



Effect of Pretreatment Methods using Citric Acid and Sodium Metabisulfite on Browning Prevention of Yacon (*Smallanthus sonchifolius*)

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Abstract

Yacon (*Smallanthus sonchifolius*) is a highly nutritious tuber rich in fructooligosaccharides (FOS), which possesses prebiotic properties and provides various health benefits. During processing, the freshly cut surface of yacon slices turns brown rapidly owing to the activity of polyphenol oxidase (PPO) and peroxidase (POD) enzymes. Therefore, controlling enzymatic browning is essential to preserve the desirable properties of yacon during processing. This research aimed to evaluate the effects of individual and combined treatments of anti-browning agents (citric acid and sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$)) on color stability and enzyme inhibition. After peeling and slicing, yacon slices were immersed in the prepared solutions at a solid-to-liquid ratio of 1:2 (w/v). Yacon tubers treated with the mixture of $\text{Na}_2\text{S}_2\text{O}_5$ (0.5%) and citric acid (0.15%) for 15 min showed the most effective pretreatment results. Under this condition, the PPO and POD activities decreased by 89% and 90%, respectively, and the browning index (BI) reached the lowest (30.75 ± 0.34). Moreover, the lightness (L^*) reached the highest (43.30 ± 0.16) compared to the other methods examined, indicating that the method retained the characteristic color of the fresh tubers. This study contributes to the development of appropriate preservation techniques to extend the shelf life of yacon and enhance its potential applications in further product processing.



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Abbreviations

AA	Ascorbic acid
AAE	Ascorbic Acid Equivalent
AOAC	Association of Official Analytical Chemists
BI	Browning index
CA	Citric acid
DNS	3,5-Dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
F-C	Folin-Ciocalteu
FOS	Fructooligosaccharides
GAE	Gallic acid equivalence
L*	Lightness
POD	Peroxidase
PPO	Polyphenoloxidase
SD	Standard deviation
SMS	Sodium metabisulfite
TPC	Total phenolic content

Introduction

Yacon (*Smallanthus sonchifolius*), native to the Andes, is currently found in many countries such as New Zealand, Japan, South Korea, and China.^{1,2} It has also gained increasing attention in Vietnam due to its outstanding health benefits. Yacon is widely cultivated in Bat Xat, Lao Cai province of Vietnam, and is considered a specialty of this mountainous region.

Yacon tubers contain high levels of fructooligosaccharides (FOS),² which promotes the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. in the gut, hence improving digestion and the immune system.³⁻⁶ Additionally, yacon tuber contains inulin and powerful antioxidants, mainly flavonoids and phenolic compounds,⁶ which contribute to its anti-cancer, antioxidant, antidiabetic, and weight management properties.^{4,7-10} Several research studies^{9,11,12} have reported that yacon extract can inhibit cancer cell growth and support colon cancer prevention. Yacon has also shown antidiabetic effects by regulating blood glucose levels and improving insulin resistance.^{8,10,13} Furthermore, studies on humans indicate that consuming yacon powder helps to reductions in weight, body fat, and waist circumference.⁷

Despite their numerous benefits, yacon tubers have not been processed efficiently. One of the main reasons is their rapid oxidation, which leads

to discoloration, reduces aesthetics, and negatively impacts the quality of processed products. During processing, yacon turns brown quickly after peeling, primarily due to the activity of polyphenol oxidase (PPO) and peroxidase (POD). These enzymes catalyze the oxidation of phenolic compounds, resulting in darkening on the fruit slice's surface.¹⁴⁻¹⁷

In plant cells, polyphenol oxidase (EC 1.10.3.1) is found in plastids, while phenolic substances are found in the vacuoles. The enzymatic browning reaction is initiated when PPO and phenolic compounds come into contact at damaged cell areas.¹⁸ PPO catalyzes the oxidation of o-diphenols to o-quinones and the o-hydroxylation of monophenols to o-diphenols when molecular oxygen is present. Quinones are highly reactive, electrophilic compounds that can polymerize with phenols or other quinones to generate pigments that are either black or brown.¹⁹ Consequently, controlling enzymatic browning is important in processing to fully utilize the potential of yacon.

One of the conventional methods to prevent enzymatic browning is the use of chemical inhibitor agents such as citric acid and sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$). The inhibitory effect of these substances on fruits and vegetables has been reported in previous studies.^{17,20,21} Among the acids, citric acid is widely used to prevent enzymatic browning of various food products owing to its affordable,

ease of use and wide range of applications. Citric acid inhibits enzymatic browning by reducing the pH and forming complexes with metal ions at the active sites of PPO.¹⁹ Meanwhile, sulfites are also economical and efficient anti-browning agents. Sodium metabisulfite prevents browning by reacting with quinones and inhibiting their polymerization into brown pigments.¹⁵ Although sulfites are highly effective and cost-efficient, concerns about their allergic reactions in some individuals, particularly in asthmatic patients, may arise.¹⁹ Therefore, it is essential to study the use of this substance at low concentrations to competently preserve the products while minimizing potential risks.

The study aims to support future processing of yacon by assessing pretreatment methods to prevent enzymatic browning. For this purpose, physico-chemical properties of raw yacon tubers were determined, followed by the evaluation of anti-browning effects using citric acid and sodium metabisulfite, individually and in combination.

Materials and Methods

Materials

Yacon tubers, grown for a period of 6 to 8 months, were collected in late September from Bat Xat, Lao Cai, Vietnam. After harvesting, the yacon tubers were promptly transported to the laboratory within 24 hours to ensure the freshness of the raw material.

Sample Preparation

Yacon tubers were washed, peeled and sliced to a thickness of 1 ± 0.05 cm before determining optimal pretreating parameters (concentration, time) of chemical agents, individually and in combination. These parameters were identified based on the analysis of color and PPO, POD activity.

Yacon tubers were washed, peeled, and then pressed using a Sharp KS-888 screw press to collect the juice and separate the pulp. Juice samples were used to analyze some physical and chemical parameters.

Identification of Each Agent's Optimal Concentration and Processing Time

The concentration and immersion time of the agents can be considered as critical points in the processing, since very high concentrations and

prolonged immersion can lead to significant losses of FOS due to the hydrolysis of this carbohydrate into mono- and disaccharides, such as glucose, fructose and sucrose.²² Therefore, the study conducted the following experimental procedures:

Yacon slices were individually immersed in different concentrations of chemical solutions: citric acid (0%, 0.1%, 0.15%, 0.2%, 0.25%, 0.3%) and $\text{Na}_2\text{S}_2\text{O}_5$ (0%, 0.1%, 0.3%, 0.5%, 0.7%) in the ratio of 1:2 (w/v) for 20 min to determine the most effective treatment concentration of each chemical agent. At the identified optimal concentration of each agent, yacon slices were immersed in different durations (10, 20, 30 min) before conducting analysis of color and enzyme activity.

Identification of the optimal mixed agent composition These two chemicals were mixed at different ratios and then assessed for their efficiency on color stability, enzyme activity, and immersed duration as above. The optimal concentration of citric acid was fixed while gradually reducing the concentration of $\text{Na}_2\text{S}_2\text{O}_5$ (0.15% citric acid + 0.5% $\text{Na}_2\text{S}_2\text{O}_5$; 0.15% citric acid + 0.3% $\text{Na}_2\text{S}_2\text{O}_5$; 0.15% citric acid + 0.1% $\text{Na}_2\text{S}_2\text{O}_5$; 0.15% citric acid + 0% $\text{Na}_2\text{S}_2\text{O}_5$) for 20 min to determine the optimal concentration of $\text{Na}_2\text{S}_2\text{O}_5$. At the optimal ratio, yacon slices were immersed in different durations before conducting an analysis of color and enzyme activity. The immersion durations depended on the optimal time of each agent, as previously demonstrated. The shorter durations enhance the meaning of this study in practical applications.

Analytical Methods

Determination of Weight, Length and Diameter

A sample of 5 tubers was randomly selected and measured using a vernier caliper, with the average length and diameter recorded in centimeters. The weight of the tubers was determined using an electronic balance and expressed in grams.

Moisture Content

Moisture content was measured using the techniques outlined in AOAC (2010).²³ It was calculated based on the difference between the initial and final dry mass, according to the formula:

$$\text{Moisture (\%)} = [(W1 - W2)/W1] \times 100$$

where W1 is weight (g) of sample before drying, W2 is weight (g) of sample after drying.

Total Sugars and Reducing Sugars

The Miller method was used to quantify the reducing sugars.²⁴ The reaction mix, consisting of 0.3 mL of DNS reagent and 0.5 mL of the diluted sample, was heated for 5 min at 100°C and then rapidly cooled. The value of absorbance at 540 nm was measured by a UV-vis spectrophotometer (PD-3000UV, Apel Co., Japan). The standard curve used glucose (0.1–1 g/L). Determine total sugars similarly after hydrolyzing the juice sample with 2% HCl for 45 min at 100°C, followed by neutralizing the solution with 10% NaOH.

Total Phenolic Content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu (F-C) method²⁵ with a modification. A test tube was prepared including 1 mL of F-C reagent and 0.2 mL of juice sample. After vortexing and incubating for three min, 0.8 mL of 7.5% Na₂CO₃ was added, and the mixture was reacted for a further hour at ambient temperature without light. A UV-vis spectrophotometer (PD-3000UV, Apel Co., Japan) was used to measure the absorbance of the solution at 765 nm. To produce the standard curve, gallic acid was utilized at concentrations between 25 and 125 mg/L.

Antioxidant Activity and Scavenging

The scavenging activity was evaluated using the DPPH method.²⁶ After the sample and the DPPH solution mixture reacted for one hour in the dark, the absorbance was measured at 517 nm by a UV-vis spectrophotometer. The scavenging percentage (%SP) was calculated as follows:

$$\%SP = [(Abl-Asa)/Abl] \times 100$$

where Abl represents the absorbance of the blank, and Asa denotes the absorbance of the sample.

The antioxidant capacity, expressed as IC₅₀, was determined from the scavenging percentage and a standard curve constructed with vitamin C at the point where y = 50%.

The Quantity of Vitamin C

Iodometric titration was used to measure the quantity of vitamin C, based on the principle of

oxidation-reduction reaction between ascorbic acid and iodine.²⁷ Starch indicator solution was prepared by dissolving 0.5 g of starch in 100 mL of boiling water and letting it cool. Dissolving 2.50 g of KI and 0.134 g of KIO₃ in 100 mL of purified water, 15 mL of 3M H₃SO₄ was added to create a 0.01N iodine solution. The solution was then made up to 250 mL. Standardization was performed using 5 mL of 0.5% ascorbic acid along with 3 drops of starch indicator. The same was done with a 5 mL juice sample. The content of vitamin C was calculated as follows:

$$\text{Vitamin C} = (V_1/V_2) \times C_0 \times (100/W_s) \text{ (mg/100g)}$$

where V₁ is volume (mL) of iodine used for the sample, V₂ (mL) is volume (mL) of iodine used for the standard, and C₀ is concentration of the standard ascorbic acid (mg/mL), W_s is fresh weight equivalent to the 5 mL juice sample used for titration.

Total Acidity

The AOAC 942.15 method was used to determine titratable acidity.²⁸ For each analysis, 5 mL of the juice sample was mixed with 20 mL of distilled water. The mixture was titrated with 0.1 N NaOH using 3 drops of 1% phenolphthalein solution prepared in ethanol (60%) as an indicator. Titration was completed when a pale pink appeared and remained visible for around 30 seconds. The total titratable acidity was expressed as % citric acid equivalent.

Evaluation of the PPO and POD Activities

Enzyme extract of yacon was obtained by homogenizing 10 g of tubers with 20 mL of 0.05 M phosphate buffer at pH = 7 for 2 min. The mixture was then centrifuged for 13 min at 3500 rpm. The supernatant obtained was the crude enzymatic extract.

Determination of PPO activity: According to Rodrigues ORL, Asquieri ER and Orsi DC,¹⁹ the PPO activity was determined by measuring the increase in absorbance at 420 nm at 25°C using a UV-Vis spectrophotometer. The substrate solution included 0.01 M catechol in 0.1 M phosphate buffer, pH = 7.0. Prepare the reaction sample containing 0.1 mL of enzyme extract and 2.9 mL of substrate solution. The blank sample included 0.1 mL of phosphate buffer and 2.9 mL of substrate solution. In a 30°C water bath, the tube containing the reaction sample was reacted for 5 min. The absorbance was monitored

for 5 min at 420 nm, recording the results once every minute. The quantity of enzyme that increased absorbance by 0.001 per minute per milliliter was considered one unit of enzyme activity.¹⁹

Determination of POD activity: Based on the test according to the method of Nagle and Hard,²⁹ guaiacol is used as the substrate. Prepare a 2.5 mL reaction sample consisting of: 0.05 mL guaiacol 0.1%, 2.3 mL sodium acetate buffer (pH=6.0), 0.05 mL H₂O₂ 0.1% and 0.1 mL enzyme extract. The blank sample consisted of 0.05 mL of 0.1% guaiacol, 2.3 mL of sodium acetate buffer (pH=6.0), 0.05 mL of 0.1% H₂O₂. The tube containing the reaction sample was reacted for 5 min in a 30°C water bath, and the absorbance was measured at 470 nm. POD activity unit was defined as the same as the PPO activity unit.

Color Measurement

The surface color of the yacon slice was randomly determined at 3 different points. The color change

was measured in the CIE-Lab color system with the three-axis L*, a*, b*. In which, the L* axis represented the lightness with values from black (0) to white (100), the a* axis represented the chroma from green (-120) to red (+120), the b* axis also represented the chroma from blue (-120) to yellow (+120).³⁰

To determine the level of browning during processing, the L*, a*, b* values were used to calculate the browning index according to the following equation:³⁰

$$BI = (100 \cdot (x - 0,31)) / 0,172, \text{ while } x = (a^* + 1,75 \cdot L^*) / (5,645 \cdot L^* + a^* - 3,012 \cdot b^*)$$

Statistical Analysis

All experiments were performed in five repeated measurements. The data were presented as mean ± standard deviation (SD) and as percentage relative activity. One-way ANOVA was used to determine statistically significant differences with SNK test ($p < 0.05$) by SPSS Statistics 20.

Table 1: Physico-chemical characteristics of raw yacon tubers

Physical characteristics	Value
Length (cm)	20.6 ± 3.6
Diameter (cm)	7.4 ± 0.8
Weight (g)	465 ± 10.77
Moisture (%)	87.43 ± 1.02
Chemical characteristics	Value
Reducing sugars (%)	3.79 ± 0.02
Total sugars (%)	6.67 ± 0.19
Total phenolic content (mg GAE/100 g)	30.67 ± 0.88
Vitamin C (mg/100 g)	2.30 ± 0.14
Titrateable acidity (% citric acid equivalent)	0.063 ± 0.001
Scavenging activity (mg AAE/100 g)	1475.67 ± 8.65

Results

Chemical and Physical Characteristics of Raw Yacon Tubers

The physical evaluation of raw yacon tubers showed an average length, diameter, and weight of tuber as 20.6 ± 3.6 cm, 7.4 ± 0.8 cm, and 465 ± 107.65 g, respectively (Table 1). The tubers had a high moisture content of 87.43 ± 1.02% and a low titrateable acidity of 0.063 ± 0.001%. The reducing sugars and total sugars were 3.79 ± 0.02% and 6.67 ± 0.19%, respectively. The ascorbic acid content was

2.30 ± 0.14 mg/100 g. The total phenolic content averaged 30.67 ± 0.88 mg GAE/100 g, and the DPPH radical scavenging activity was 1475.67 ± 8.65 mg AAE/100 g.

Effect of Citric Acid Concentration and Immersion Time on The Enzyme Activities and The Discoloration of Yacon Tubers

Figure 1 illustrates the activities of PPO and POD during treatment with citric acid at different concentrations and immersion times. POD activity

tended to remain lower than PPO activity under all treatment conditions.

Figure 1A demonstrated that the initial PPO and POD activities of raw yacon were 22.00 ± 1.41 U/mL/min and 18.80 ± 1.09 U/mL/min, respectively. After treatment, the activities of both enzymes decreased significantly. At 0.15% citric acid, the enzyme activities decreased to the lowest of 7.60 ± 0.89 U/mL/min for PPO activity and 7.20 ± 1.09

U/mL/min for POD activity, corresponding to a 65.45% and 61.70% decrease in PPO, POD activity compared to the sample treated with 0% citric acid. When the citric acid concentration further exceeded 0.15%, PPO activities tended to slightly increase while no significant differences in POD activities were observed. This suggested that 0.15% citric acid is the optimal concentration for impeding the enzyme activities.

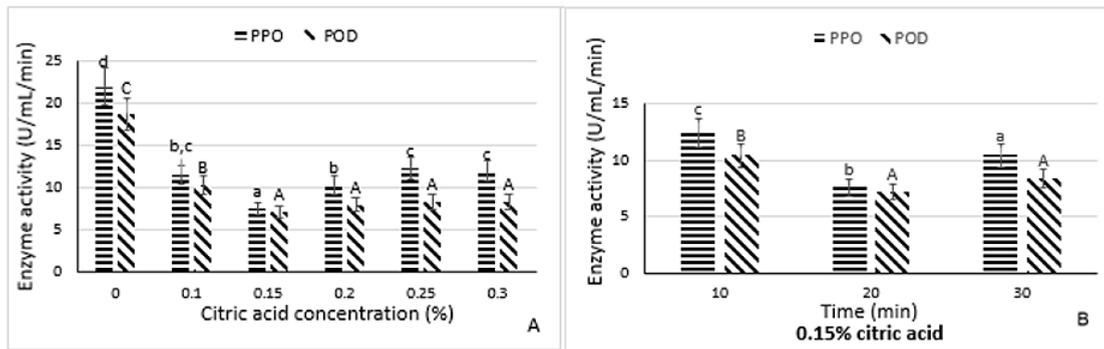


Fig.1: PPO and POD activities influenced by the citric acid concentration (A) and immersion time (B)

At this concentration, the immersion time was studied. Figure 1B showed that enzyme activities reached their minimum values at 20 min, followed by a slight increase with longer immersion time. Specifically, at 30 min, the PPO and POD activities were 10.4 ± 0.89 U/mL/min and 8.4 ± 0.89 U/mL/min, respectively, representing increases of 26.92% and 14.29% compared to the sample treated for 20 min. Therefore, treatment with 0.15% citric acid for 20 min was identified as the optimal condition for reducing the enzyme activities.

The color parameters under varying citric acid concentration and immersion times were presented in Table 2. The results indicated that untreated yacon slices (0% citric acid) browned quickly with the highest BI value (43.21 ± 0.34). As the citric acid concentration increased, the L^* value also increased, indicating higher lightness, while the BI value decreased, suggesting effective inhibition of browning. In addition, the increase in the positive b^* value indicated a brighter yellow color. Notably, the sample treated with 0.15% citric acid showed the lowest BI value (34.64 ± 0.18) and the highest

L^* value, indicating it as the optimal concentration. The evaluation of immersion times (10, 20, and 30 min) at the chosen concentration showed that 20 min was the most effective duration with the highest L^* and the lowest BI value, with significant differences compared to other immersion times. At 30 min, there was a slight decline in L^* value and a minor increase in BI value; however, the differences were not substantial. Therefore, treatment with 0.15% citric acid for 20 min was identified as the most effective method to preserve the original color of yacon slices.

Effect of sodium metabisulfite concentration and immersion time on the enzyme activities and the discoloration of yacon tubers

In the control sample (0% $\text{Na}_2\text{S}_2\text{O}_5$), POD activity (18.80 ± 1.09 U/mL/min) was significantly lower than PPO activity (22.00 ± 1.41 U/mL/min). This trend persisted across all treatments, with POD consistently exhibiting a lower activity than PPO when the $\text{Na}_2\text{S}_2\text{O}_5$ concentration increased. At the concentration of 0.1 and 0.5% $\text{Na}_2\text{S}_2\text{O}_5$, the PPO and POD enzyme activity was relatively similar.

Table 2: Color of yacon tubers influenced by the citric acid concentration and immersion time

Sample	L*	a*	b*	BI
Citric acid (0%)	28.09 ± 0.13 ^a	-0.91 ± 0.07 ^a	10.66 ± 0.02 ^a	43.21 ± 0.34 ^e
Citric acid (0.1%)	35.90 ± 0.09 ^a	-0.23 ± 0.03 ^a	11.55 ± 0.04 ^b	36.83 ± 0.18 ^d
Citric acid (0.15%)	37.08 ± 0.08 ^e	-0.26 ± 0.03 ^a	11.35 ± 0.03 ^a	34.64 ± 0.18 ^a
Citric acid (0.2%)	36.60 ± 0.02 ^d	-0.25 ± 0.02 ^a	11.53 ± 0.02 ^b	35.91 ± 0.10 ^b
Citric acid (0.25%)	36.07 ± 0.07 ^b	-0.29 ± 0.03 ^a	11.50 ± 0.04 ^b	36.31 ± 0.18 ^c
Citric acid (0.3%)	36.21 ± 0.01 ^c	-0.24 ± 0.04 ^a	11.50 ± 0.01 ^b	36.27 ± 0.11 ^c
At 0.15% citric acid				

Time (min)	L*	a*	b*	BI
10	35.82 ± 0.03 ^b	-0.22 ± 0.03 ^a	11.54 ± 0.07 ^b	36.92 ± 0.24 ^c
20	37.08 ± 0.08 ^a	-0.26 ± 0.03 ^a	11.35 ± 0.03 ^a	34.64 ± 0.18 ^a
30	36.55 ± 0.03 ^b	-0.25 ± 0.02 ^a	11.48 ± 0.03 ^b	35.78 ± 0.08 ^b

Within a single column, various superscript letters show significant differences ($p < 0.05$).

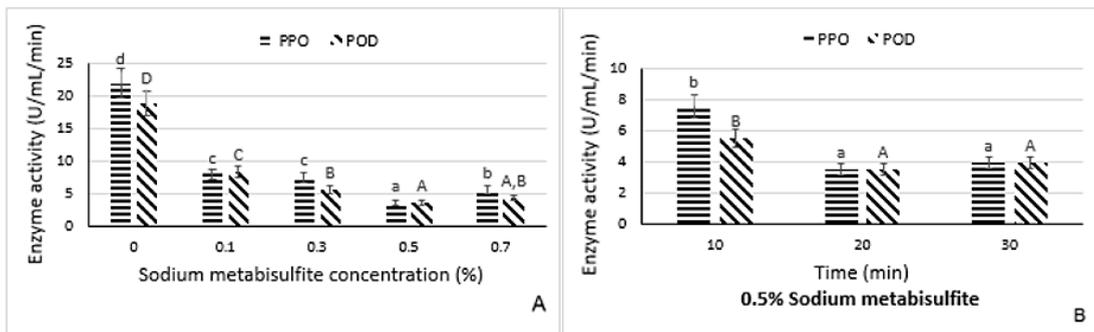


Fig. 2: PPO and POD activities influenced by the sodium metabisulfite concentration (A) and immersion time (B). Significant differences in values were indicated by different lowercase and uppercase letters ($p < 0.05$).

In general, the treated samples showed a significant reduction in both enzyme activities with the increase of $\text{Na}_2\text{S}_2\text{O}_5$ concentration (Figure 2A). Notably, at 0.5% $\text{Na}_2\text{S}_2\text{O}_5$, the activities of the PPO and POD decreased remarkably to 3.6 ± 0.89 U/mL/min. These results were statistically significant in comparison to other concentrations. However, when the concentration was further raised to 0.7%, PPO activity tended to increase slightly (5.6 ± 0.89 U/mL/min), suggesting potential limitations in enzyme inhibition at higher concentrations.

The results of Figure 2B indicated that immersion time also affected the enzyme inhibition efficiency in

the tubers. The longer the immersion time resulted in greater the reduction in the PPO activity. In the sample treated with 0.5% $\text{Na}_2\text{S}_2\text{O}_5$ for 10 min, the activities of PPO and POD were 7.60 ± 0.89 U/mL/min and 5.60 ± 0.89 U/mL/min, respectively. Increasing the immersion time to 20 min, the treatment efficiency was recorded to nearly double, specifically PPO and POD activity were both 3.60 ± 0.89 U/mL/min. However, from 20 min onwards, the enzyme inhibition efficiency was not significantly different. The findings indicated that immersion in a 0.5% $\text{Na}_2\text{S}_2\text{O}_5$ solution for 20 min was the optimal condition.

The color parameters recorded after 20 min of Na₂S₂O₅ immersion are presented in Table 3. The

data showed that treatment by Na₂S₂O₅ can retain the color characteristic of the raw yacon slice.

Table 3: Color of yacon tubers influenced by the sodium metabisulfite concentration and immersion time

Samples	L*	a*	b*	BI
Na ₂ S ₂ O ₅ (0%)	28.09 ± 0.13 ^a	-0.91 ± 0.07 ^a	10.66 ± 0.02 ^a	43.21 ± 0.34 ^d
Na ₂ S ₂ O ₅ (0.1%)	37.52 ± 0.03 ^b	-0.56 ± 0.06 ^b	11.69 ± 0.07 ^b	34.78 ± 0.29 ^c
Na ₂ S ₂ O ₅ (0.3%)	38.10 ± 0.08 ^c	-0.46 ± 0.03 ^c	11.70 ± 0.01 ^b	34.38 ± 0.07 ^b
Na ₂ S ₂ O ₅ (0.5%)	39.41 ± 0.04 ^e	-0.39 ± 0.05 ^c	11.81 ± 0.03 ^c	33.54 ± 0.01 ^a
Na ₂ S ₂ O ₅ (0.7%)	39.05 ± 0.07 ^d	-0.44 ± 0.06 ^c	11.80 ± 0.03 ^c	33.79 ± 0.26 ^a

At 0.5% Na ₂ S ₂ O ₅				
Time (min)	L*	a*	b*	BI
10	38.55 ± 0.04 ^a	-0.4 ± 0.11 ^a	11.78 ± 0.03 ^a	34.32 ± 0.29 ^b
20	39.41 ± 0.04 ^c	-0.39 ± 0.05 ^a	11.81 ± 0.03 ^a	33.54 ± 0.01 ^a
30	39.18 ± 0.11 ^b	-0.44 ± 0.03 ^a	11.82 ± 0.04 ^a	33.71 ± 0.26 ^a

Within a single column, various superscript letters show significant differences ($p < 0.05$).

All samples showed an increase in L* value compared to the control sample and a decrease in the BI as the Na₂S₂O₅ treatment concentration increased (Table 3). The sample treated with 0.5% Na₂S₂O₅ recorded the highest value for L* (39.41 ± 0.04) and the lowest BI (33.54 ± 0.01). No significant differences were obtained in the 0.7% sample for L* (39.05 ± 0.07) and the BI (33.79 ± 0.26) compared with 0.5%. Similarly, through the investigation of treatment time at a concentration of 0.5% Na₂S₂O₅, increasing the immersion time from 10 to 20 min improved the

color values. Yet, statistical analysis showed that no further enhancement was noted between the 20- and 30-minute immersion. Therefore, based on both enzymatic activity and color retention, the method of immersion in 0.5% Na₂S₂O₅ for 20 min gave the best result in maintaining the color and limiting the enzyme activity of the tubers.

Effect of citric acid in combination with sodium metabisulfite and immersion time on the enzyme activities and the discoloration of yacon tubers

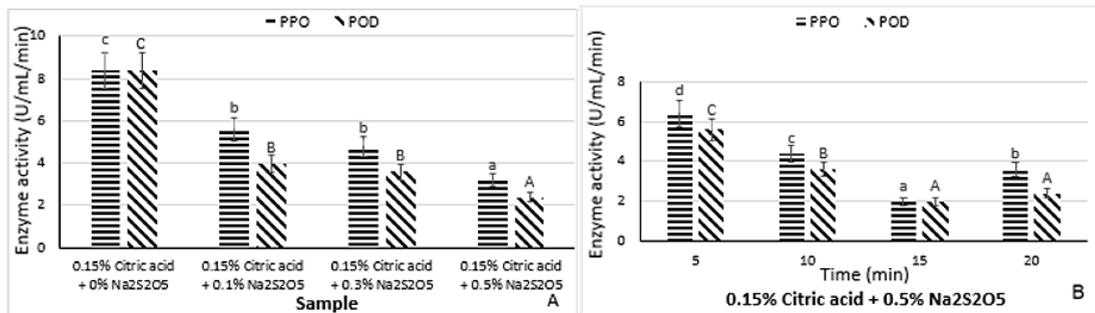


Fig. 3: PPO and POD activities influenced by the concentration of citric acid and sodium metabisulfite (A) and immersion time (B). Significant differences in values were indicated by different lowercase and uppercase letters ($p < 0.05$)

To investigate the synergistic effect of sodium metabisulfite and citric acid, the optimal concentration of citric acid (0.15%) while gradually reducing the concentration of Na₂S₂O₅ (0.5%, 0.3%, 0.1%, 0%). Once the optimal concentration of Na₂S₂O₅ based on the enzyme activity inhibition was determined, further experiments was conducted to identify the ideal immersed times (5, 10, 15, 20 min) for processing yacon tubers.

The results of Figure 3 demonstrated that enzyme activities declined gradually when the concentration of Na₂S₂O₅ increased. The combination of 0.5% Na₂S₂O₅ and 0.15% citric acid was identified as the most effective treatment ratio. At this concentration, the enzyme activities decreased to the lowest of 3.20 ± 1.10 U/mL/min for PPO activity and 2.40 ± 0.89 U/mL/min for POD activity. In comparison to the individual treatments of citric acid (Figure 1A) and Na₂S₂O₅ (Figure 2A), using combinations of chemical agents brought superior inhibitory effects. PPO and POD activities were reduced by 5.60 ±

0.89 and 4.00 ± 0.00 U/mL/min, respectively, at 0.1% Na₂S₂O₅ concentration; and enzyme activities were reduced by 4.80 ± 1.10 and 3.60 ± 0.89 U/mL/min, respectively, at 0.3%Na₂S₂O₅ concentration.

Subsequently, at the optimal chemical combination (0.5% Na₂S₂O₅ + 0.15% citric acid), different immersion durations were investigated. As illustrated in Figure 3B, 15-min immersion was the most effective time to inhibit the PPO and POD enzyme activity, representing a relatively similar value, at 2.00 ± 0.00 U/mL/min. They were reduced by roughly 90% compared to the control sample. The enzyme activities decreased to 6.40 ± 0.89 in PPO and 5.60 ± 0.89 U/mL/min in POD at 5 min, followed by a further reduction to 4.40 ± 0.89 in PPO and 3.60 ± 0.89 U/mL/min in POD at 10 min. At 20 min, the enzyme activities reached 3.20 ± 1.10 and 2.40 ± 0.89 U/mL/min, in terms of PPO and POD, respectively. Across all treatments, POD activity tended to remain consistently lower than PPO activity.

Table 4: Color of yacon tubers influenced by the concentration of citric acid and sodium metabisulfite and immersion time

Samples	L*	a*	b*	BI
Citric acid (0.15%) + Na ₂ S ₂ O ₅ (0%)	37.08 ± 0.08 ^a	-0.26 ± 0.03 ^c	11.35 ± 0.03 ^a	34.64 ± 0.18 ^c
Citric acid (0.15%) + Na ₂ S ₂ O ₅ (0.1%)	39.04 ± 0.13 ^a	-0.42 ± 0.07 ^{ab}	11.78 ± 0.03 ^a	33.75 ± 0.22 ^b
Citric acid (0.15%) + Na ₂ S ₂ O ₅ (0.3%)	39.37 ± 0.13 ^b	-0.37 ± 0.03 ^b	11.81 ± 0.03 ^a	33.63 ± 0.24 ^b
Citric acid (0.15%) + Na ₂ S ₂ O ₅ (0.5%)	41.16 ± 0.11 ^a	-0.46 ± 0.02 ^a	11.91 ± 0.03 ^b	32.04 ± 0.18 ^a

At 0.15% citric acid and 0.5% Na ₂ S ₂ O ₅				
Time (min)	L*	a*	b*	BI
15	38.34 ± 0.09 ^a	-0.81 ± 0.06 ^a	11.90 ± 0.14 ^a	34.11 ± 0.58 ^d
10	40.23 ± 0.05 ^b	-0.36 ± 0.03 ^b	11.96 ± 0.03 ^{ab}	33.28 ± 0.14 ^c
15	43.30 ± 0.16 ^d	-0.42 ± 0.02 ^b	12.07 ± 0.09 ^b	30.75 ± 0.34 ^a
20	41.16 ± 0.11 ^c	-0.46 ± 0.02 ^b	11.91 ± 0.03 ^a	32.04 ± 0.18 ^b

Within a single column, various superscript letters show significant differences (p < 0.05).

Corresponding color attributes, compared with the sample treated with 0.15% citric acid for 20 min, the b* value of the combined chemical treatment showed a tendency to gradually enhance the yellow color of yacon slices. Conversely, the L* value was higher and the BI value was lower, indicating the

combined treatment samples were lighter and less brown in color.

As summarized in Table 4, it can be seen that the color of yacon slices was moderately impacted by the immersion time. The optimal time was 15 min,

producing the most favorable color outcomes, with the highest L* value (43.30 ± 0.16) and the lowest BI value (30.75 ± 0.34). This result was consistent with the enzyme activities in Figure 3B. These indicators showed a considerable improvement in comparison with the sample treated with 0.15% citric acid only.

Discussion

Chemical and Physical characteristics of Raw Yacon Tubers

The physico-chemical characteristics and antioxidant properties of raw yacon tubers are shown in Table 1. Regarding the physical properties analysis, the yacon tubers' average dimensions of length, diameter, and weight recorded in this study were notably higher than the results for yacon cultivated in Nauni, Solan, India (as 12.15 ± 1.38 cm, 3.39 ± 0.53 cm, 131.2 ± 26.83 g, respectively).²¹ Comparing the moisture attribute of raw yacon tubers, a significantly higher moisture content was observed in this study (87.43%) than the findings of Lago CC²⁰ and Gangta R²¹ (corresponding to 81.70 and 83.33%). Similarly, the total titratable acidity of yacon was slightly higher, with 0.063% citric acid equivalent, as opposed to those obtained by Prati P³¹ (0.05%). These minor discrepancies may be attributed to the plants' age, cultivation altitude, harvesting time, environmental conditions, and soil moisture at the growing site. Meanwhile, the values of vitamin C (2.30 mg/100g), reducing sugar (3.79%), and total sugar content (6.67%) in this research is generally consistent with the previous published studies.^{3,6,21,31} According to Gangta R²¹ and Prati P;³¹ the yacon tubers presented a relatively similar vitamin C value (2.20 mg/100g) and reducing sugar content (3.94%) with a mildly higher total sugar content (7.86%). The literature has highlighted that yacon's elevated moisture content contributed to its perishability, while its significant levels of phenolics and sugars, combined with low acidity, enhanced its susceptibility to enzymatic browning processes.

The chemical analysis indicated that yacon tubers had a TPC of 30.67 ± 0.88 mg GAE/100 g and a DPPH free radical scavenging capacity of 1475.67 ± 8.65 mg AAE/100 g. These values demonstrated that yacon was a rich source of natural antioxidants, with levels comparable to or exceeding those found in several commonly consumed fruits. Specifically, TPC in apples ranged from about 377 to 839 mg GAE/100 g fresh, pears from 6.4 - 17.7 mg GAE/100

g, and ripe bananas from 42 - 54 mg GAE/100 g, depending on the variety.³²⁻³⁵

In addition, the relatively high total sugar and reducing sugar content ($6.67 \pm 0.19\%$ and $3.79 \pm 0.02\%$) also reflected the potential for outstanding biological value. In particular, total sugar included a substantial number of FOS, a functional carbohydrate with prebiotic effects. Meanwhile, reducing sugar, primarily glucose and fructose, contributed to a natural sweetness, was easy to absorb, and was favorable for fermentation-based applications. The characteristics of yacon have emphasized its potential, which is not only limited to a prebiotic source but also has high antioxidant properties and natural sweetness, supporting its application in the development of nutritious functional food products, such as syrup, fermented drinks, or jam.^{36,37}

Effect of Citric Acid Concentration and Immersion Time on the Enzyme Activities and The Discoloration of Yacon Tubers

According to Figure 1, it is observed that POD activity was consistently lower than PPO activity in all treatments, which aligns with the role of PPO as the primary enzyme responsible for the browning process. PPO catalyzed phenolic compounds in the presence of oxygen through the o-hydroxylation reaction to form o-quinone, which had a distinctive brown color. POD also contributed to browning; however, its activity depends on the presence of hydrogen peroxide (H_2O_2), an essential substrate for POD oxidizes phenols to quinones. Notably, some studies suggest that these enzymes may function synergistically. While PPO directly oxidizes phenolic compounds using readily available oxygen in the tuber tissue, especially after mechanical damage, POD requires H_2O_2 , by-products of the PPO-catalyzed oxidation reactions. This functional relationship helps explain the consistently higher PPO activity compared to POD observed in this study and may accelerate browning reactions, leading to more pronounced browning effects.³⁸

With individual treatments, citric acid was used to form complexes with metal cations (copper) presented in the active sites of PPO, POD and maintain the pH ($pH < 3$) below the optimal value for the catalytic activity of these enzymes.^{19,39} The treatment using 0.15% citric acid for 20 min was demonstrated as the most effective method to

retain the original color of yacon tubers. This result is consistent with those found by Gangta R,²¹ which showed that yacon powder treated with 0.15% citric acid for 20 min retained the best color and flavor characteristics.

When the citric acid concentration and immersion time were further increased beyond 0.15% and 20 min, respectively, cellular damage may occur, which could result in a slight increase of enzyme activities and partial offset of browning inhibitory. During shorter treatment (in the range of 0 to 20 min), citric acid primarily acted on the cell surface and the outer cell layers, lowering the surface pH and inhibiting PPO, POD activity. However, with prolonged exposure, citric acid can penetrate deeper into the cells, reducing the intracellular pH, denaturing membrane proteins and causing disruption of the cell membrane and vacuolar membrane.⁴⁰ Consequently, PPO enzymes in plastids may be released, allowing direct contact with phenolic substrates in the vacuoles, leading to increased enzyme activity and browning index.

Effect of Sodium Metabisulfite Concentration and Immersion Time on the Enzyme Activities and the Discoloration of Yacon Tubers

According to Rodrigues ORL, Asquiere ER and Orsi DC,¹⁹ the color change in yacon tubers was related to the activity of endogenous polyphenol oxidase and peroxidase enzymes and the content of phenolic compounds. As illustrated in Figure 2 and Table 3, a clear correlation was observed between the browning index and PPO and POD enzyme activity. Specifically, the higher the PPO activity, the greater the increase in BI value. This supported the mechanism in PPO and POD catalyzed the oxidation of phenolic substrates to unstable quinones, which then polymerized into brown or black pigments. The accumulation of these pigments results in an increasing value in browning intensity. The more of these pigments formed, the higher the browning index was measured.⁴¹

The findings of this study, presented in Figure 2 has demonstrated that treating yacon tuber slices with 0.5% Na₂S₂O₅ for 20 min effectively inhibited enzymatic browning. This was evidenced by the significant reduction in the activities of PPO and POD by 84% and 81% compared to the control, respectively. Concurrently, the 0.5% Na₂S₂O₅

treated slices exhibited the highest L* value and lowest BI value, as shown in Table 3, indicating the optimal treatment. This is consistent with the study of Krishnan JG and Padmaja G16 where the 0.5% concentration of Na₂S₂O₅ treatment on yam flour has significantly enhanced brightness and reduced browning index, compared to the 0.25 and 1.00% concentration.

The enzymatic browning inhibition was explained by Na₂S₂O₅'s strong antioxidant properties. When dissolved in water, Na₂S₂O₅ generates sulfurous acid (H₂SO₃) and sulfite ions (SO₃²⁻). While H₂SO₃ reduced the pH value below the optimal range for the catalytic activity of PPO and POD enzymes,¹⁹ sulfite ions acted as a reducing agent, preventing the enzymatic oxidation of phenolic compounds. In more specific terms, this compound blocks oxidation by reacting with o-quinones to convert them back into phenolic form, preventing colored pigment formation.¹⁵ In addition, sulfite consumed dissolved oxygen and reacted directly with H₂O₂ to form sulfate (SO₄²⁻), thereby depleting oxygen and H₂O₂ needed for PPO and POD activity.¹⁴

Moreover, it can be observed that POD activity remained consistently lower than PPO activity across all treatments. As mentioned above, Na₂S₂O₅ acts as a potent antioxidant that disrupts the oxidation reactions of both PPO and POD by competing with oxygen (needed by PPO) and H₂O₂ (required by POD). However, the limited availability of H₂O₂ in yacon tubers' tissues may explain the consistently lower POD activity. Supporting these findings, a previous study on banana slices indicated that the 0.1% Na₂S₂O₅ solution inhibited 92.60% of PPO and 76.04% of POD after 10 min, whereas 96.74% PPO and 87.50% POD inhibition occurred by increasing the concentration to 0.2%.⁴⁵ Both treatments showed more pronounced inhibition of PPO activity than POD. This aligns with the findings in this study on yacon tubers, where Na₂S₂O₅ also exhibited a stronger inhibitory effect on PPO than POD.

Effect of Citric Acid in Combination with Sodium Metabisulfite and Immersion Time on The Enzyme Activities and The Discoloration of Yacon Tubers

To date, in addition to conventional heat treatment methods, researchers have paid much attention to the use of chemical agents—such as organic

acids (tartaric acid, citric acid, malic acid, oxalic acid and ascorbic acid), sodium metabisulfite, potassium metabisulfite, L-cysteine, EDTA —to control enzymatic browning in fruits and vegetables. Different chemical agents affected enzymes' activity through a distinct mode of action, and application of a mixture of these agents created a synergistic effect, resulting in more efficient inhibition of enzymes and improvement of color retention, while reducing the physicochemical alterations and sensory deterioration of fresh-cut products.

The effectiveness of the combination of these agents has been documented on a variety of fruit and vegetable products. For example, ascorbic acid (AA) and sodium metabisulfite (SMB) significantly inhibited the browning process in minimally processed apples, which was confirmed to be superior to ascorbic acid alone (Cort WR *et al.*),⁴⁶ while their combined use was also shown to be effective in reducing enzymatic browning in potatoes at a ratio of 0.25% SMB + 0.25% AA (Nascimento RFD and Canteri MHG, 2019).³⁹ Similarly, treatments with sodium metabisulfite and citric acid (CA) prolonged the shelf life and improved the color retention of sweet potatoes treated with 2% SMB adjusted to pH 2.91 with CA (Sgroppo SC, Vergara LE, Tenev MD),⁴⁷ and chilli treated at 0.3% SMB + 1% CA suggested a positive effect on preserving color and functional components including ascorbic acid, carotenoids and phenolics (Chaethong K and Pongsawatmanit R),⁴² while banana powder reported a ratio of 0.1% SMB + 0.5% CA to be more effective in browning inhibitory but still retention of the physico-chemical properties compared to using SMB alone (Kamal MM, Ove TA *et al.*).⁴⁸ These findings suggest that the optimal concentrations and ratios of chemical agents vary depending on the characteristics of each food. Therefore, it is necessary to study and tailor processing strategies according to the specific tissue structure and enzymatic characteristics of each product.

In the study of Gangta R, Thakur NS, Hamid, Gautam S, Thakur A,²¹ the role of 3% citric acid and 0.5% sodium metabisulfite was independently examined on minimally processed yacon. Both agents were found to exhibit strong inhibition of browning enzyme activity (POD). However, the findings revealed that

citric acid was more effective in preserving color (L^* values), texture, and physicochemical stability and possessed higher sensory scores, while sodium metabisulfite received lower sensory acceptability due to the sulfurous off-flavor. However, the research paper did not investigate the combination of these agents, therefore, leaving a gap in evaluating whether synergistic effects might improve browning inhibitory while reducing undesirable sensory effects.

Addressing this gap, the present study emphasized yacon tubers with the combination treatment of chemical agents. It was demonstrated from Figures 1 and 2 that both citric acid and sodium metabisulfite were effective in inhibiting the browning of yacon slices by significantly impeding the activity of PPO and POD. Moreover, their simultaneous application showed greater inhibitory effects, as they reduced the enzyme activity up to 90% with 0.5% $\text{Na}_2\text{S}_2\text{O}_5$ + 0.15% citric acid treatment, illustrated in Figure 3. Besides, the optimal immersion time was shortened to 15 min compared with using each agent individually (at 20 min), demonstrating the improved effectiveness in industrial utilization. As shown in Table 4, the highest L^* value and the lowest BI value were obtained with this combination ratio, proving a clear correlation between the browning index and PPO and POD enzyme activity. These findings support the synergistic role of these agents and establish an effective, non-thermal approach to maintaining the postharvest quality of yacon tubers.

Conclusion

This study evaluated the effectiveness of inhibitors in preventing enzymatic browning and maintaining the color of yacon. The results showed that the treatment method of immersing yacon slices in 0.15% citric acid or 0.5% sodium metabisulfite for 20 min was efficient in controlling browning caused by PPO and POD. However, the most effective method was achieved by immersing yacon slices in a solution of 0.15% citric acid and 0.5% sodium metabisulfite for 15 min. Future studies may focus on developing bioactive products from yacon, such as FOS-rich juice or syrup, after treating yacon slices with a combination of citric acid and sodium metabisulfite, based on the findings. These products may benefit patients suffering from chronic diseases such as diabetes and obesity.

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Author Contributions

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