

Storage and Preservation Study of Tropical Fruits by Kojic Acid

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Abstract: Kojic acid is a kind of weak acid compound produced by aerobic fermentation by some strains of aspergillus using glucose, fructose, sorbitose and sugar alcohol and other raw materials. Kojic acid was widely used as a food additive for preventing enzymatic browning. In this paper, we studied the preservation of tropical fruits papaya and wax apple with kojic acid. The kojic acid-treated papaya could be stored for 8~10d, which was prolonged 4~5d than the untreated. The same preservation effective exists when using kojic acid painted wax apple. In addition, results showed that kojic acid could effectively prevent banana juice from browning.

Key words: Kojic acid, Tropical fruits, Storage and preservation

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I. Introduction

Kojic acid, its chemical name is 5-hydroxy-2-hydroxymethyl-pyrano-4-ketone, which is a kind of weak acid compound produced by aerobic fermentation by some strains of aspergillus using glucose, fructose, sorbitose and sugar alcohol and other raw materials. It was first discovered by Saito in 2007 from fermented rice.

Kojic acid has a certain antibacterial activity. Gram-negative bacteria are more sensitive to sodium kojate than gram-positive bacteria, while most other antibacterial substances are the opposite. The antibacterial activity of kojic acid is bacteriostatic rather than bactericidal. When kojic acid is diluted 1000 times, it can inhibit *Clostridium botulinum*, *S. aertrycke*, *S. pullorum*. When diluted 500 times, it can completely inhibit *Aerobaerogenes*, *Alcaligenes faecalis*, *Bacillus anthracis*, *Bacillus megaterium*, *B. megatherium*, and so on. When diluted 100 times, it can kill human white blood cells¹.

Because kojic acid is bacteriostatic, anti-oxidative and has chelated properties with metal ions, it can be used as a preservative and color protection agent in foods; researchers use kojic acid to inhibit common contaminating bacteria and prevent corrosion. According to the results of Japanese scholars, kojic acid has good preservation effect in food preservation and color protection, and has been applied for many patents in compound food preservatives².

In addition, Suet al. compared the antibacterial effects of kojic acid and sodium benzoate. The results also showed that kojic acid had better antibacterial effect than sodium benzoate³. Chen et al. compared kojic acid with ascorbic acid, phytic acid and other preservatives when studying the application of natural preservatives in clean vegetable processing. The results show that kojic acid has a good antiseptic and fresh-keeping effect⁴. Qiao et al. proved that 20% kojic acid has extensive antibacterial effects on *Bacillus subtilis*, *Streptococcus aureus*, *E. coli*, *Saccharomyces cerevisiae* and *Candida utilis*. The results confirmed that kojic acid does have a wide antibacterial effect as a food preservative⁵.

II. Materials and method

2.1 Materials

Strain: *Aspergillus oryzae* M34, preserved in the laboratory; Agar slant culture-medium (PDA medium); Seed medium and fermentation medium refer to the literatures⁶.

2.2 Kojic acid detection and fruit preservation effect

(1) Kojic acid detection

Within a certain range, the absorbance of kojic acid with a certain amount of Fe³⁺ has linear relation with the concentration of kojic acid. The maximum absorption peak was scanned by WFZ800-D3B UV and visible spectrophotometer.

(2) Method for measuring fruit storage and preservation effect

Weight loss rate = fruit loss weight / fruit original weight
Determination of surface rot rate: A transparent film

having a square area of 0.25 cm^2 was applied to the surface of papaya fruit, and the surface area and total area of the fruit surface were calculated.

Surface decay rate = decay area / total fruit area

(3) Determination of hardness:

Hardness was measured for different orientations of the fruit using a durometer. The experiment selected three parts of the fruit from the top, middle and bottom, and each part was tested repeatedly for 5 times. The data is finally recorded and averaged.

(4) Determination of the activity of polyphenol oxidase (PPO):

1.8 mL phosphate buffer (pH 6.5) and 0.01 mol/L catechol 1.0 mL were added to the cuvette, and then 0.2 mL of crude enzyme solution was added and measured at 420 nm absorbance. The amount of enzyme that increases the absorbance value per minute is an enzyme activity unit (U)^{7,8}.

(5) Determination of peroxidase (POD) activity:

2.6 mL phosphate buffer (pH 7.0), 0.1 mL of 1% o-phenylenediamine-ethanol solution and 0.2 mL of 0.3% hydrogen peroxide were added to the cuvette, respectively. 0.1 mL of the crude enzyme solution was added, and the change in absorbance was measured at 430 nm. The amount of enzyme that increases the absorbance value per minute is an enzyme activity unit (U)^{7,8}.

2.5 Fruit treatment and preservation

(1) Treatment of papaya by kojic acid

Papaya of the same variety and maturity is picked to ensure that the papaya is not damaged during the harvesting process and the maturity is similar. The experiment was divided into 2 groups of 30, each requiring a total of 60 papayas. One group of papaya was treated with kojic acid, and the other group was control group. Starting from the 3rd day, each group was randomly selected from each group for parallel determination, and the average was taken as the final data. Take 3 each day, and observe data from 3rd day to 12th day.

Two gram of the crude kojic acid product was weighed, and distilled water 100 mL to obtain a 2% kojic acid solution. Apply evenly on the surface of papaya fruit with a soft fine brush, then place it in a 30°C incubator and put a proper amount of water to control the humidity. The total acid, total sugar, Vc, weight loss rate, surface rot rate and hardness of papaya fruit were observed and measured every 24 hours.

(2) Treatment of wax apple by kojic acid

The preservation experiment was carried out by selecting non-destructive of wax apple the same variety and the same maturity.

2% kojic acid solution was also prepared. Apply evenly on the surface of the wax apple fruit with a fine brush, then place it in a 30°C incubator and put a proper amount of water to control the humidity. Observe every 24h and record the fruit rot. Try not to touch the fruit during the observation process, and then put it into the incubator to continue the preservation. Photographs were used to record fruit surface preservation effects.

(3) Treatment of banana juice by kojic acid

At first, banana juice was carried out as following process.

Raw materials → peeling → sectioning (thickness about 0.5cm) → soaking (adding coloring agent 1% kojic acid solution, 1% kojic acid fermentation broth, 0.1% L-cysteine, 0.5% Vc, 1% NaCl, 0.5% citric acid soak for 20min) → beating → pectinase enzymatic hydrolysis (treatment for 100min) → inactivation of enzyme (85°C, 10min) → separation of slag juice (3600r/min, 20min) .

The resulting supernatant was immediately assayed for its OD at 420 nm; the remainder was poured into a Petri dish. It was placed at room temperature (30°C) to sufficiently oxidize browning, and its OD value was measured again after 30 minutes. The difference between the two (Δ OD value) indicates the degree of browning of the banana juice. Each experiment was repeated 3 times and the average was taken.

Take 50g of fresh bananas and soaked bananas with different color-protecting agents respectively, and add 100mL of pre-cooled 0.2mol/L phosphate buffer (pH 7.0) containing 1% insoluble polyvinylpyrrolidone (PVP) and 1% TritonX-100, rapid mixed within 30s, and the homogenate was centrifuged at 7800 r/min for about 20 minutes at 4°C. The enzyme is retained in the supernatant. The PPO and POD activities of the crude enzyme solution were measured separately.

III. Results and Analysis

3.1 Study on the preservation effect of kojic acid on tropical fruits

(1) Preservation effect of kojic acid on papaya

Papaya is a typical subtropical fruit. It is a high-breathing fruit and is susceptible to anthrax. Since papaya suffers from chilling below 6°C, it is not suitable for the refrigeration method. Irradiation to kill anthrax requires hundreds of thousands of rads of radiation dose, which could not be afforded. Therefore, the photo method is not good either. It is now optimistic about the gas adjustment method, but the relative cost is high. In this study, 2% kojic acid was applied to the surface of papaya using a chemical method to observe the storage effect: the control group began to rot on the 5th day and completely rotted on the 10th day; but the kojic acid treatment group began to rot on the 10th day, partially rotted on the 12th side, and the surface became hard and the epidermis became thick.

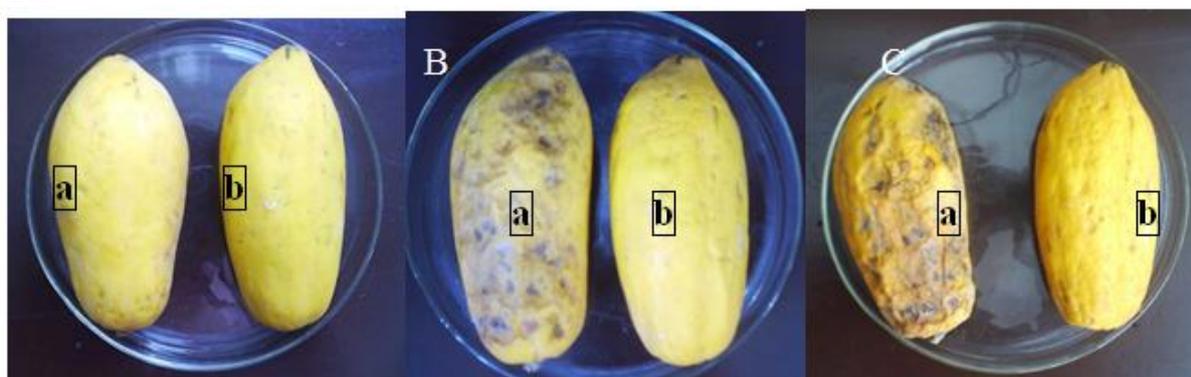


Figure 1. The preservation of papaya: pre-experiment (A), preserved 6d (B), preserved 10d (C). **a** is control group, **b** is kojic acid treated group.

Figure 1 shows two samples randomly selected and saved to the end of the experiment for photo recording. On the left side, a papaya is the control group, and on the right side, the papaya is the kojic acid treatment group. The good preservation effect of the kojic acid treatment group can be clearly seen in B and C of Figure 1.

Table 1 shows the physiological changes during storage. It can be seen that the decrease of total acid, total sugar and Vc content in the kojic acid treated group is lower than that in the control group; weight loss rate and the surface rot rate was significantly better than the control group. After 12 days, the weight loss rate of the kojic acid treatment group was about 7% lower than that of the control group. The decay rate was about 35% lower than that of the control group. It can be seen from the hardness measurement data that the hardness of the kojic acid treated papaya was significantly greater than that of the control group, and the epidermis layer became thicker and the pulp slightly hardened.

It can be seen from the above analysis that the acid preservation effect of kojic acid on papaya fruit is obvious. However, it can be found from the relevant data that the papaya epidermis preserved by kojic acid becomes thicker and harder, which affects the taste of the fruit to some extent. Therefore, in the in-depth study of this problem, we should start from maintaining the good quality of the fruit on the basis of prolonging the storage period, for example, compounding kojic acid, adjusting the concentration of kojic acid, changing the treatment, and so on.

Table 1. The data of papaya preservation

	Detect time days	Totle acid H ⁺ concentration mol/100g	Totlesuger mg/100g	Vc mg/100g	Weight-loss ratio			Surface decay rate %	Hardness (×10 ³ Pa)							
					Original weight	Sample weight	Weight-loss ratio		Detect point	1	2	3	4	5	average	Totle average
Original sample	—	14.51	15.16	88.46	—	—	—	0	top	8.4	9.7	8.3	8.9	9.3	8.92	8.77
									bottom	8.6	8.4	9	9.4	9.2	8.92	
									middle	7.9	8.2	9.1	8.7	8.5	8.48	
Control group	6d	12.25	7.688	63.54	183.83	165.87	9.77%	28.6	top	3.6	4.8	3.9	3.5	2.8	3.72	3.77
									bottom	3.3	5	4.8	3.2	2.9	3.84	
									middle	3.4	4.6	3	4.1	3.7	3.76	
	8d	17.75	6.983	46.93	198.74	155.94	21.54%	34.3	top	2.4	3.4	3.3	3.1	3.4	3.12	2.69
									bottom	2.4	2.2	2.6	2	2.4	2.32	
									middle	2.2	2.1	2	4.2	2.6	2.62	
	10d	12.54	6.851	41.35	181.92	128.97	29.11%	57.7	top	2.7	2.3	3.7	2	4.3	3	2.35
									bottom	2.3	1.9	3	0.3	0.6	1.62	
									middle	2.6	0.7	1.9	3	4	2.44	
	12d	12.12	5.626	32.98	190.71	120.34	36.9%	68.3	top	3.2	2.4	3.1	5.4	3.8	3.58	1.74
									bottom	0	0	0	0	0	0	
									middle	1.3	1	2.5	0.4	3	1.64	
KA treated group	6d	12.75	7.471	69.64	197.79	178.95	9.53%	4.0	top	7.5	8.4	8	11.4	5	8.1	8.71
									bottom	7.5	8	9.5	8	5	7.6	
									middle	9	11	10.4	12.2	9.8	10.5	
	8d	17.25	7.657	50.38	203.68	174.69	14.23%	15.5	top	12	13	10.8	5.4	6.2	9.48	8.30
									bottom	3.9	5.5	4.6	5.1	6.1	5.04	
									middle	12.3	4.4	11.4	13	10.8	10.4	
	10d	17.89	6.339	47.56	234.39	172.33	26.48%	19.3	top	5.2	5.5	4.6	5.5	5.3	5.22	5.19
									bottom	3.1	3.9	4.6	3.5	5.2	4.06	
									middle	5.5	5.6	5.2	8	7.1	6.28	
	12d	14.22	6.231	38.31	244.92	171.87	29.83%	33.9	top	4.8	4.8	4.6	4.9	5.2	4.86	4.47
									bottom	3.9	3.1	3.6	5.1	3.8	3.9	
									middle	4.2	6.3	4.9	3.8	4.1	4.66	

(2) Preservation effect of kojic acid on wax apple

Wax apple is a tropical fruit with very thin skin and high moisture content. It is not resistant to storage. It can only be stored for about 1 week at room temperature. Most of them are currently used as fresh fruit in the local market. In this paper, the use of kojic acid for preservation research, as shown in Figure 2, the effect is better.

Control group: After 8 days, the surface began to fade, the surface became soft and the water loss seriously; on the 9th day, it began to rot, and the surface appeared dark spots; on the 10th day, it lost the edibility. More than 40% of the surface became rust on the 12th day.

Kojic acid treatment group: the water retention was good on the 10th day, the skin was smooth, the color was bright, the skin color was still similar to that before the experiment, and a small amount of black spots appeared on the surface of the 12th day, but the color and water content of other parts were not affected, and the whole fruit showed healthy, bright color, and still edible.

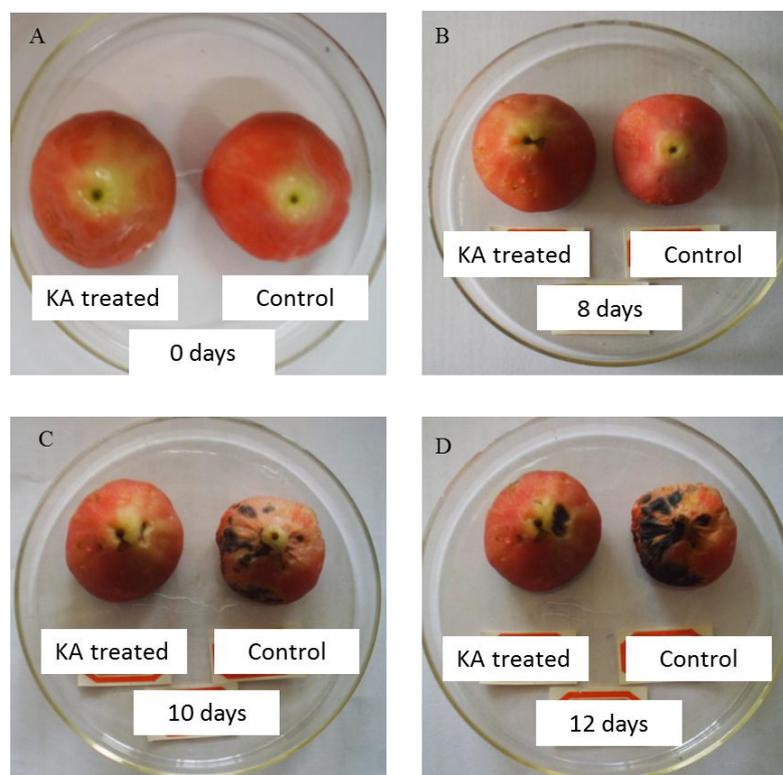


Figure 2. The preservation of wax apple: pre-experiment(A), preserved 8d(B), preserved 10d(C), preserved 12d(D)

(3) Color protection effect of kojic acid on banana juice

Banana is an important tropical fruit produced in southern China. The banana juice or banana mixed juice has potential development prospects. However, during the processing of banana juice, the browning phenomenon is prominent, which seriously affects its nutritional quality and appearance quality. Therefore, how to effectively inhibit its browning becomes a key issue that must be solved for the development of clarifying banana juice.

Polyphenol oxidase (PPO) and peroxidase (POD) are present in the banana. Both are the main enzymes that cause enzymatic browning of bananas. The inhibition of enzymatic browning caused by kojic acid on PPO is controlled by various factors. First, its structure is similar to phenolic substrate and has competitive inhibition; secondly, it can chelate copper ions necessary for PPO activity; third, kojic acid can interfere with oxygen in PPO enzymatic reaction by removing oxygen free radicals. Absorption; Fourth, kojic acid can reduce the formation of melanin substrate steroids to prevent the formation of melanin.

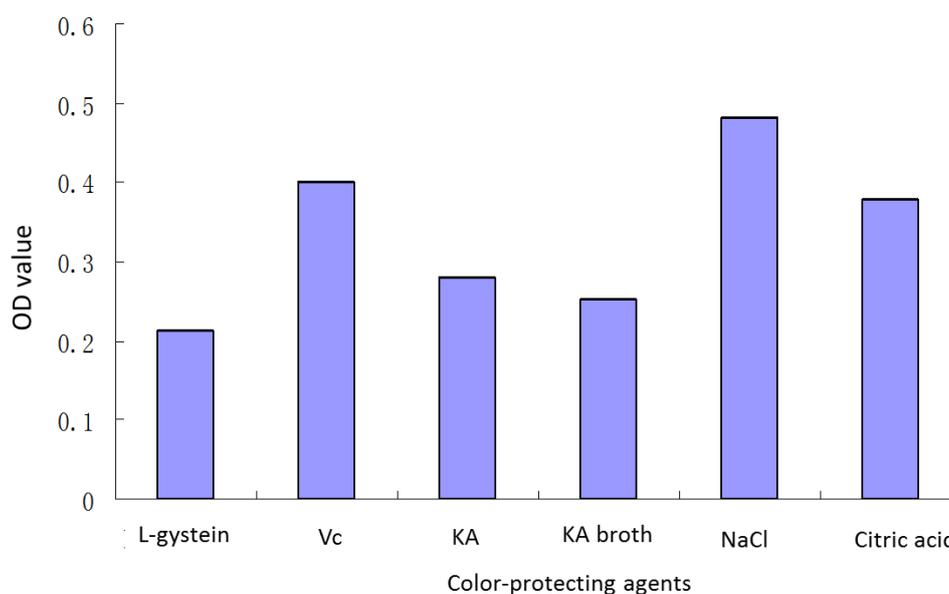


Figure 3. The preventing banana juice from browning

Ascorbic acid, L-cysteine, citric acid, and sodium chloride were used as color-protecting agents as literature. They were selected for comparison with kojic acid and referenced to the optimal amount reported in the literature^{9,10}. The experimental results are shown in Figure 3.

The experiment shows that the inhibition of enzymatic browning by L-cysteine on banana juice is more obvious than other effects. The color protection effect of NaCl and citric acid is not satisfactory. This is basically consistent with the literature. Vc is a typical non-competitive inhibitor, but the effect of Vc inhibiting browning is sometimes unstable.

Comparing the above several color-protecting agents, kojic acid has a good browning inhibition effect. 1% of the amount shows a good color protection effect. And kojic acid is colorless and odorless, and it is an excellent color-protecting agent without adding its taste to the juice. If kojic acid is compounded with other color-protecting agents to prevent enzymatic browning, so that it exerts a synergistic effect, it will have a better color-protecting effect.

In the experiment, the color protection effect of kojic acid fermentation broth was slightly better than that of kojic acid. The possible reason is that in addition to kojic acid, the fermentation broth also contains a variety of other active ingredients, including polypeptides, free amino acids, polysaccharides, polysaccharides, B vitamins, active factors, etc., may also promote inhibition of browning.

Table 2. The residual activity of PPO and POD after color protection

Enzyme	PPO/POD	Control	L-Cys	Vc	KA	KA broth	NaCl	Citric acid
PPO	enzyme activity (U)	6.62	0.81	1.24	0.79	0.81	1.82	1.09
	Relative enzyme activity	100.00	12.24	18.73	11.93	12.24	27.49	16.47
POD	enzyme activity (U)	3.76	0.35	1.15	0.45	0.40	1.57	1.31
	Relative enzyme activity	100.00	9.31	30.59	11.97	10.64	41.76	34.84

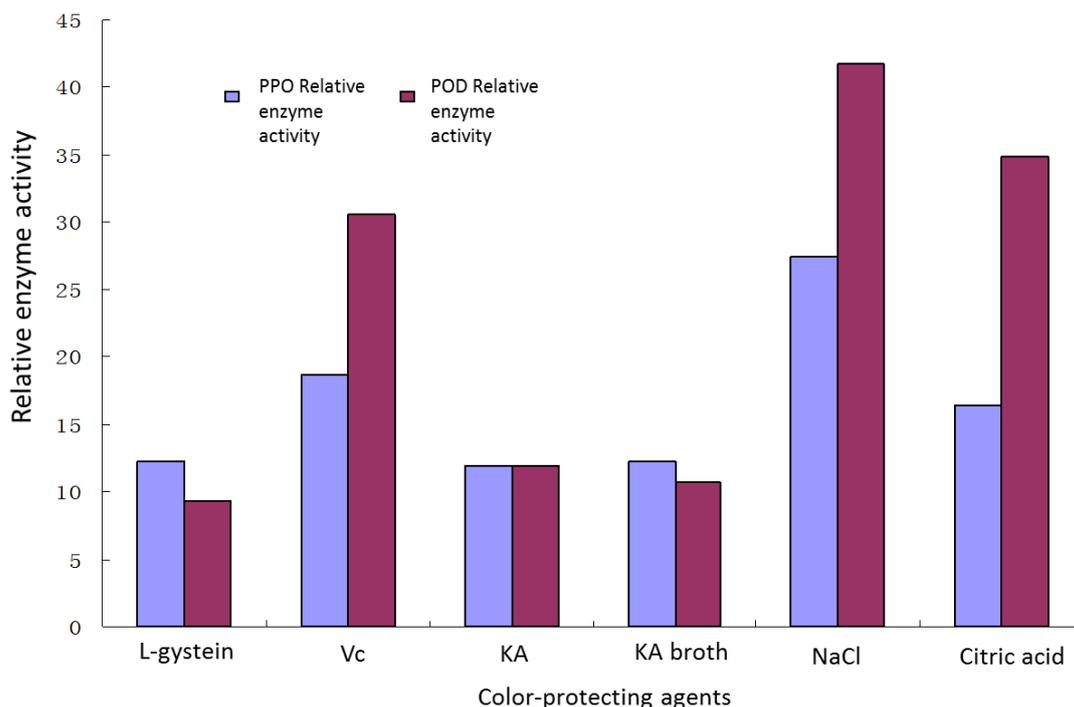


Figure 4. The residual activity of PPO and POD after color protection

The PPO and POD residual activities of banana juice after color treatment were also determined. The experimental results are shown in above results. It can be seen from the experimental results that Vc, NaCl and citric acid treated banana juice have higher PPO and POD residual activities. Both L-cysteine and kojic acid-treated banana juice had lower PPO and POD activity. It is consistent with the results of the browning experiment. The experiment not only proved that the main cause of browning of banana juice was enzymatic browning of PPO and POD, but also revealed that the mechanism of kojic acid inhibiting browning is mainly to inhibit the activity of PPO and POD.

IV. Summary

In this paper, the research on the preservation of tropical fruits, it is found that kojic acid has obvious preservation effect on papaya and wax apple. Among them, the kojic acid-treated papaya had less loss of total acid, total sugar, Vc and water than the control group; the rot rate was about 35% lower than that of the control group; however, from the hardness index, kojic acid treatment The papaya skin hardness increases and the skin layer becomes thicker. The kojic acid treated wax apple has better water retention performance. Under the condition that the 12d control group has completely rotted, the kojic acid treated wax apple is still smooth in the epidermis, the color is bright, and the whole fruit shows a healthy state and can still be eaten. In the study of the color protection of banana juice, it was found that kojic acid had a good inhibitory effect on PPO and POD activities. Both 2% kojic acid solution and kojic acid fermentation broth can achieve a better browning inhibition effect.

For the application study of kojic acid, fewer tropical fruit varieties were selected. Especially for deep processing products of some tropical fruits, there is without deep application research on kojic acid. As a natural additive, kojic acid has the advantages of high efficiency, safety and stability. It will be widely used in the preservation of tropical fruits. Therefore, strengthening relevant research can provide corresponding technical support for the application of kojic acid in the preservation of tropical fruits.

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