Responses of GP3 and MD2 Pineapple Clones to Postharvest Applications of ABA, Chitosan, and Decrowning on the Severity of Internal Browning and Other Fruit Qualities

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Abstract

Internal browning (IB) is one of pineapple fruits' main postharvest physiological disorders in response to cold storage. The purpose of the research was to study the response of GP3 and MD2 clones to postharvest treatment with the application of ABA, chitosan, and decrowning on the IB severity and other fruit qualities. The experimental design used a Completely Randomized Design with 3 factors of clone (GP3 and MD2), decrowning (crown and crownless), and fruit coating [chitosan 1%, ABA 50 mg/L, ABA+chitosan mix, and control (H_2O)]. The fruits were kept at 7 ^oC and observed at 0, 3, 6, 9, 16, 23, 30, and 37 days. The results showed that the MD2 was significantly lower IB than GP3. IB severity was negatively correlated with ascorbic acid (AsA) content. MD2 had lower fruit weight loss (FWL), higher AsA, soluble solid content (SSC), and SSC/titratable acidity (STA) ratios compared to GP3. The crown+ABA treatment decreased IB severity of GP3, with an IB severity level of 0.75% after 37 days which was lower than that of crown+H_2O by 9.17% and crownless+H_2O by 8.42%. ABA treatment also showed higher skin dehydration and FWL and AsA, SSC, titratable acidity, and STA were not significant to the control.

Keywords: ***Pineapple, postharvest, internal browning***

I. INTRODUCTION

Pineapple is a fruit that is one of Indonesia's leading products. Reference [1] shows Indonesia's pineapple production in 2019, the 4th largest in the world after Costa Rica, the Philippines, and Brazil, which was 2,196,456 tons. Internal browning (IB) issues are particularly important in the global pineapple industry where their incidence can result in substantial losses in canning or after shipping in refrigerated ocean containers. The loss to the Australian pineapple industry due to IB is US$1.3 million per year out of a total production value of around US$30 million [2].

Reference [3] shows that the IB severity on MD2 is smaller than that of cayenne pepper cultivars, this confirms that MD2 cultivars are resistant to IB induction [4]. The initiation of IB incidence through an enzymatic reaction mechanism depends on the enzyme activity of phenolic compounds, polyphenol oxidase (PPO) and peroxidase (POD), and O_2. Browning incidences as a result of the reaction between oxidized phenolic compounds in the presence of PPO and/or POD enzymes that form highly reactive o-quinones to combine with their counterparts or with other phenolic compounds [5]. Symptoms are incidence in the flesh of the fruit close to the core with the initial appearance of translucent flesh [6]. Symptoms develop with changes in color to brown and black, and symptoms incidence internally without any external signs of the fruit [7].

In the experiment on the protrusion of crowned and uncrowned pineapples. The highest content of gibberellic acid (GA) was found in the pineapple with no crown, it was concluded that the crown was one of the media that translocated abscisic acid (ABA) in the pineapple. Reference [8] showed that crowned pineapple fruit with a maturity level of 70% had a positive correlation with an increase in the IB incidence, GA content, reactive oxygen species (ROS), malondialdehyde (MDA), phenolic content, phenylalanine ammonia-lyase (PAL), and soluble solid content/fatty acid ratios (SFA) and there was no relationship with PPO activity in pineapple after 9 days of storage at 20°C.

ABA infiltration research was carried out by spraying ABA solution on pineapples to determine the IB severity and GA levels in pineapples. ABA 380 µM was positively correlated in reducing the IB severity, GA after 9 days of storage, and PPO after 6 days of storage [9]. The combination

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treatment of 200 mg/L ABA and storage temperature of 5 °C can reduce the IB severity and GA_4 [10].

The IB severity after storage of susceptible cultivar pineapple (Trad Sri Tong) at 10°C started on the 10th day of storage and accelerated on the 8th day after being transferred to 25°C for a day. In the tolerant cultivar (Pattavia), IB severity started after 19 days of storage at 10°C, and IB severity accelerated to 15 days after being transferred to 25°C for a day [11].

Long storage at cold temperatures can initiate IB, wax treatment can reduce the IB incidence by 87.5% at day 20 [12]. The IB incidence was reported in several pineapple cultivars in Malaysia, such as Mauritius, Sarawak, Gandol, Babagon, and Maspine. Mauritius cultivars previously stored at 8°C and 12°C for up to 4 weeks followed by a week-long storage period at room temperature (28°C) can induce IB events [13].

II. METHOD

This research was conducted at Great Giant Food Co. Ltd. (GGF), East Lampung, Lampung, Indonesia in August-September 2022. Fresh pineapple freshly harvested from GGF with a weight of 825-1,124 g/fruit with an export standard maturity level with yellow skin color 0 %. The experiment used a 2 x 2 x 4 factorial in a Completely Randomized Design. The 3 factors were clones (GP3 and MD2), Decrowning (Crown and Crownless), Coating [(chitosan 1%, ABA (Phytotechlab, Lenexa, Kansas, USA) 50 mg/L, ABA 50 mg/ L+chitosan 1% mix, and control (H_2O)]. Observations were made (destructive and non-destructive) 7 times (days 3, 6, 9, 16, 23, 30, and 37) with 3 replicates.

The treatment of the application of H_2O and chitosan was done by soaking the fruit in H_2O and single chitosan, and the application of ABA and the combination of ABA+chitosan by spraying a solution of ABA or ABA+chitosan all over the surface of the fruit until the entire surface of the fruit skin was wet. In the ABA+chitosan treatment, the fruit was first sprayed with ABA, dried for 30 minutes, then sprayed with chitosan. Pineapple fruit is air-dried for 30 minutes before being packaged in cardboard with a capacity of 10-11 sheets per carton that has been perforated before (PT GGF packaging cardboard). All fruits were stored at 7°C for 37 days.

Observations were made on the characteristics of destructive and non-destructive. Destructive observations with variables: 1) the IB severity, namely by observing the fruit score, by observing the surface area of the transverse fruit pieces that experienced a change in color from transparent to blackish brown, a score of 1 category was mild (< 5%), a score of 2 was moderate (6-10%), 3 moderately severe (11-20%), a score of 4 weight categories (> 20%), with the formula:

IB severity (%) = [(Σ no. of the IB in category. category value) / (total no. of fruits. the highest category value)]. 100%

Scoring of IB severity follows United States Standards for Grades of Pineapples with modification [14], 2) Ascorbic acid (AsA), pineapple juice or ascorbic acid (as Dye factor) as much as 2 ml, added 5 ml of metaphosphoric acid (HPO3), then shaken until homogeneous. The solution was titrated using 2,6-dichlorophenol indophenol (2,6-D). The AsA value is calculated by the formula:

AsA (ppm) = [(Σ actual 2,6-D volume. Dye-factor) / (sample volume)]. 1000

3) Soluble solid content (SSC), fruit juice is dropped on a digital refractometer prism glass, the value shown is the Brix value. 4) Titratable Acidity (TA), 5 ml of fruit juice, added 5 drops of phenolphthalein indicator, then shaken until homogeneous. The solution is titrated using 1 N NaOH compound. The TA value is calculated by the formula:

TA (%) = [(Σ NaOH volume. N NaOH. BE CH_3COOH) / (sample volume)]. 100%

5) and SSC/TA ratio (STA), the comparison between SSC and TA values. Non-destructive observations with variables: 1) Fruit weight losses by weighing the fresh weight of the fruit with a digital scale and 2) Dehydration of the fruit skin by observing fruit scoring, by observing 3 sides of the fruit skin with 10 skin samples around the open eyeball, each skin around the eyeball was observed for its shrinkage level, a score of 1 in the mild category (< 25%), a score in 2 in the moderate category (26-50%), and a score in 3 in the heavy category (> 26%). The fruit has calculated the severity of skin dehydration (SD) with the formula:

SD (%) = [(Σ no. of the skin around the eye in the category. category value) / (total no. of skin eyes. the highest category value)]. 100%

The data were processed by comparing the mean with the 95% confidence interval (mean±CI) (α=0.05) for each treatment group and post hoc. test with Duncan's Multiple Distance Test (DMRT). Data is displayed on bar and line charts with CI bars and tables with letter notation to compare their significance. Statistical data was processed using the IBM SPSS Statistics Version 26 program.

III. RESULTS DAN DISCUSSION

The results in this experiment of pineapple clones gave different responses to long shelf life at 7°C for 37 days on the IB severity. MD2 clones were more resistant to IB severity than GP3 clones. The IB severity of the MD2 clone was 1.40%, lower than that of the GP3 clone which was 11.63% on day 37. The MD2 has resistance to IB severity, this can be seen from the results of research that MD2 cultivars have higher resistance than cayenne pepper cultivars [3]. The IB incidence stored at a temperature of 7°C was initiated from the fruit's shelf life of 23 days, the shelf life of 16 days and below indicated that there was no IB incidence (see Fig. 1.A).

There was a significant difference in AsA content in GP3 and MD2 clones. AsA content in MD2 clones was higher than GP3, this was negatively correlated with the IB severity (see Fig. 1.B). Ascorbic acid is an acid compound that can decrease the activity of the PPO enzyme, thereby decreasing the IB severity [5]. Pineapple genetic factors have a considerable influence on the IB severity, this is supported by research [11], that the pineapple sclerenchyma cultivar MD2 observed using scanning electron microscopy, has a thicker sclerenchyma fiber layer structure and is twice as large as susceptible cultivars (Trad Sri Thong) and tolerant (Pattavia),

MD2 sclerenchyma cells form concentric rings around the phloem and xylem.

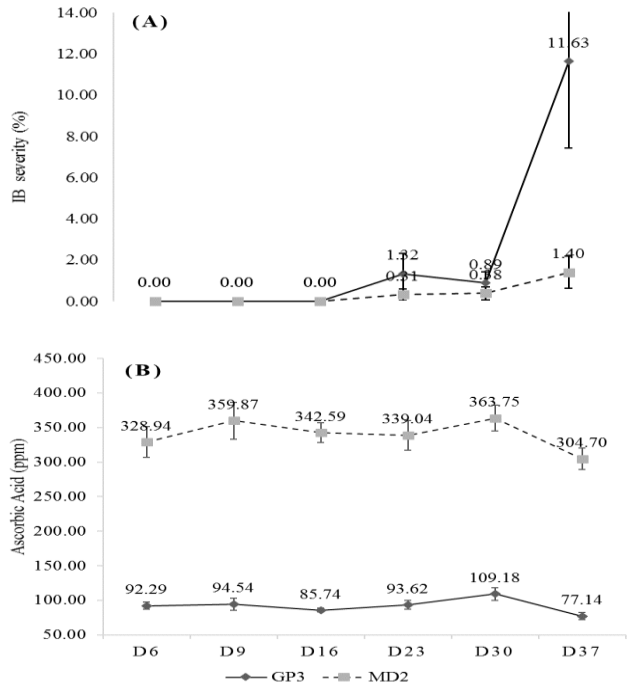

Fig. 1. Comparative response between the development of GP3 and MD2 clones stored at 7°C for 37 days on the IB severity (A) and ascorbic acid (B). The values are mean±CI.

In all treatments of MD2 clone showed no significant difference in the IB severity. In clone GP3, the interaction of crown and ABA 50 mg/L (D) showed a significant effect on the IB severity compared to other treatment interactions in clone GP3, except GP3 x Crownless x Chitosan treatment (F), but it was not significant with the others (see Fig. 2).

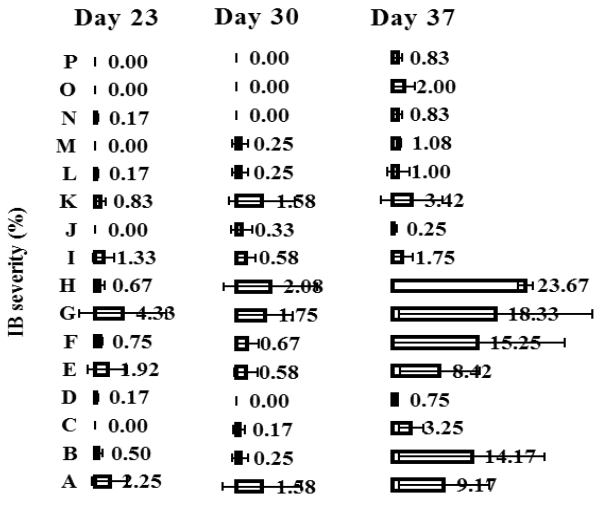

Fig. 2. Response of pineapple clones GP3 and MD2 to postharvest treatment on the development of IB severity stored at 7°C for 37 days (A: GP3 x Crown x H_2O, B: GP3 x Crown x Chitosan, C: GP3 x Crown x ABA+Chitosan, D: GP3 x Crown x ABA, E: GP3 x Crownless x H_2O, F: GP3 x Crownless x Chitosan, and G: GP3 x Crownless x ABA+Chitosan, H: GP3 x Crownless x ABA, I: MD2 x Crown x H_2O, J: MD2 x Crown x Chitosan, K: MD2 x Crown x ABA+Chitosan, L: MD2 x Crown x ABA, M: MD2 x Crownless x H_2O, N: MD2 x Crownless x Chitosan, O: MD2 x Crownless x ABA+Chitosan, P: MD2 x Crownless x ABA). The values are mean±CI.

The Crown x ABA treatment was included in the category with mild severity, and the others were in the moderate category, except for the Crown x ABA+chitosan treatment. Crown x ABA treatment can be a reference for the storage of GP3 clones in reducing the IB severity at a temperature of 7°C up to shelf life of 37 days. Crown is a source of exogenous ABA in pineapple, and exogenous ABA contributes to ABA donors, where ABA compounds are antagonistic to GA compounds. Based on research shows GA positively correlated with IB severity [8]-[10].

There was no significant difference between the treatment of clones on AsA levels, this indicates that there was no effect of decowning and coating treatments on AsA levels (see Fig. 3). In this experiment, it was concluded that AsA was formed by the respective genetic clones (GP3 and MD2). According to [15], the IB severity was not correlated with ascorbic acid, the IB-tolerant Pattavia cultivar had lower AsA content than the Trad Sri Tong cultivar which was more susceptible to IB.

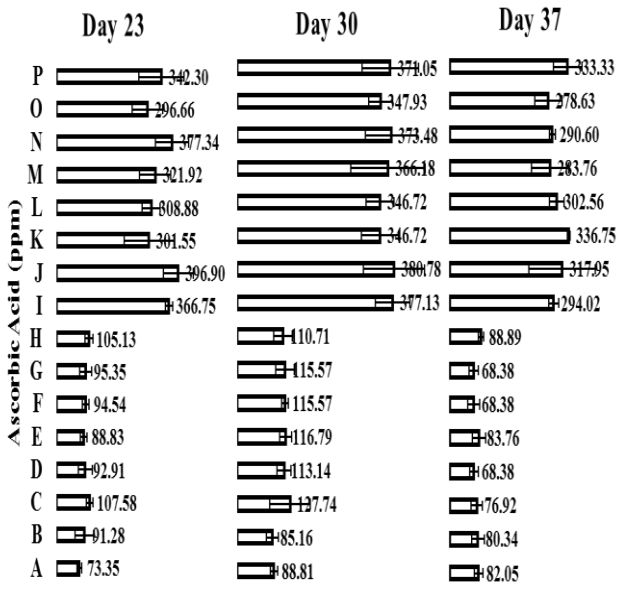

Fig. 3. Response of pineapple clones GP3 and MD2 to postharvest treatment on the development of ascorbic acid stored at 7°C for 37 days (A: GP3 x Crown x H_2O, B: GP3 x Crown x Chitosan, C: GP3 x Crown x ABA+Chitosan, D: GP3 x Crown x ABA, E: GP3 x Crownless x H_2O, F: GP3 x Crownless x Chitosan, and G: GP3 x Crownless x ABA+Chitosan, H: GP3 x Crownless x ABA, I: MD2 x Crown x H_2O, J: MD2 x Crown x Chitosan, K: MD2 x Crown x ABA+Chitosan, L: MD2 x Crown x ABA, M: MD2 x Crownless x H_2O, N: MD2 x Crownless x Chitosan, O: MD2 x Crownless x ABA+Chitosan, P: MD2 x Crownless x ABA). The values are mean±CI.

Based on the AsA value in MD2 clones which was quite high, it was positively correlated in decreasing the IB severity, this could be a reference for the AsA compound infiltration experiment in decreasing the IB severity in pineapple clones GP3 or other clones that naturally produce little AsA. The increase in endogenous AsA can be obtained

from the combination treatment of the application of AsA or isoascorbic acid (IAA) and plastic bagging on pineapple slices in decreasing the IB severity [16]. The application of methyl jasmonate (MJ) can prevent the decrease in AsA content in pineapple storage on PPO activity, incidence and severity of IB [17].

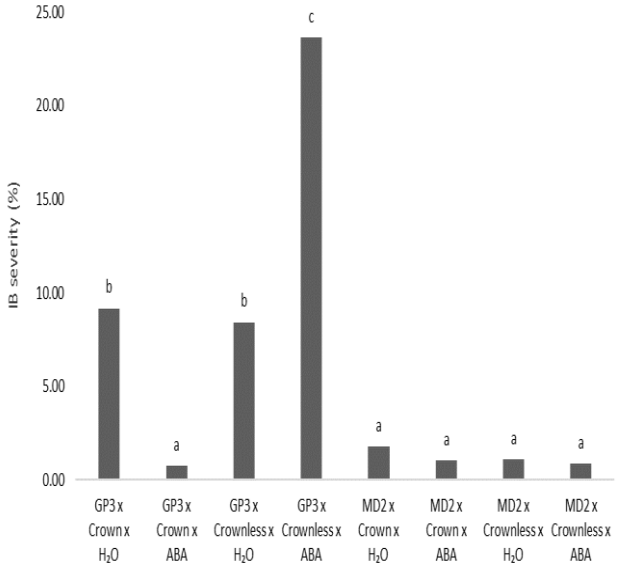

Fig. 4. Effect of 50 mg/L ABA application on full crown pineapple on ABA treatment and control of IB severity stored at 7°C for 37 days. Lowercase letters indicate statistically significant differences by DMRT ($p \leq 0.05$).

The GP3 x Crown x ABA treatment was not significant compared to the ABA treatment and the control MD2 clone on the IB severity and significant to the other GP3 treatments during 37 days of storage (see Fig. 4). This treatment has the same resistance as the MD2 clone which has a fairly high AsA content, it is suspected that the Crown and ABA treatment as a source of endogenous ABA in decreasing GA and phenolic biosynthesis that can trigger IB induction. Crown and ABA treatment in pineapple storage decreased GA biosynthesis and phenolics compound, and PAL activity [6], decreased GA biosynthesis and PAL and PPO activity [9], and decreased GA biosynthesis [10] compared to crownless treatment.

Table I. Effect of ABA 50 mg/L application on crown intact pineapple on fruit quality stored at 7ºC for 37 days.

Treatment	SSC (%)	TA (%)	STA	FWL (%)	SD (%)
GP3 x Crown x H_2O	12.03abc	0.49ab	24.82a	13.40e	28.87a
GP3 x Crown x ABA	10.77a	0.37ab	29.75abc	14.17e	34.85bc
GP3 x Crownless x H_2O	11.33ab	0.55b	20.92a	10.27cd	28.52a
GP3 x Crownless x ABA	12.30abc	0.47ab	26.27ab	10.77d	37.11c
MD2 x Crown x H_2O	13.03abcd	0.34a	39.71c	9.52bc	31.16ab
MD2 x Crown x ABA	14.90cd	0.51ab	29.69abc	10.10cd	32.86b
MD2 x Crownless x H_2O	16.40d	0.45ab	37.66bc	7.14a	27.82a
MD2 x Crownless x ABA	14.40bcd	0.50ab	29.49abc	8.44b	34.48bc

Lowercase letters indicate statistically significant differences by DMRT ($p \leq 0.05$).

The soluble solids content in the GP3 x Crown x ABA treatment at a shelf life of 37 days at 7°C was not significant with other treatments on the GP3 clone, lower than all MD2 treatments except for the Crown x H_2O treatment. TA and Sweetness levels (STA) in the GP3 x Crown x ABA treatment showed no significant difference to all treatments, weight loss was higher than the other treatments except for the GP3 x Crown x H_2O treatment. Skin dehydration (SD) was higher than the control treatment on GP3 and MD2 clones except for the MD2 x Crownless x H_2O treatment (see Table I).

CONCLUSION

MD2 clones had resistance to IB severity compared to GP3, AsA content was positively correlated to decrease IB severity. ABA treatment with intact crown on GP3 clones had a significant effect on IB severity compared to control (H_2O) on fruit with intact or pruned crowns. ABA treatment on fruit with intact crowns also maintained fruit quality, such as AsA, SSC, TA, STA, but had higher FWL and SD. ABA treatment by maintaining the integrity of the crown on GP3 clones stored at 7°C for 37 days can be a reference in reducing the IB severity.

REFERENCES

[1] Food and Agriculture Organization (FAO), "Production of Pineapples: top 10 producers 2019. Accessed via: http://www.fao.org/faostat/en/#data/QC/visualize, 2019.

[2] H.L. Ko, P.R. Campbell, M.P. Jobin-Decon, K.L. Eccleston, M.W. Graham, and M.K. Smith, "The introduction of transgenes to control internal browning in pineapple (*Ananas comosus* L.) cv. 'smooth cayenne' by microprojectile bombardment," Euphytica, vol. 150, pp. 387-395, 2006.

[3] C. Souleymane, Y.S.E. Salome, N.A. Laurent, K.O.K. Samuel, and K.T. Hilaire, "Effect of potasium fertilization for pineapple on internal browning of fruit in post-harvest conservation," Journal of Agriculture and Crops, vol. 5, no. 6, pp. 100-108, 2019.

[4] K. Luengwilai, D.M. Beckles, and J. Siriphanich, "Postharvest internal browning of pineapple fruit originates at the phloem," Journal of Plant Physiology, vol. 202, pp. 121–133, 2016.

[5] C. Queiroz, M.L.M. Lopes, E. Fialho, and V.L. Valenta-Mesquita, "Polyphenol oxidase: Characteristics and mechanisms of browning control," Food Reviews International, vol. 24, pp. 361-375, 2018.

[6] R.E. Paull and K.G. Rohbach, "Symtom development of chilling injury in pineapple fruit," J. Am. Soc. Hortic. Sci, vol. 110, no. 4, pp. 100-105, 1985.

[7] R.J. Stewarts, B.J.B. Sawyer, and S.P. Robinson, "Blackheart development following chilling in fruit of susceptible and resistant pineapple cultivars," Aust. J. Exp. Agric, vol. 42, pp. 195-199, 2002.

[8] J. Liu, C. He, F. Shen, K. Zhang, and S. Zhu, "The crown plays an important role in maintaining quality of harvested pineapple," Postharvest Biology and Technology, vol. 124, pp. 18–24, 2017.

[9] Q. Zhang, Y. Liu, C. He, and S. Zhu, "Postharvest exogenous application of abscisic acid reduces internal browning in pineapple," Journal of Agricultural and Food Chemistry, vol. 63, pp. 5313-5320, 2015.

[10] Q. Zhang, X. Rao, L. Zhang, C. He, F. Yang, and S. Zhu, "Mechanism of internal browning of pineapple: The role of gibberellins catabolism gene (AcGA2ox) and Gas," Scientific Reports, vol. 6, no. 33344, 11 pp, 2016.a
[11] K. Luengwilai, D.M. Beckles, U. Roessner, D.A. Dias, and V. Lui, "Identification of physiological changes and key metabolites coincident with postharvest internal browning of pineapple (Ananas comosus L.) fruit," Postharvest Biology and Technology, vol. 137, pp. 56–65, 2018.
[12] D.S. Pitadeniya and P.L.N. Lakshman, "A study of pineapple black heart disorder measures to reduce postharvest losses during transportation and storage," Procidings of the Second Academic, vol. 2004, pp. 192-195, 2004.
[13] H. Abdullah and M.A. Rohaya, "The develop-ment of internal browning disease in Mauritius pineapple (*Ananas comosus* cv. Mauritius) during storage at lower temperatures," MARDI Res. Bull, vol. 11, pp. 309–319, 1983.
[14] United States Department of Agriculture, "The United States standars for grades of pineapples: Classification of Defects," United States Department of Agriculture, pp. 1-8, May, 2008.
[15] O. Nukuntornprakit, K. Luengwilai, and J. Siriphanich, "Chilling injury in pineapple fruit is related to mitochondrial antioxidative metabolism," Postharvest Biology and Technology, vol. 170, no. 111330, 10 pp. 2020.
[16] L.E. Ayón-Reyna, L.G. Ayón-Reyna, M.E. López-López, G. López-Angulo, K.V. Pineda-Hidalgo, J.A. Zazueta-Niebla, M.O. Vega-García, "Changes in ascorbic acid and total phenolics contents associated with browning inhibition of pineapple slices," Food Sci. Technol, Campinas, vol. 39, no. 3, pp. 531-537, July-Sept. 2019.
[17] X.H. Lu, D.Q. Sun, Y.H. Li, W.Q. Shi, G.M. Sun, "Pre- and post-harvest salicylic acid treatments alleviate internal browning and maintain quality of winter pineapple fruit," Sci. Hortic, vol. 130, pp. 97-101, 2011.

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