

The Effect of Keratin Hydrolyzate Peptide Concentration and Harvest Maturity Level on the Physiological Response and Physicochemical Quality of Siam oranges (*Citrus nobilis* Lour.)

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ABSTRACT

This study evaluated the effects of Keratin Hydrolyzate Peptide (KHP) concentration, harvest maturity, and their interaction on physiological and physicochemical traits of Siam oranges. A 3 × 3 factorial randomized complete block design with four blocks (36 trees) was conducted in Kintamani from October 2025 to May 2026. KHP was foliar-applied at 0, 2, and 4 mL L⁻¹. The 2 mL L⁻¹ treatment increased relative leaf water content, chlorophyll, TSS, and fruit weight. Harvest maturity affected vitamin C, TSS, TA, TSS:TA ratio, and fruit weight. P₂M_p produced the highest total fruit moisture content (88.72%). Multi-season and multi-location validation is required before broader recommendation

INTRODUCTION

Siam orangess (*Citrus nobilis* Lour.) are one of the most widely cultivated citrus crops in Bali, with Kintamani in Bangli Regency serving as one of its major production centers (Pranata et al., 2024). Fruit quality can vary because size, juice content, sweetness, acidity, and water loss are influenced by orchard conditions and harvest timing. Citrus fruits are non-climacteric, so improvements in consumption quality after harvest are relatively limited, and the timing of harvest is crucial in determining the final quality of the fruit (Alferez et al., 2021). Therefore, preharvest management and the level of ripeness at harvest must be considered together when production goals include physicochemical quality and postharvest characteristics.

Protein hydrolysates are a group of biostimulants consisting primarily of peptides and amino acids resulting from the hydrolysis of protein-containing materials; they can influence photosynthesis, nutrient assimilation, water balance, and stress responses (Malécange et al., 2023). The magnitude of the response to protein hydrolysates can vary depending on the source material, product composition, plant species, environmental conditions, application timing, and dosage; therefore, higher concentrations do not always provide greater benefits (Sun et al., 2024). Keratin-rich materials, including poultry feathers, can also be utilized as a source of protein hydrolysate for peptide-based agricultural products (Pasković et al., 2024).

Evidence regarding the benefits of protein hydrolysates is available for several horticultural crops, but the results cannot yet be directly generalized to Siam orangess. In tomatoes, foliar application of protein hydrolysates was reported to increase yield, fruit weight, total soluble solids, and ascorbic acid compared to untreated plants (Rouphael et al., 2020). In grafted watermelons, plant-derived protein hydrolysates modified several physicochemical characteristics of the fruit and indicated that the biostimulant response may interact with the fruit ripening process (Soteriou et al., 2021). In *Citrus aurantium* under salinity stress, protein hydrolysate enhanced growth and mineral uptake; however, that study focused on stress tolerance rather than fruit quality at harvest (Lu et al., 2023). Thus, it remains unclear how keratin hydrolysate peptides applied via the leaves affect the plant physiology and fruit quality of Kintamani Siam orangess, particularly at different harvest maturity levels.

The selection of concentrations of 0, 2, and 4 mL L⁻¹ in this study formed a dose gradient encompassing a control without peptides, a medium concentration, and a high concentration. The use of 2 mL L⁻¹ has a precedent in KHP research by the same manufacturer on rice, although that concentration was reported based on recommendations for rice and not as evidence of an optimal dose for oranges (Gultom et al., 2025). The 4 mL L⁻¹ concentration was used as the upper limit because it corresponds to the foliar spray application rate listed in the FUTAY 3 technical instructions for perennial fruit crops. This range allows for testing the possibility of a non-linear concentration response without assuming that the highest dose will yield the best results.

Harvest maturity also affects orange quality because total soluble solids, titratable acidity, the TPT:AT ratio, juice content, and ascorbic acid levels change

during fruit development (Singh et al., 2023). Peel color can serve as a practical indicator of ripening progress; however, orange ripeness indices are cultivar-, environment-, and market-specific, so a robust assessment must consider the fruit's internal characteristics (Gupta et al., 2022). Therefore, the term "physiological maturity" in this study is used operationally to denote a mid-harvest stage characterized by green skin with a yellow tinge, rather than as a definitive physiological determination based solely on skin color. This stage is expected to represent a more advanced stage of development than the early green stage but has not yet reached uniform yellow skin, thereby providing a better balance between sweetness, acidity, nutritional content, and fruit water content.

A gap in the literature lies in the limited information regarding the response of Siam oranges to the application of Keratin Hydrolyzate Peptide at different harvest maturity levels. Specifically, it is not yet known whether the fruit's physicochemical characteristics in response to KHP concentration vary according to the level of maturity at harvest. Therefore, this study aims to evaluate the effect of KHP concentration on relative leaf water content, chlorophyll content, canopy light transmission, total sugars, and leaf P and K content; as well as to evaluate the effects of KHP concentration, harvest maturity stage, and their interaction on fruit weight, vitamin C, total soluble solids, titratable acidity, TPT:AT ratio, juice yield, fruit moisture content, and weight loss up to day 21. This study tested the hypothesis that a KHP concentration of 2 mL L⁻¹ yields a more favorable response compared to 0 and 4 mL L⁻¹ and that the fruit's response to KHP depends on the harvest maturity level.

LITERATURE REVIEW

Keratin Hydrolyzate Peptides and Plant Physiological Responses

Protein hydrolysates are a group of biostimulants containing peptides and amino acids that have the potential to influence various plant physiological processes. Peptides are known to contain amino acids that play a role in plant metabolism, stress protection, water status regulation, and tissue strengthening. Peptides may also be involved in cell-to-cell communication by binding to membrane receptors that activate signal transduction pathways and are associated with the regulation of stomata, turgor pressure, and cell wall structure (Liu et al., 2025). Sulfur-containing amino acids have also been reported to potentially support the plant's antioxidant system in coping with oxidative stress (Zhu et al., 2024).

Protein hydrolysates, which contain amino acids and peptides, have been reported to promote root development, nutrient uptake and assimilation, and several physiological processes in plants (Malécange et al., 2023; Pasković et al., 2024). Small signaling peptides are also involved in regulating plant growth and developmental processes, including fruiting, maturation, and responses to environmental stress (Lu & Xiao, 2024). Thus, foliar application of peptide-based biostimulants may influence plant water status and leaf chlorophyll content, which are subsequently associated with plant growth and fruit development. However, plant responses to protein hydrolysates and peptides are not

necessarily linear with increasing concentration, making the selection of an appropriate concentration important.

Evidence from maize also indicates that responses to peptide concentration may vary among physiological and yield-related traits. Nagi et al. (2026) evaluated peptide concentrations of 0, 2, and 4 mL L⁻¹ and reported significant effects on leaf area and relative water content. The 2 mL L⁻¹ treatment tended to produce higher values for several yield components than the control and the higher concentration, although most growth and yield differences were not statistically significant. These findings support the evaluation of 2 mL L⁻¹ as a moderate peptide concentration, while also indicating that it should not be considered an established optimum dose across crops and growing conditions.

H1: The concentration of Keratin Hydrolyzate Peptide affects the physiological response of plants and the physicochemical characteristics of Siam orangess, with a concentration of 2 mL L⁻¹ expected to yield more favorable responses compared to 0 and 4 mL L⁻¹.

Harvest Maturity Level and Physicochemical Quality of Siam orangess

The level of ripeness at harvest is a critical factor determining citrus fruit quality because it is related to physiological and biochemical changes during fruit development. The ripening process is characterized by changes in peel color, increased sugar content, decreased acidity, changes in vitamin content, and changes in fruit tissue structure. Fruits harvested too early generally have not yet reached optimal consumption quality, while fruits harvested when overripe are more susceptible to water loss and tissue damage. Therefore, the harvest maturity level must be determined by considering the balance between the fruit's physical and chemical properties.

Changes in the level of ripeness are closely related to the fruit's internal quality components. Vitamin C content generally increases during fruit development until it approaches a certain level of ripeness and may then decrease due to oxidation, while total soluble solids tend to increase as sweetness develops. At the same time, changes in acidity cause the ratio between total soluble solids and titratable acidity to shift, resulting in distinct flavor profiles at each stage of ripeness.

Manurung et al., (2024) reported that oranges harvested at the physiological maturity stage have better water content and texture characteristics compared to fruit that has passed physiological maturity. Conversely, overripe fruit experiences increased water loss and tissue weakening due to the aging process. At this stage, increased metabolic activity and cell wall degradation cause the tissue's ability to retain water to decline further (Kaur et al., 2024). Thus, physiological maturity is expected to provide a better balance of physicochemical characteristics compared to harvesting that is either too early or too late.

H2: The level of ripeness at harvest affects the physicochemical characteristics of Siam orangess, with harvesting at the physiological maturity stage expected to provide a more favorable balance of quality compared to harvesting before or after physiological maturity.

Interaction Between Keratin Hydrolyzate Peptides and Harvest Maturity Level

The fruit's response to peptide application is likely linked to its physiological condition at harvest. Peptide application has the potential to

influence the plant's water status, assimilate translocation, osmotic balance, and tissue integrity, while the maturity level determines the fruit's physiological and biochemical state at harvest. Therefore, the effect of peptides on fruit quality may vary at different maturity levels. The combination of peptide concentration and maturity level was indeed designed as two factors that are hypothesized to jointly influence plant and fruit characteristics.

The physiological ripening stage theoretically provides relatively balanced tissue conditions, whereas the application of peptides at appropriate concentrations is expected to support the plant's physiological status and the development of fruit quality. Conversely, in unripe fruit, the fruit filling process has not yet been fully completed, whereas in overripe fruit, the aging process and tissue degradation have already increased. Based on this, the combination of peptide concentration and harvest maturity level has the potential to produce different responses regarding the physicochemical characteristics of the fruit.

H3: There is an interaction between the concentration of Keratin Hydrolyzate Peptide and the maturity level at harvest on the physicochemical characteristics of Siam oranges.

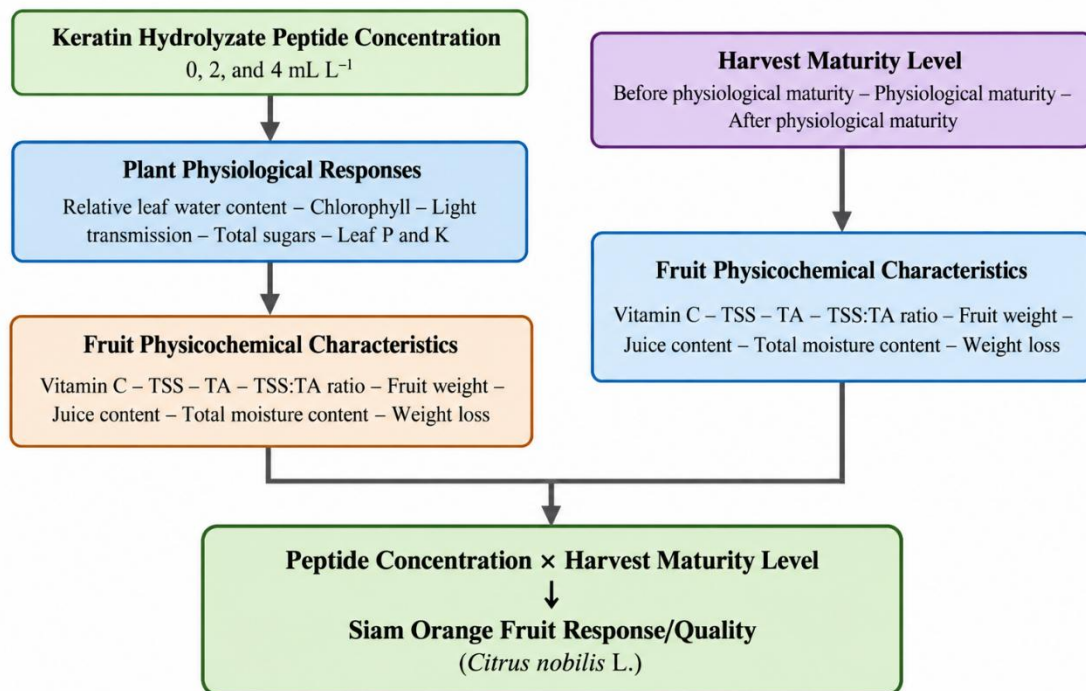


Figure 1. Conceptual Framework

METHODOLOGY

Research Location, Time, and Materials

The study was conducted from October 2025 to May 2026 at a farmer-owned Siamese orange orchard in Bayung Gede Village, Kintamani Subdistrict, Bangli Regency, Bali. Laboratory analyses were performed at the Agronomy and Horticulture Laboratory and the Chemistry and Soil Fertility Laboratory, Faculty

of Agriculture, Udayana University; the Analytical Laboratory, Udayana University; and the Food Analysis Laboratory, Faculty of Agricultural Technology, Udayana University. The plants used were approximately 12-year-old Siam orangess (*Citrus nobilis* Lour.) from a single orchard plot with a consistent maintenance history. Trees were selected based on uniformity in plant size and canopy to minimize initial variation among experimental units.

The treatment material was the commercial *Keratin Hydrolyzate Peptide* (KHP) Futay 3 produced by CH Biotech Co., Ltd., Taiwan. All plants received the same cultivation management, including weed control, organic and inorganic base fertilization, pest and disease control, and pruning of water sprouts and dead branches.

Experimental Design

The study employed a 3×3 factorial randomized complete block design (RCBD) with four blocks with a two-factor factorial design and four blocks. The first factor was the concentration of *Keratin Hydrolyzate Peptide* (KHP), consisting of $P_0 = 0 \text{ mL L}^{-1}$, $P_2 = 2 \text{ mL L}^{-1}$, and $P_4 = 4 \text{ mL L}^{-1}$. The second factor was harvesting maturity level, namely M_i = before physiological maturity (shiny green skin), M_p = physiological maturity (green skin with a yellow tinge), and M_o = after physiological maturity (uniformly yellow skin). The combination of the two factors resulted in nine $P \times M$ treatments.

Four blocks were formed based on the uniformity of the plants' initial conditions and their positions in the sloped orchard. Each block consisted of nine trees and contained all treatment combinations, which were randomly assigned. Thus, 36 trees were used as 36 experimental units, and each treatment combination had four biological replicates. One tree constitutes one experimental unit, receives one treatment combination, and is harvested at only one predetermined maturity level. Observations of multiple leaves, measurement points, or fruits on a single tree constitute subsampling and are not considered additional biological replicates.

Materials and Equipment

The equipment used in the study included an analytical balance, a penetrometer, a titrator, a juice press, Petri dishes, measuring cups, pipettes, spatulas, scissors, and a knife. The materials used were *Keratin Hydrolyzate Peptide* (a product of CH Biotech) or peptides, a borer, distilled water, 0.01 N NaOH solution, Ca(OH)_2 solution, 0.01 N iodine solution, starch solution, 0.1% phenolphthalein, label paper, Siamese orange leaves and fruit, plastic bags, and plastic wrap.

KHP Application and Harvest

The treatment material was the commercial KHP Futay 3 (CH Biotech Co., Ltd., Taiwan). The KHP solution was prepared at concentrations of 0, 2, and 4 mL L^{-1} with a spray volume of 2 L per tree. Thus, P_0 received 2 L of water without KHP, while P_2 and P_4 received 4 mL and 8 mL of KHP, respectively, in 2 L of water. The solution was applied evenly to the entire canopy using a sprayer. The control treatment received the same volume of water and followed the same spraying procedure.

Applications were carried out four times based on the plants' phenological development: October 19, 2025, during the flowering phase; November 23, 2025,

at *fruit set*; January 25, 2026, approximately 60 days after *fruit set*; and March 22, 2026, approximately 120 days after *fruit set*. Spraying procedures and volumes were standardized across all experimental units.

Harvesting was conducted selectively according to pre-determined maturity levels. A total of 15 fruits were harvested from each tree, selected based on appropriate maturity level, relatively uniform size, normal condition, and freedom from mechanical damage and signs of pest infestation. Five fruits were used for destructive testing and physicochemical analysis, while ten fruits were used to measure weight loss. The five fruits from one tree used for destructive testing were homogenized into a single composite sample so that each tree would yield a single value for each variable in the statistical analysis.

Variable Observations

The observed physiological responses of the plants included leaf chlorophyll content, canopy light transmission, relative leaf water content, total leaf sugar content, and leaf P and K content. Chlorophyll content was measured using a SPAD-502 on two leaves each from the upper, middle, and lower parts of the canopy. Each leaf was measured at ten points, and all readings were averaged to produce a single value per tree. Light transmission was measured using a UBER *lux meter* at the top and bottom of the canopy at a height of 50 cm above the ground. Chlorophyll and light measurements were taken twice after the third and fourth KHP applications, and the results at the tree level were used as experimental unit values. Relative water content (RWC) of the leaves was determined based on fresh weight (FW), turgid weight (TW), and dry weight (DW) using the equation:

$$\text{RWC (\%)} = (\text{fresh weight (FW)} - \text{dry weight (DW)}) / (\text{turgid weight (TW)} - \text{dry weight (DW)}) \times 100\%.$$

Leaf samples were hydrated for 5 hours to obtain turgid weight and dried at 70°C for 24 hours until the weight stabilized. Total leaf sugar was analyzed using the Anthrone method as described by Yemm and Willis (1954), while leaf P and K were analyzed after plant tissue preparation as described by Eviati et al., (2023). P content was determined spectrophotometrically, and K was determined using *Atomic Absorption Spectrophotometry* (AAS).

The physicochemical characteristics of the fruit analyzed included fruit weight, vitamin C, total soluble solids (TSS), titratable acidity (TA), the TSS:TA ratio, juice content, and total moisture content. Fruit weight was determined by individual weighing using a digital scale. Vitamin C was analyzed by titrimetry and expressed as mg of ascorbic acid per 100 g of fresh weight. TSS was measured using a *handheld refractometer* and expressed in °Brix. TA was determined by titration using standardized NaOH and expressed as a percentage of citric acid equivalents, while the TSS:TA ratio was calculated from the ratio of these two values.

Juice content was calculated as the percentage of juice weight relative to the initial fruit weight, while total moisture content was determined gravimetrically by drying the samples at 105°C until a constant weight was reached.

Weight loss was observed in ten identical fruits from each tree during storage under ambient conditions of approximately 28°C and RH $\pm$ 75%. The fruits were weighed on days 0, 7, 14, and 21, and weight loss was calculated using the equation: Weight loss (%) = $[(B_0 - B_t) / B_0] \times 100$, where B_0 is the fruit weight on day 0 and B_t is the fruit weight at the time of observation. The values for ten fruits were averaged to obtain a single value for each tree.

Data Analysis

Data were analyzed with the tree as the experimental unit and four blocks as biological replicates. Data derived from multiple leaves, measurement points, fruits, or technical replicates within a single tree were first averaged to obtain a single value for each experimental unit.

Preharvest physiological variables namely, chlorophyll content, canopy light transmission, relative leaf water content, total sugars, and leaf P and K were analyzed for the KHP (P) concentration factor because the maturity level constituted the harvest treatment and was not yet applicable at the time of physiological measurements. The physicochemical characteristics of the fruit were analyzed using a 3 $\times$ 3 factorial ANOVA in a randomized complete block design (RCBD) to test the effects of KHP (P) concentration, harvest maturity level (M), and the P $\times$ M interaction at the 5% significance level.

If the P $\times$ M interaction was significant, the treatment combination means were compared using *Duncan's Multiple Range Test* (DMRT) at the 5% significance level. If the interaction was not significant but the main factors were significant, the main factor means were compared using the Least Significant Difference (LSD) test at the 5% significance level. The weight loss analysis in this article uses the cumulative value on day 21 as *the endpoint*; therefore, the interpretation does not treat the four observation times as independent biological replicates.

RESEARCH RESULTS

Table 1. Significance of the Effects of *Peptide* Concentration (P) and Fruit Maturity Level (M) and Their Interaction (P $\times$ M) on All Variables

No	Variable	Treatment		
		P	M	P $\times$ M
1.	Leaf chlorophyll content (SPAD)	**	-	-
2.	Percentage of light transmission reaching the canopy floor (%)	**	-	-
3.	Relative leaf water content (%)	**	-	-
4.	Leaf phosphorus (P) content (%)	ns	-	-
5.	Leaf K content (%)	ns	-	-
6.	Total sugar content (%)	ns	-	-
7.	Weight per piece (g)	*	**	ns
8.	Total moisture content (%)	ns	**	**
9.	Fruit juice content (%)	ns	ns	**
10.	Vitamin C content (mg/100g)	ns	**	ns
11.	Total soluble solids ($^{\circ}$ Brix)	**	**	ns

12. Total titratable acidity (%)	ns	**	ns
13. TSS:TA ratio	ns	**	ns
14. Percentage of weight loss	ns	**	**

Note: ns = not significant ($P \geq 0.05$);

* = significant ($P < 0.05$);

** = highly significant ($P < 0.01$)

- = not analyzed

Table 2. Effect of peptide concentration on plant physiological variables, including relative water content, leaf chlorophyll content, light transmission to the canopy base, total sugars, and P and K nutrient content in leaves.

Treatment	RWC (%)	Chlorophyll (SPAD)	Light transmission (%)	Total Sugar (%)	Leaf P (%)	Leaf K (%)
P ₀	72.79 c	61.37 c	43.25 a	12.99 a	0.78 a	0.11 a
P ₂	80.91 a	65.30 a	26.94 c	12.22 a	0.79 a	0.12 a
P ₄	74.79 b	62.96 b	36.68 b	10.87 a	0.81 a	0.16 a
5% LSD	0.75	0.55	1.89	4.27	0.04	0.52

Note: Numbers followed by different letters in the same column indicate significant differences based on the 5% LSD test at the 5% significance level.

Table 3. Effect of keratin hydrolyzate peptide concentration and fruit maturity level at harvest on physicochemical variables

Treatment	Vitamin C (mg per 100 g)	TSS (°Brix)	TA (%)	TSS:TA	Fruit weight (g)
<i>Peptide concentration (P)</i>					
P ₀	130.53 a	8.61 b	1.06 a	10.61 a	88.35 b
P ₂	137.87 a	9.22 a	1.28 a	11.01 a	98.09 a
P ₄	137.87 a	8.71 b	1.03 a	11.49 a	96.07 a
5% LSD	43.03	0.37	0.39	2.84	6.93
<i>Harvest maturity (M)</i>					
Mi	137.80 a	8.31 b	1.77 a	6.51 c	82.10 b
Mp	161.33 a	8.86 ab	1.00 b	10.99 b	102.90 a
Mo	71.13 b	9.38 a	0.60 c	15.61 a	97.52 a
5% LSD	43.03	0.62	0.39	2.84	6.93

Note: Within each factor and column, mean values with the same letter are not significantly different at the $P < 0.05$ level according to the LSD test. Mi, before

physiological maturity; Mp, physiological maturity; Mo, after physiological maturity; TSS, total soluble solids; TA, titratable acidity.

Table 4. Interaction effects of keratin hydrolyzate peptide concentration and harvest maturity level on fruit juice content, total moisture content, and weight loss percentage after 21 days

Treatment	Juice content (%)	Total water content (%)	Weight loss (%)
P ₀ Mi	42.32 bc	88.15 ab	14.28 bc
P ₀ Mp	43.90 ab	86.54 bc	13.02 c
P ₀ Mo	39.93 bc	84.19 e	20.27 a
P ₂ Mi	38.12 c	86.52 bc	17.55 ab
P ₂ Mp	44.13 ab	88.72 a	14.24 bc
P ₂ Mo	47.43 a	84.69 de	16.81 ab
P ₄ Mi	41.86 bc	86.70 bc	14.08 bc
P ₄ Mp	43.10 b	86.79 bc	18.25 a
P ₄ Mo	41.58 bc	86.20 c	19.18 a

Note: Within a column, values followed by the same letter across all treatment combinations are not significantly different at the $P < 0.05$ level according to the DMRT test. Treatment codes represent combinations of peptide concentration (P₀, P₂, P₄) and maturity level at harvest (Mi, Mp, Mo).

DISCUSSION

Pre-Harvest Physiological Responses

Peptide concentration significantly affected relative leaf water content (RWC), chlorophyll content, and canopy light transmission, but had no significant effect on total sugars, P content, or K content in the leaves (Table 1). The P₂ treatment produced the highest RWC of 80.91% and the highest chlorophyll content of 65.30 SPAD, as well as the lowest light transmission to the lower canopy at 26.94%. All three peptide concentrations showed significant differences in these variables based on the results of the 5% BNT test. Increasing the peptide concentration from 2 to 4 mL L⁻¹ did not result in any further increase in RWC or chlorophyll content. These results indicate that, based on the measured variables, a peptide concentration of 2 mL L⁻¹ produced a more beneficial physiological response compared to concentrations of 0 and 4 mL L⁻¹. However, this response represents the primary effect of peptide concentration and does not indicate that the P₂Mp combination specifically caused an increase in RWC or chlorophyll content. Data on RWC, chlorophyll, and light transmittance for each peptide concentration are presented in Table 2 of the manuscript.

Biologically, the increase in RWC and chlorophyll content in the P₂ treatment may be related to the plant's physiological response to the application of protein hydrolysate, although this causal relationship was not directly tested in

this study (Colla et al., 2017). Protein hydrolysates are known to contain amino acids and peptides that can influence various physiological and metabolic processes in plants (Pasković et al., 2024). Therefore, the increases in RWC and chlorophyll content observed in this study should be interpreted as an empirical response to peptide concentration, rather than as direct evidence of a specific biochemical mechanism. The increase in SPAD values accompanied by a decrease in light transmission indicates differences in canopy characteristics under the P2 treatment. However, these results cannot be used to conclude an increase in photosynthetic rate because leaf area index and photosynthetic rate were not measured in this study. The limitations of these physiological measurements were also identified in the research manuscript.

When these physiological results are considered together with the measured fruit characteristics, the P2Mp combination can be viewed as a promising treatment because it exhibits a favorable combination of several observed traits; however, it cannot be declared a formally optimized treatment. Specifically, P2 produced favorable responses in RWC and chlorophyll content as the main effects of peptide concentration, whereas Mp exhibited favorable fruit quality characteristics as the main effects of maturity level. For variables showing a significant $P \times M$ interaction, the P2Mp combination produced the highest total fruit moisture content at 88.72%. Therefore, P2Mp is more appropriately described as a promising treatment combination based on a number of measured traits, rather than as the optimal treatment combination for all observed variables.



Figure 2. Appearance of the canopy of citrus plants treated with peptide treatments P0, P2, and P4

Visually, Figure 2 shows that the application of keratin hydrolyzate peptides elicits different responses in the plant leaves. Plants in the P0 (no peptide) treatment exhibited a sparse canopy with slightly yellowish leaves, whereas plants treated with P2 and P4 displayed a dense canopy with green leaves; however, in the " " plants treated with P4, the number of leaves was not as abundant as in those treated with P2.

Physicochemical Quality Of Fruit At Harvest

Peptide concentration had no significant effect on vitamin C content, TA, or the TSS:TA ratio, but it did have a significant effect on TSS and fruit weight (Table 3). Treatment P2 produced the highest TSS of 9.22 °Brix, which was significantly

different from P0 and P4, while fruit weight in P2 and P4 was not significantly different but was higher than the control in both cases. These results indicate that, based on the measured variables, a peptide concentration of 2 mL L⁻¹ produced a beneficial response in terms of TSS and fruit weight but did not affect all fruit quality characteristics (Table 3).

Biologically, the responses in TSS and fruit weight to peptide application can be attributed to the ability of protein hydrolysate-based biostimulants to influence crop yield and quality components, although these responses depend on the crop type and application conditions (Rodrigues et al., 2020). Therefore, the increase in TSS and fruit weight observed in P2 should be viewed as an empirical response to peptide concentration rather than as direct evidence of a specific physiological or biochemical mechanism. The effects of protein hydrolysates on fruit quality can indeed vary depending on concentration, plant species, and the application protocol used (Dlačić et al., 2026).

The harvest maturity stage had a significant effect on vitamin C content, TSS, TA, the TSS:TA ratio, and fruit weight. The highest vitamin C content was obtained at the physiological maturity (Mp) stage, namely 161.33 mg 100 g⁻¹, which was not significantly different from Mi but higher than Mo by 71.13 mg 100 g⁻¹. The decrease in vitamin C in fruits harvested after physiological maturity may be related to metabolic changes and the degradation of ascorbic acid during the development and ripening of citrus fruits (Magwaza et al., 2017). However, since the interaction between peptide concentration and ripeness level on vitamin C was not used as the basis for this conclusion, the high vitamin C content in Mp should be interpreted as a primary effect of ripeness level and not specifically attributed to the P2Mp combination.

TSS increased from 8.31 °Brix in Mi to 8.86 °Brix in Mp and 9.38 °Brix in Mo, although the difference between Mp and Mo of 0.52 °Brix was smaller than the BNT ripeness value of 0.62. Conversely, TA decreased from 1.77% at Mi to 1.00% at Mp and 0.60% at Mo, with all three maturity levels showing significant differences. The decrease in acidity, accompanied by changes in TSS, caused the TSS:TA ratio to increase from 6.51 at Mi to 10.99 at Mp and 15.61 at Mo. Changes in TSS and TA during ripening are characteristics commonly used to describe the development of quality and the ripeness level of citrus fruits (Hervalejo et al., 2021).

Based on the measured parameters, Mp exhibited a favorable combination of characteristics, including high vitamin C content, high fruit weight, moderate TSS, and lower acidity compared to Mi. In contrast, Mo exhibited the highest TSS:TA ratio but was accompanied by lower vitamin C content compared to the other ripeness stages. These findings indicate differences in fruit quality characteristics among ripeness stages but do not provide a basis for concluding that any single ripeness stage has been formally optimized.

When the main effects of peptide concentration and maturity level are considered together with variables showing a P × M interaction, P2Mp can be viewed as one of the promising treatment combinations because it exhibits a favorable combination of several measured traits. For variables that did show a significant P × M interaction, P2Mp produced the highest total fruit moisture

content of 88.72%. However, traits such as vitamin C which are primarily driven by the main effect of maturity level should not be attributed to a specific effect of P2Mp. Therefore, P2Mp is more appropriately described as a promising treatment based on a combination of several measured traits, rather than as the optimal treatment for all physiological and physicochemical traits of the fruit.

Interaction Between KHP Concentration and Harvest Maturity Level on Fruit Juice Yield, Fruit Moisture Content, and Weight Loss

The interaction between peptide concentration and maturity level ($P \times M$) had a significant effect on juice yield, total fruit moisture content, and weight loss accumulation on day 21 (Table 4). Since all three variables showed a significant $P \times M$ interaction, differences among treatment combinations can be specifically interpreted based on the results of the 5% DMRT test (Table 4).

The P2Mo combination yielded the highest juice yield of 47.43% and fell into the highest DMRT group. Meanwhile, P2Mp yielded a juice yield of 44.13% and the highest total fruit moisture content of 88.72%. These results indicate that the responses of juice yield and total moisture content to peptide concentration depend on the fruit's maturity level, as evidenced by the significant $P \times M$ interaction.

The high vitamin C content at the Mp maturity level should not be treated as a specific characteristic of the P2Mp combination because, in this study, vitamin C was driven by the main effect of maturity level, not by the interaction between the $P \times M$ treatment combinations. Similarly, the high fruit weight at the Mp maturity stage is a response to the maturity level and cannot be directly attributed to a specific P2Mp effect if the $P \times M$ interaction for fruit weight is not significant. Thus, vitamin C and fruit weight must be clearly distinguished from juice yield, total moisture content, and weight loss, which do exhibit a $P \times M$ interaction.

Regarding cumulative weight loss on day 21, P0Mp produced the lowest numerical value of 13.02%, while P2Mp showed a weight loss of 14.24%. Both combinations fell within overlapping DMRT groups, so the difference in their numerical values cannot be interpreted as a significant difference. Relatively higher weight loss values were found in P0Mo (20.27%), P4Mo (19.18%), and P4Mp (18.25%). Therefore, the weight loss results are more accurately explained as variations in response among peptide combinations and maturity levels during the 21-day observation period, without extending the interpretation to claims regarding the fruit's shelf life or marketability.

When all measured traits are considered collectively, P2Mp can be regarded as a promising treatment combination because it exhibits a favorable combination of several measured traits, particularly a juice yield of 44.13%, the highest total moisture content of 88.72%, and a weight loss on day 21 of 14.24%. However, P2Mp cannot be formally declared the optimal treatment, as not all observed variables showed superiority in this combination, and not all responses were supported by a significant $P \times M$ interaction.

Biologically, the high leaf RWC at the P2 concentration and the high total fruit water content in the P2Mp combination may indicate a relationship between plant water status and fruit water status. However, this relationship is interpretive, as water movement from the leaves to the fruit was not directly tracked in this

study. Therefore, the research data only support the existence of a consistent pattern between these two measurements and cannot yet prove the physiological mechanism that directly links them. Based on the overall results, H1 and H2 are partially supported because the effects of KHP and maturity level were significant only for some of the observed variables. H3 is also partially supported, as the KHP $\times$ maturity level interaction was found for juice yield, fruit water content, and weight loss, but not for all physicochemical characteristics

CONCLUSIONS AND RECOMMENDATIONS

Under these experimental conditions, the application of 2 mL L⁻¹ KHP produced favorable responses in several plant and fruit characteristics, particularly relative leaf water content, chlorophyll content, total dissolved solids, and fruit weight compared to the control. The harvest maturity level influenced vitamin C, TPT, AT, the TPT:AT ratio, and fruit weight. The KHP $\times$ maturity level interaction had a significant effect on juice yield, fruit moisture content, and weight loss on day 21, with the P2Mp combination yielding the highest fruit moisture content at 88.72%. Although P2Mp showed a promising combination of responses, this treatment cannot yet be declared as the optimal dose and harvest time for all observed traits. Multi-season and multi-location validation is required before it can be widely adopted as a cultivation recommendation.

FURTHER RESEARCH

Future research should validate the peptide concentration of 2 mL L⁻¹ and harvesting at physiological maturity across different seasons, orchards, and environmental conditions to test the consistency of the response. A narrower concentration range around 2 mL L⁻¹ also needs to be tested to obtain a more precise dose. Physiological observations can be expanded to include measurements of photosynthetic rates and leaf area index to elucidate the mechanisms underlying the plant's response to peptide application. Further postharvest evaluations should utilize respiration data at the replicate level and incorporate sensory acceptance parameters, decay rates, and microbiological aspects. Multi-season and multi-location trials are required before this treatment can be more widely recommended for Siamese orange cultivation.

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