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A cold-resistant preservative based on γ -aminobutyric acid and its preparation method and application

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ABSTRACT

The invention discloses a cold-resistant preservative based on γ -aminobutyric acid (GABA) and its preparation method and application. GABA and sodium alginate are the effective components of the anti-cold preservative, and their contents are 100 ~ 500 $\mu\text{g/mL}$ and 0.01 ~ 0.05 g/mL respectively.

The anti-cold preservative of the invention can effectively reduce the cold injury symptoms of the cold sensitive fruit, improve the cold resistance performance of the fruit, delay the increase of the permeability of the cell membrane of the fruit and the process of membrane lipid peroxidation, thereby better maintaining the quality of the fruit and prolonging the storage and preservation period of the fruit. The anti-cold preservative in the invention has the advantages of safety, non-toxicity and good preservative effect, and can avoid potential hazards to human beings and the environment caused by the use of chemical preservative. The preparation method of the anti-cold preservative is simple, easy to operate, convenient to use, better conforms to the current design and research direction of the anti-cold preservative, and has better practical value and popularization and application prospect.

A cold-resistant preservative based on γ -aminobutyric acid and its preparation method and application

TECHNICAL FIELD

[01] The invention belongs to the technical field of fresh-keeping, in particular to an anti-cold preservative based on γ -aminobutyric acid combined with sodium alginate and its application in fruit fresh-keeping.

BACKGROUND

[02] After harvest, the fruit is still a living body, carrying out vigorous life activities. Low temperature can inhibit the respiratory metabolism of fruits and reduce the activities of microorganisms, thus delaying the senescence process and prolonging the shelf life of fruits. Therefore, low temperature storage is one of the most effective techniques to store and preserve postharvest fruits. However, for cold-sensitive fruits such as bananas, passion fruits and mangoes, inappropriate low temperature is easy to cause physiological metabolic disorders and cell membrane structure damage, and then causing chilling injury symptoms. This symptom usually does not appear until the fruit is transferred from low temperature to normal temperature, so it is not easy to be found and controlled in time. Chilling injury will lead to serious deterioration of fruit edible quality and even failure to mature normally, thus limiting the application of low-temperature storage and transportation in cold-sensitive fruits. With the wide application of cold chain transportation, it is of great significance to explore the prevention and control methods of cold damage of cold sensitive fruits during low temperature storage.

[03] At present, the methods to control chilling injury of refrigerated fruits include physical methods, chemical methods and biological methods. Physical methods mainly include heat treatment, cold shock, ultra-high pressure, irradiation, ice temperature storage, low temperature stress, etc. This kind of method has good effect, but it has the disadvantages of large investment, high energy consumption, and is not conducive to large-scale popularization and use. Biological methods mainly refer to the use of antagonistic bacteria, natural extracts and transgenic technology to control the

chilling injury of fruits and vegetables, which has the disadvantages of immature, high cost, and is not easy to be accepted. Chemical method is a widely used treatment method in China, which has the advantages of low cost and simple operation. There are many kinds of chemical preservatives such as monomethyl cyclopropene, methyl jasmonate vinegar, betaine, and so on, which can well avoid the health hazards and environmental pollution caused by traditional chemical preservatives. However, the effect of the above-mentioned fruit chilling injury control technology on cold-sensitive fruits still needs to be improved.

[04] GABA is a kind of four-carbon non-protein amino acid which exists widely in the free state in animals, plants and microorganisms. As a new food functional factor, it has been approved for use in food, and the U.S. Environmental Protection Agency (EPA) has also confirmed that this substance has no toxic or side effects on the environment and human health when used on plants or fruits. Therefore, the research and development of GABA has become a new hot spot. In recent years, there have been a few reports on GABA regulating postharvest fruit quality. Studies have shown that GABA treatment has the effects of delaying the ripening and senescence process of postharvest fruits, inducing and improving the disease resistance of fruits, and reducing the occurrence of browning and chilling injury of fruits. Chinese invention patent "A SPECIAL PRESERVATIVE FOR LOTUS PLUMS" (ZL201310486815.0) and "A METHOD FOR PRESERVING LOTUS PLUMS" (ZL201310486816.5) disclose a preservative for lotus plums consists of chitosan, GABA, loquat leaf extract, calcium lactate and food grade glycerol, wherein the content of GABA is 2-5g/L. Chinese invention patent "A SPECIAL ANTISTALING AGENT FOR CHERRY" (ZL201310498239.1) disclose a special antistaling agent for cherry, which mainly comprises chitosan, GABA, honeysuckle, chrysanthemum and pomegranate peel, wherein the content of GABA is 3-5g/L. A Chinese invention patent application "METHOD FOR KEEPING PEACH FRESH AT LOW TEMPERATURE AND APPLICATION OF Γ -AMINOBUTYRIC ACID IN KEEPING PEACH FRESH AT LOW TEMPERATURE" (CN 103300141A) discloses a method for keeping peach fresh at low temperature by soak peach fruit in 1-10 mM GABA solution for 5-30min, which can significantly inhibit that occurrence of chilling injury of peach fruit and maintain the quality of fruit postharvest. The Chinese invention authorization patent "A

STRAWBERRY PRESERVATIVE CONTAINING FUNCTIONAL AMINO ACIDS" (ZL201310076524.4) discloses a strawberry preservative composed of GABA, arginine, ascorbic acid, calcium chloride, acetic acid and chitosan, wherein the content of GABA is 0.2-0.5 g/L. Chinese invention application patent "STRAWBERRY FRUIT FRESH-KEEPING LIQUID, STRAWBERRY FRUIT FRESH-KEEPING BOX AND ITS USE METHOD " (CN104222264A) discloses a strawberry fruit preservative consists of strawberry leaf digestive juice, calcium chloride, ascorbic acid, citric acid, sucrose, arginine, GABA, proline and water, wherein the content of GABA is 0.1-0.2 g/L. Chinese patent application "A FRESH-CUT YAM PRESERVATIVE" (CN105104505A) discloses a fresh-cut yam preservative composed of GABA, calcium lactate, zinc acetate, garlic extract and loquat leaf extract, with that maximum content of GABA being 1g/L. The apricot fresh-keeping solution disclosed in the Chinese invention application patent "A BIOLOGICAL FRESH-KEEPING METHOD FOR APRICOT" (CN105454412A) consists of spermidine, olive leaf extract, GABA, arginine, ascorbic acid, calcium chloride and chitosan, wherein the GABA content is 1-2g/L. The Chinese invention authorization patent "BIOLOGICAL FRESH-KEEPING LIQUID BASED ON Γ -AMINO BUTYRIC ACID COMBINED WITH BIOCONTROL YEAST AND ITS USE" (ZL201410085393.0) discloses a prevention and control method for controlling pear fruit penicillium disease by using γ -aminobutyric acid combined with biocontrol yeast, and the use concentration of GABA is 90-110g/L. The method comprises the following steps: firstly, spraying GABA solution on the surface of pear fruit, inducing at room temperature for 22-26 hours, and then spraying the same volume of *cryptococcus laurenensis* or *rhodozyma hodophylla* cell suspension on the surface of the obtained induced pear fruit, so as to improve the antagonistic activity of biocontrol yeast and achieve the effect of preventing and controlling pear fruit penicillium.

[05] Sodium alginate is a natural polysaccharide extracted from algae, which can form edible membrane and has the characteristics of high strength and good isolation. Sodium alginate can obviously reduce the respiration and browning reaction of food, and has been widely used in fruit and vegetable preservation. A Chinese patent entitle "A METHOD FOR PRESERVING FRESH LOTUS CANOPY" (ZL201510674982.7) discloses a color protection and preservative agent composed of citric acid, EDTA-2Na

and sodium alginate wherein the concentration of sodium alginate is 2.50-4.50% by mass percentage.

[06] The antistaling agents disclosed in the invention patents "SELENIUM-CONTAINING FILM AGENT FOR BLUEBERRY PRESERVATION" (ZL201310455171.9) and "SELENIUM-CONTAINING FILM AGENT FOR BIG CHERRY PRESERVATION" (ZL201310455353.6) authorized by China are all composed of chitosan, sodium alginate, tea polyphenol, calcium chloride, vitamins, fructose, sodium chloride, ethanol, acetic acid and loofah water, wherein the content of sodium alginate is 0.05-0.25% and 0.1-0.3%, respectively. Chinese invention application patent "A PASSION FRUIT PRESERVATIVE AND ITS APPLICATION" (CN108208147A) discloses a passion fruit preservative and its application, which is prepared by compounding ten substances such as sodium alginate, wherein the content of sodium alginate is 2.4-3.5%. Chinese invention patent application "A PASSION FRUIT ANTISTALING AGENT AND ITS PREPARATION METHOD" (CN107361133A) discloses an antistaling agent with modified sodium alginate as the main component, and its content reaches more than 40%. Chinese invention application patent "A FRUIT AND VEGETABLE PRESERVATIVE AND ITS PREPARATION METHOD AND APPLICATION" (CN108157494A) discloses a fruit and vegetable preservative prepared by compounding seven substances and its preparation method and application, wherein the content of sodium alginate is 1-3%. Chinese patent application for invention, "A GRANULATED SUGAR ORANGE ANTISTALING AGENT AND ITS PREPARATION METHOD" (CN107801772A), discloses an antistaling agent composed of five plant extracts and three film-forming substances, wherein the content of sodium alginate is 5-10%. Chinese invention application patent "A LONGAN PRESERVATION METHOD" (CN107494ed sodium 717A) discloses a longan preservative, which consists of two modified sodium alginate and several plant essential oils. Chinese invention patent application "A FRUIT TRANSPORTATION PRESERVATIVE" (CN106172725A) discloses a composite preservative composed of 13 substances, of which sodium alginate content is only 0.2-0.4%. Chinese patent application for invention "COMPOSITE PRESERVATIVE FILM BASE ON PLANT-DERIVED EXTRACTANT AND PREPARATION METHOD AND APPLICATION THEREOF" (CN106720282A) discloses a composite preservative film, based on plant-

derived extract, which characterized in that the preservative film is composed of an extract extracted by ultrasonic wave from plant leaves and a composite solution of sodium alginate, citric acid and sucrose ester wherein the content of sodium alginate is 0.4%. Chinese invention application patent "GREEN EDIBLE FRUIT AND VEGETABLE PRESERVATIVE BASED ON SEAWEED POLYSACCHARIDE AND ITS PREPARATION METHOD" (CN105918449A) discloses a green edible fruit and vegetable preservative based on seaweed polysaccharide, which is characterized in that fruit and vegetable preservative is mainly prepared by reaction of sodium alginate, amidation accelerator and L# cysteine. Chinese invention application patent "A SIRAITIA GROSVENORII ANTISTALING AGENT AND ITS ANTISTALING METHOD" (CN107897357A) describes an antistaling agent which consists of six plant extract and four film-forming substances, wherein the concentration of sodium alginate is 2.5-4.0%. Chinese invention patents "STRAWBERRY PRESERVATION METHOD AND ANTISTALING AGENT" (CN108208150A) and "EDIBLE COMPOSITE FILM ANTISTALING AGENT AND ANTISTALING PROCES METHOD FOR AGARICUS BISPORUS" (CN104054818A) disclose an edible composite film antistaling agent for *Agaricus bisporus*, which is characterized in that it consists of sodium alginate, ascorbic acid and glycerol, wherein the content of sodium alginate is 2-3%. To sum up, sodium alginate is usually used as a film-forming agent to compound with antioxidant substances, preservatives or some plant extracts to make preservatives. At present, most of the disclosed preservatives containing sodium alginate have relatively complex components, and there are few reports on fruits and vegetables cold-resistant preservatives containing sodium alginate.

[07] Judging from the application of the above-mentioned γ -aminobutyric acid and sodium alginate, the dosage of γ -aminobutyric acid and sodium alginate has certain differences according to different uses and formulas, and lacks certain universality and reference, and there is no relevant report on the common use of the two in the prior art.

SUMMARY

[08] The invention aims to provide a cold-resistant preservative based on γ -aminobutyric acid combined with sodium alginate, which is designed to meet the

preservation requirements of cold-sensitive fruits, so as to achieve the application purpose of prolonging the storage period of cold-sensitive fruits.

[09] The technical scheme adopted in this application is described in detail as follows.

[010] The invention relates to a cold-resistant preservative based on γ -aminobutyric acid combined with sodium alginate, and its effective components are γ -aminobutyric acid and sodium alginate.

[011] Among them, the concentration of γ -aminobutyric acid is 100 ~ 500 $\mu\text{g/mL}$, the concentration of sodium alginate is 0.01 ~ 0.015 g/mL, the concentration of xanthan gum is 0.005 g/mL, and the concentration of glycerol is 0.005 g/mL.

[012] The invention also provides a preparation method of the above-mentioned anti-cold preservative based on γ -aminobutyric acid combined with sodium alginate, which specifically comprises the following steps:

- (1) Sodium alginate is dissolved into sol by adding water;
- (2) Adding xanthan gum into sodium alginate solution for blending;
- (3) Adding γ -aminobutyric acid into the blended sodium alginate-xanthan gum solution and mixing evenly;
- (4) Adding glycerol and stirring evenly;
- (5) Adding water to make up to the corresponding volume;
- (6) Ultrasonic treatment of the above mixed solution and vacuum standing to obtain the anti-cold preservative.

[013] The invention also provides an application of the above-mentioned anti-cold preservative based on γ -aminobutyric acid combined with sodium alginate in cold-sensitive fruits, wherein the application is operated according to the following methods:

- (1) Select fresh fruits with uniform size, consistent color, no diseases and insect pests, integrity and no damage;
- (2) At normal temperature, after the fruit is completely soaked in the anti-cold preservative for 5-10 min, the fruit is taken out and the water on its surface is drained;
- (3) After the fruit surface is dried, store it at suitable low temperature.

[014] Compared with the prior art, the invention has the advantages that:

1. The γ -aminobutyric acid used by the invention is a natural regulating substance widely existing in animals and plants, has the effects of sedation, anxiety resistance, blood pressure reduction, brain vitality enhancement, anti-aging, disease pain relief, obesity prevention and the like, and is beneficial to human health; sodium alginate is a natural polysaccharide extracted from algae, which can form edible film and has the characteristics of high strength and good isolation.

Experiments show that the anti-cold preservative prepared by using γ -aminobutyric acid combined with sodium alginate in the invention can effectively reduce the chilling injury symptoms of cold-sensitive fruits, improve the cold-resistant performance of fruits, delay the increase of cell membrane permeability of fruits and the process of membrane lipid peroxidation, thus better maintaining the quality of fruits and prolonging the storage and preservation period of fruits; in addition, for the natural source characteristics of γ -aminobutyric acid and sodium alginate, the preservative in the invention has the advantages of safety, non-toxicity and good preservative effect, can not only avoid potential hazards to human beings and the environment caused by the use of chemical preservative, but also can enhance the nutritional and health-care effects of fruits;

2. That preparation method of the antistaling agent of the invention is simple, the operation is simple, the use is convenient, and the antistaling agent better conforms to the current antistaling agent design, research and

development direction, so that the antistaling agent has better practical value and commercial popularization and application prospect.

BRIEF DESCRIPTION OF THE FIGURES

[015] FIG. 1 shows that relative conductivity of banana peel treated by the method of Example 1 and Comparative Example;

[016] FIG. 2 shows the relative conductivity of passion fruit peels treated by the methods of Example 2 and Comparative Example;

[017] FIG. 3 shows the malondialdehyde content of banana pericarp treated by the method of Example 1 and Comparative Example;

[018] FIG. 4 shows the malondialdehyde content of passion fruit peel treated by the method of Example 2 and Comparative Example;

[019] Fig. 5 show that chilling injury index of banana fruit treated by the method of Example 1 and Comparative Example;

[020] FIG. 6 shows the chilling injury index of passion fruit treated by the method of Example 2 and Comparative Example.

DESCRIPTION OF THE INVENTION

[021] The present application will be further explained in connection with examples below. Before introducing specific examples, some materials in the following examples will be briefly introduced as follows.

[022] Experimental Material:

[023] Food grade γ -aminobutyric acid, purity 98%.

[024] Green bananas, purchased from the farmers market in Yanshan Town, Guilin City.

[025] Passion fruit, 70% ripe, purchased from Dongyang Village, Guanyang County, Guilin City and transported directly to the laboratory for treatment after harvest.

[026] EXAMPLE 1

[027] The formula of the anti-cold preservative based on γ -aminobutyric acid combined with sodium alginate provided in this example is as follows:

[028] γ -aminobutyric acid 500 μ g/mL, sodium alginate 0.01 g/mL, xanthan gum 0.005 g/mL, glycerol 0.005 g/mL.

[029] The preparation of the anti-cold antistaling agent comprises the following steps: firstly, sodium alginate is dissolved into a sol state by adding water, then xanthan gum, γ -aminobutyric acid and glycerol are sequentially added and mixed evenly. Water is added for constant volume, ultrasonic treatment is carried out, and finally vacuum standing is carried out to obtain the anti-cold antistaling agent.

[030] The application method of the anti-cold preservative is to soak banana fruits in the above-mentioned anti-cold preservative for 10 min, then take out, drain the water on the surface of the fruits, put them into a plastic basket lined with preservative film, and store at 4 ± 1 °C.

[031] EXAMPLE 2

[032] The formula of the anti-cold preservative based on γ -aminobutyric acid combined with sodium alginate provided in this example is as follows:

[033] Aminobutyric acid 300 μ g/mL, sodium alginate 0.015 g/mL, xanthan gum 0.005 g/mL, glycerol 0.005 g/mL.

[034] The preparation method of the anti-cold preservative was the same as that of Example 1

[035] Application method of anti-cold preservative: soak passion fruit in the above-mentioned anti-cold preservative for 5 min, then take it out, drain the water on

the surface of the fruit, put it into a plastic basket lined with preservative film, and store it at 4 ± 1 °C.

[036] Comparative proportion

[037] The anti-cold preservative in the example was changed to clean water, and the rest of the operations were the same as in the example.

[038] Test:

[039] The fruits were sampled regularly during storage and then divided into two parts, one part was used to measure the permeability of pericarp cell membrane and membrane lipid peroxidation, and the other part was used to investigate the occurrence of chilling injury after being stored at normal temperature for one day.

1. Pericarp cell membrane permeability: relative conductivity method.

The test results are shown in FIGS. 1 and 2. FIG. 1 shows the relative conductivity of banana peel treated by the method of Example 1 and Comparative Example. FIG. 2 shows the relative conductivity of passion fruit peels treated by the methods of Example 2 and Comparative Example.

2. Determination of lipid peroxidation: the content change of malondialdehyde was used to evaluate the degree of lipid peroxidation in fruit sebum.

The test results are shown in FIGS. 3 and 4. FIG. 3 shows the malondialdehyde content of banana peel treated by the method of Example 1 and Comparative Example. FIG. 4 shows the malondialdehyde content of passion fruit peel treated by the method of Example 2 and Comparative Example.

3. Incidence and index of chilling injury.

Investigate the occurrence of chilling injury of fruits in different storage periods according to the classification standard of chilling injury,

and then calculate the chilling injury index of pericarp according to the following formula.

[040] Chilling injury index = $\frac{\sum(\text{chilling injury series} \times \text{number of fruits corresponding to chilling injury series})}{(\text{total number of fruits} \times 4)} \times 100\%$

[041] Classification standard for chilling injury of fruits

Series number	Symptoms
0	The pericarp is bright green and has no symptoms of chilling injury.
1	The pericarp became dark and lost luster, with small brown sunken spots. The chilling injury area accounted for less than 25% of the total pericarp area.
2	Browning of pericarp, enlargement of sag area, chilling damage area accounting for 25-50% of total pericarp area
3	The pericarp became black, the area of sunken spots expanded and showed water stains, and the chilling damage area accounted for 50-75% of the total pericarp area.
4	Most of the pericarp turned black and the water-stained area expanded and became one piece, and the chilling damage area accounted for more than 75% of the total pericarp area.

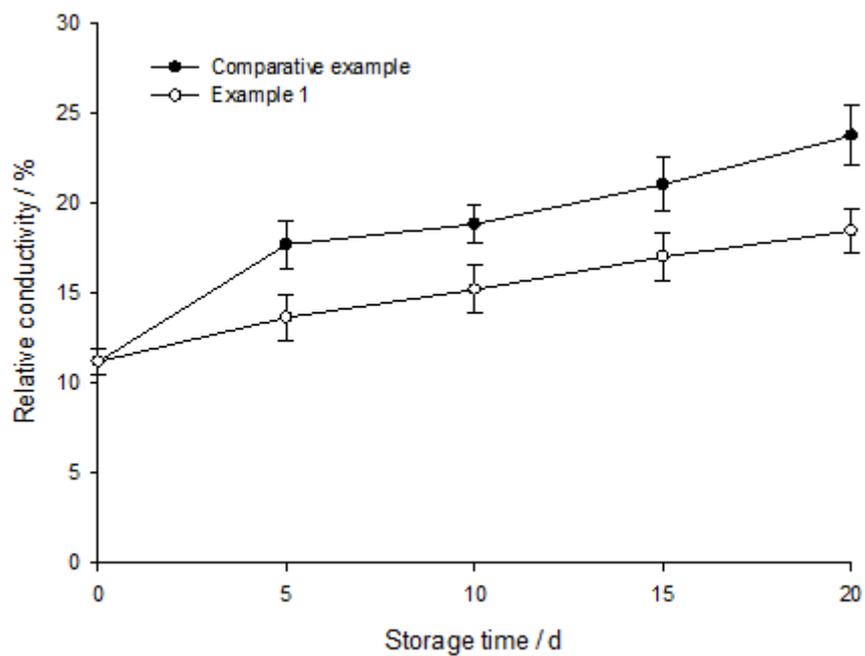
[042] The test results are shown in FIGS. 5 and 6. FIG. 5 shows the chilling injury index of banana fruits treated by the method of Example 1 and Comparative Example. FIG. 6 shows the chilling injury index of passion fruit treated by the method of Example 2 and Comparative Example.

[043] Although the invention has been described with reference to specific examples, it will be appreciated by those skilled in the art that the invention may be embodied in many other forms, in keeping with the broad principles and the spirit of the invention described herein.

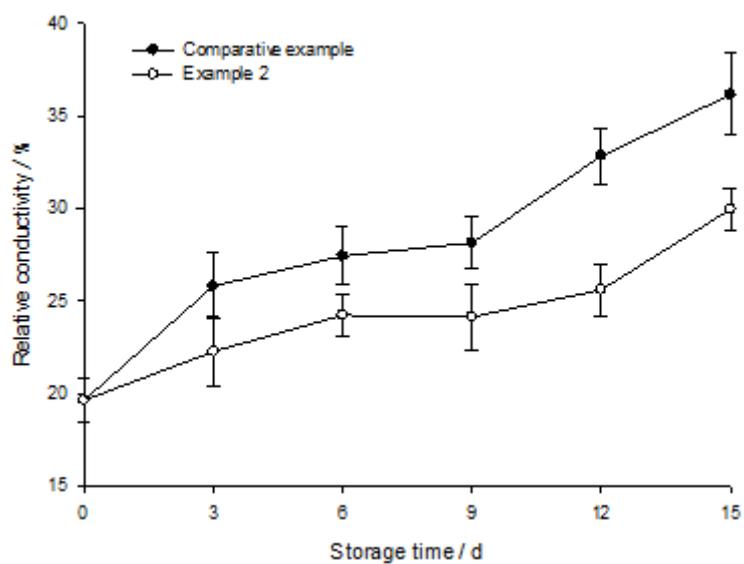
[044] The present invention and the described embodiments specifically include the best method known to the applicant of performing the invention. The present invention and the described preferred embodiments specifically include at least one feature that is industrially applicable

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. The invention relates to a cold-resistant preservative based on γ -aminobutyric acid, which is characterized in that the effective components are γ -aminobutyric acid and sodium alginate.
2. The anti-cold preservative based on γ -aminobutyric acid according to claim 1 is characterized in that the concentration of γ -aminobutyric acid is 100 to 500 $\mu\text{g/mL}$, the concentration of sodium alginate is 0.01 to 0.015 g/mL, the concentration of xanthan gum is 0.005 g/mL, and the concentration of glycerol is 0.005 g/mL.
3. A method for preparing a γ -aminobutyric acid-based cold-resistant preservative according to any one of claim 1 and 2 is characterized in that that method comprises the following steps:
 - (1) Sodium alginate is dissolved into sol by adding water;
 - (2) Adding xanthan gum into sodium alginate solution for blending;
 - (3) Adding γ -aminobutyric acid into the blended sodium alginate-xanthan gum solution and mixing evenly;
 - (4) Adding glycerol and stirring evenly;
 - (5) Adding water to make up to the corresponding volume;
 - (6) Ultrasonic treatment of the above mixed solution and vacuum standing to obtain the anti-cold preservative.
4. That application of the γ -aminobutyric acid-based cold-resistant antistaling agent according to any one of claims 1 and 2 in cold-sensitive fruit is characterized in that the application is carried out according to the following method:
 - (1) Select fresh fruits with uniform size, consistent color, no diseases and insect pests, integrity and no damage;
 - (2) At normal temperature, after the fruit is completely soaked in the anti-cold preservative for 5-10 min, the fruit is taken out and the water on its surface is drained;
 - (3) After the fruit surface is dried, store it at a suitable low temperature.



FIG, 1



FIG, 2

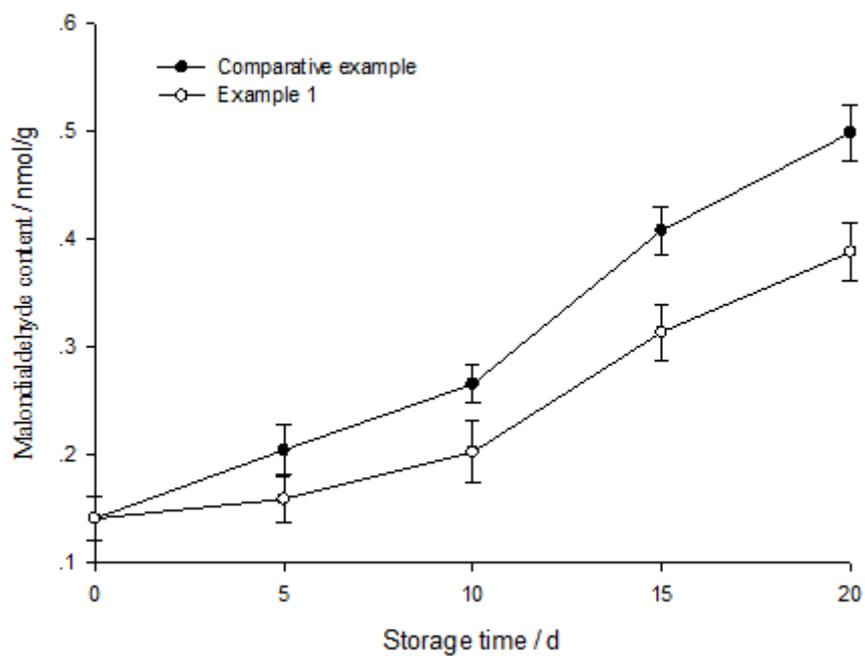


FIG. 3

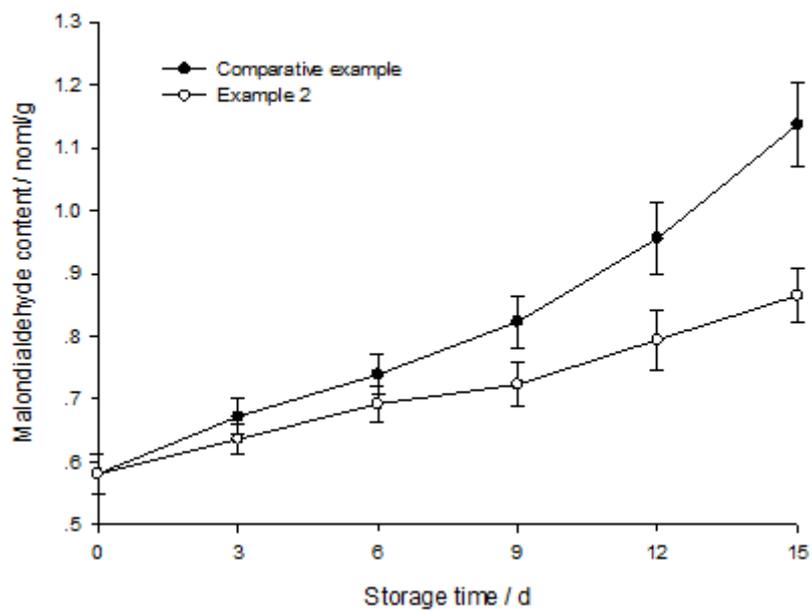


FIG. 4

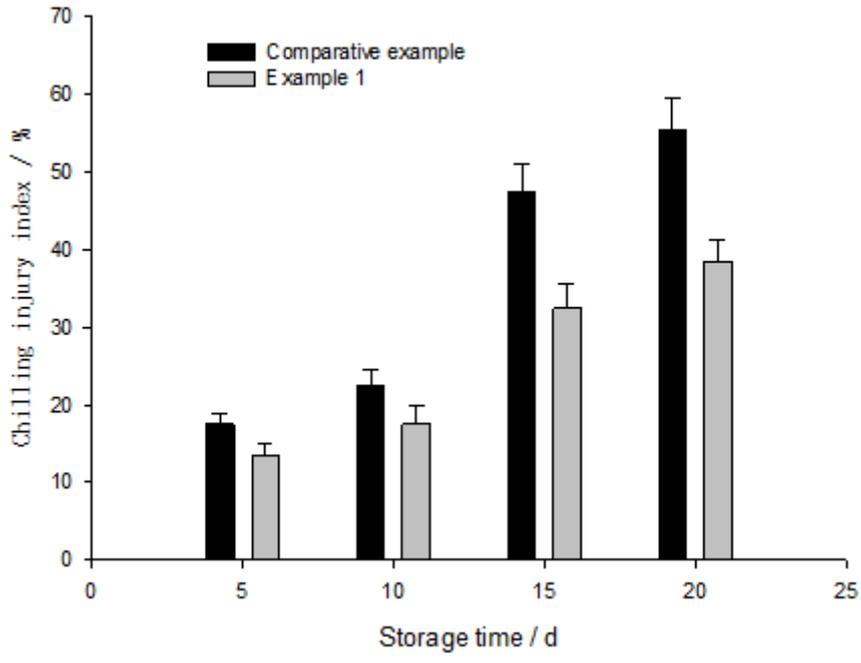


Fig. 5

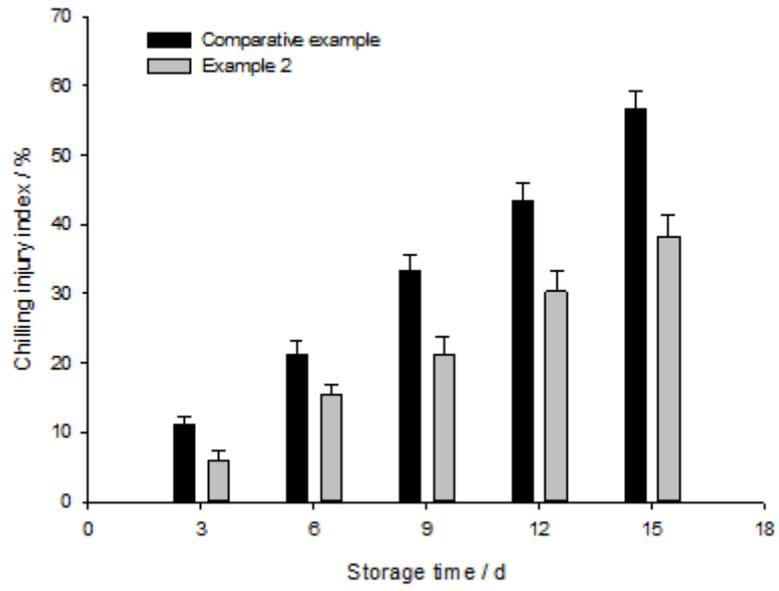


Fig. 6