iodometri [Analysis of vitamin C levels in citrus fruits using the iodometric titration method]," *J. Sainteks*, vol. 17, no. 1, pp. 27–32, 2020.

[5] S. Almatsier, *Prinsip Dasar Ilmu Gizi [Basic Principles of Nutrition Science]*. Jakarta: Gramedia Pustaka Utama, 2009.

[6] E. Rusiani, S. Junaidi, H. S. Subiyono, and S. Sumartiningsih, "Suplementasi vitamin C dan E untuk menurunkan stres oksidatif setelah melakukan aktivitas fisik maksimal [Vitamin C and E supplementation to reduce oxidative stress after maximum physical activity]," *Media Ilmu Keolahragaan Indones.*, vol. 9, no. 2, pp. 32–37, 2019.

[7] S. A. Nugroho, "Analisis kandungan asam askorbat pada tanaman kangkung (Ipomoea reptana Poir), bayam (Amaranthus spinosus), dan ketimun (Cucumis sativus L) [Analysis of ascorbic acid content in on kale (Ipomoea reptana Poir), spinach (Amaranthus spinosus), and cucumber (Cucumis sativus L)]," *J. TAMBORA*, vol. 4, no. 1, pp. 26–31, 2020, doi: 10.36761/jt.v4i1.567.

[8] A. C. Guyton and S. Widodo, *Biokimia untuk Pertanian [Biochemistry for Agriculture]*. Medan: Universitas Sumatera Utara Press., 2007.

[9] P. E. S. K. Yuda and N. M. D. S. Suena, "Pengaruh suhu penyimpanan terhadap kadar tablet vitamin C yang diukur menggunakan metode spektrofotometri Uv-Vis [The effect of storage temperature on the concentration of vitamin C tablet were measured using UV-VIS spectrophotometry]," *Mediamento*, vol. 2, no. 1, pp. 23–27, 2016.

[10] Y. A. M. Anggraini, "Analisis Pengaruh Suhu Penyimpanan Terhadap Kadar Vitamin C [Analysis of the Effect of Storage Temperature on Vitamin C Levels]," Akademi Farmasi Surabaya., 2021.

[11] N. Angraini and P. Desmaniar, "Optimasi penggunaan High Performance Liquid Chromatography (HPLC) untuk analisis asam askorbat v [Optimizing the use of High Performance Liquid Chromatography (HPLC) for ascorbic acid analysis to support Marine Biotechnology Practicum activities]," *J. Penelit. Sains*, vol. 22, no. 2, pp. 69–75, 2020, doi: 10.56064/jps.v22i2.583.

[12] H. Susanti, N. P. M. Araaf, D. Gunanto, and A. Kusbandari, "Perbandingan metode spektrofotometri UV dan HPLC pada penetapan kadar kafein dalam kopi [Comparison of UV spectrophotometry and HPLC methods for determining caffeine levels in coffee]," *Maj. Farmasetika*, vol. 4, no. 1, pp. 28–33, 2019, doi: 10.24198/mfarmasetika.v4i0.25887.

[13] R. Fauziyah, "Analisis Rhodamin-B Pada Lip Cream Yang Beredar Di Aplikasi Belanja Online Secara Kromatografi Cair Kinerja Tinggi [Analysis of rhodamine-B in lip cream circulating in online shopping applications using high performance liquid chromatography]," Stikes Bhakti Husada Mulia Madiun, 2021.

[14] J. Jubahar, Y. Astuti, and N. Suharti, "Penetapan kadar vitamin C dari buah cabe rawit (Capsicum frutescens L.) dengan metode kromatografi cair kinerja tinggi (KCKT) [Determination of vitamin C levels from cayenne pepper (Capsicum frutescens L.) using High Performance Liquid Chromatography (HPLC)]," *J. Farm. Higea*, vol. 7, no. 2, pp. 208–217, 2015.

[15] M. Sanuddin, N. Andriani, and D. S. Ramadhani, "Penetapan kadar vitamin C pada Ekstrak Paprika (Capsicum annuum var . grossum Sendtn.) di Supermarket menggunakan Metode KCKT [Determination of vitamin C level in paprica (Capsicum annuum var. grossum Sendtn.) Extract Sold in Supermarket using the HPLC]," *Pharm. J. Farm. Indones. (Pharmaceutical J. Indones.*, vol. 18, no. 2, pp. 207–212, 2021.

[16] D. Rubiyanto, *Metode Kromatografi: Prinsip Dasar, Praktikum dan Pendekatan Pembelajaran Kromatografi [Chromatography Methods: Basic Principles, Practicum and Learning Approaches to Chromatography]*. Yogyakarta: Deepublish, 2017.

[17] U. R. P. Putri and R. Rismaya, "Penentuan kadar merkuri (Hg) pada kerang hijau (Perna viridis) di pasar Serpong menggunakan mercury analyzer metode direct thermal decomposition [Determination of mercury (Hg) levels in green mussels (Perna viridis) in Serpong market using a mercury analyzer direct thermal decomposition method]," *Food Sci. J. Food Sci. Technol.*, vol. 3, no. 1, pp. 27–39, 2023, doi: 10.33830/fsj.v3i1.4911.2023.

[18] A. M. Ibrahim, Yunianta, and F. H. Sriherfyna, "Pengaruh suhu dan lama waktu ekstraksi terhadap sifat kimia dan fIsik pada pembuatan minuman sari jahe merah (Zingiber officinale var. Rubrum) dengan kombinasi penambahan madu sebagai pemanis [Effect of Temperature and Extraction Time on Physicochemical Properties of Red Ginger (Zingiber officinale var. Rubrum) Extract with The Additional of Honey Combination as Sweetener for Functional Drink]," *J. Pangan dan Agroindustri*, vol. 3, no. 2, pp. 530–541, 2015.

[19] R. Rachmawati, R. M. Defiani, and N. L. Suriani, "Pengaruh suhu dan lama penyimpanan terhadap kandungan vitamin C pada cabai rawit putih (Capsicum frustescens) [Effect of temperature and length of storage on vitamin c concentration of chilli (Capsicum frustescens)]," *J. Biol.*, vol. 13, no. 2, pp. 36–40, 2009.

[20] I. J. Tambunan, S. Sulasmi, and S. M. Julianty, "Kajian kinetika penentuan laju reaksi penetapan kadar vitamin C pada brokoli (Brassica oleracea L) dengan metode titrasi titrimetri [Kinetic study of determination the reaction rate of vitamin C in brokoli (Brassica oleracea L) using titrimetric titration method]," *J. Pharm. Sci.*, vol. 6, no. 1, pp. 1–7, 2023, doi: 10.36490/journal-jps.com.v6i1.1.

[21] K. T. Hok, W. Setyo, W. Irawaty, and F. E. Soetaredjo, "Pengaruh suhu dan waktu pemanasan terhadap kandungan vitamin A dan C pada proses pembuatan pasta tomat [The effect of temperature and heating time on the content of vitamins A and C in the process of making tomato paste]," *Widya Tek.*, vol. 6, no. 2, pp. 111–120, 2017, [Online]. Available: http://journal.wima.ac.id/index.php/teknik/article/view/1237

[22] R. Andalia, Raihannaton, and V. Ulfa, "Uji kuantitatif vitamin C pada sayuran hijau akibat pemanasan secara spektrofotometri UV- VIS [Quantitative test of vitamin C in green vegetables due to heating using UV-VIS spectrophotometry]," *J. Sains dan Kesehat. Darussalam*, vol. 1, no. 2, pp. 67–72, 2021, doi: 10.56690/jskd.v1i2.13.

[23] A. Auliya, "Studi Stabilitas Dan Fortifikasi Vitamin C pada Pembuatan Konsentrat Jeruk Pontianak [Stability and Fortification Study of Vitamin C in Making] Pontianak Orange Concentrate]," Fakultas Teknologi Pertanian Institut Pertanian Bogor, 2008.

[24] R. Ameliya, Nazaruddin, and D. Handito, "Pengaruh lama pemanasan terhadap vitamin C, aktivitas antioksidan dan Sifat sensoris sirup kersen (Muntingia calabura L.) [The effect of

boiling time on vitamin C, antioxidant activity and sensory properties of Singapore cherry (Muntingia calabura L.) syrup],” *Pro Food (Jurnal Ilmu dan Teknol. Pangan)*, vol. 4, no. 1, pp. 290–297, 2018, [Online]. Available: http://www.profood.unram.ac.id/index.php/profood