

## Research Article

### Research of Stability Influencing Factors of Rebaudioside A Powder

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**Abstract:** It was found that the purity of non-crystal Rebaudioside A (Reb-A) decreased almost 11.48% along with time at room temperature conditions by long term tracking and detection experiment for 33 months. While the purity of crystal Reb-A kept almost the same. The influence of heating, oxygen, sunlight, UV and  $\gamma$  ray on the stability of both crystal and non-crystal was studied by experiments. The results indicated that all these factors expect oxygen could precipitate the degradation of Reb-A powder. And the stability of crystal Reb-A was much better than non-crystal Reb-A. The divergence between the stability of crystal and non-crystal Reb-A demonstrated that the existence of crystal structure in Reb-A powder was the key factor which would affect its stability.

**Keywords:** Crystal, non-crystal, Reb-A powder, stability influencing factors

## INTRODUCTION

Steviosides is a new type of natural sweeteners extracted from leaves of stevia (Madan *et al.*, 2010) and belongs to tetracyclic diterpene compounds (Xu, 2004). It has been found about 11 kinds of sweet ingredients from leaves of stevia, known as Stevioside (STV), Rebaudioside A~F (Reb-A~F), Duke glycosides (Du-A), Rubusoside (Rub) and steviol (Geuns, 2013; Shizhen, 1989). Rebaudioside A (Reb-A) is the one of the main ingredients in steviosides which has the highest sweetness and the cleanest taste. Rebaudioside A is used in food, medicine, wine, daily chemical and other industries widely.

It has been reported that Reb-A featured a high stability. Firstly with thermal stability, sweetness of steviosides remained almost the same after heating for 2 h at 95°C, even for 9 h, in addition with better performance of acid and alkali resistance compared with other sweeteners. The survival rate was 90% after saved for 6 months at pH condition was 3.1 to 4.1. And it was almost unchanged after heating for 1 h at 100°C when pH condition was 3 to 9 (FeiSheng *et al.*, 2009). While, there were some reports indicated that Reb-A would have a certain loss in aqueous solution and pH was 2 to 8 (Prakash *et al.*, 2008; Schiffinana *et al.*, 2000). And when pH was more than 9 or less than 3, Reb-A would be decomposed after heating for a long time and the sweetness would reduce accordingly (Kroyer, 1999). It was very stable to sunlight both powder and solution of steviosides. The survival rate of 0.11% steviosides solution was more than 90% after

exposed in the sun. Chang and Cook (1983) researched the stability of steviosides and Reb-A in carbonated soft drinks and found that it kept almost the same both steviosides preserved for 5 months and Reb-A preserved for 3 months at room temperature. But Reb-A lost nearly 20% and steviosides still kept unchanged after illumination for a week.

However it was found that Reb-A powder would have different stability due to the difference of molecular arrangement. Tracking and detection for 33 months of the non-crystal Reb-A indicated that content of Reb-A reduced gradually from 97.53% originally to 86.26 finally. It decreased more than eleven percent in the whole process. Therefore, factors that would affect the stability of Reb-A powder were studied.

## MATERIALS AND METHODS

**Materials:** Samples of Reb-A, Tianjin Meilun Medical Co., Ltd; acetonitrile, HPLC, TIANJIN guangfu chemical institute; standard substance of Reb-A, Wako Pure Chemical Industries, Ltd.; N<sub>2</sub> and O<sub>2</sub>, TIANJIN shunhe gas sales co., Ltd.; electro-thermostatic blast oven, TIANJIN zhonghuan experiment electric co., Ltd.; cobalt irradiation units and UV irradiation units, TIANJIN institute of technical physics; LC-10A HPLC, Shimadzu, Japan; SPD-10A UV, Shimadzu, Japan; BS110S electronic scales, Sartorius.

**Detection method by HPLC:**

**Preparation of the standard solutions:** The standard of stevioside was dried for 2 h at 105°C before used.

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Dissolved 120, 90, 60 and 30 mg of this standard in acetonitrile/water (80/20) solution, diluted to 25 mL.

**Conditions for HPLC:** Chromatographic column: Kromasil 100-5 NH<sub>2</sub> (250×4.6 mm, 5 μm); mobile phase: acetonitrile/water = 80/20; column temperature: 30°C; flow rate: 1.2 mL/min; sample size: 15 μL; determine wavelength: 210 nm.

**Computational formulas:** Detected the four standard solution of stevioside and drew standard curve between peak area A and concentration C, firstly. Then detected the samples, recorded peak areas of Reb-A, Reb-C, STV, Du-A and Rub as Aa, Ac, As, Ad and Ab. Aa, Ac, As, Ad and Ab multiplied 1.20, 1.18, 1.00, 0.98 and 0.80, respectively could get Aa', Ac', As', Ad' and Ab'. The content of these five compounds P<sub>i</sub> (%) could be reached by calculation as the formula below:

$$P_i = \frac{A_i'}{k} \times \frac{25}{m} \times 100 \quad (1)$$

In formula (1),

i = a, b, s and d

m = Quality of samples

k = The slope of calibration curve

k = A/C

The sum of Pa, Pc, Ps, Pd and Pb was the content of total glycosides.

#### INFLUENCE OF STORAGE TIME ON PURITY OF CRYSTAL AND NON-CRYSTAL REB-A

It was found that the purity of non-crystal Reb-A decreased along with time at conventional storage conditions. Therefore, we did a tracking and detection experiment for almost 3 years as follows. Dissolved crystal Reb-A with water to prepare Reb-A solution (mass concentration was 45%), destroyed the crystal structure by spray drying and got the non-crystal Reb-A. Saved both the crystal and non-crystal Reb-A routinely at room temperature. Determined the content of Reb-A every 3 months to inspect the variation trend of content for both crystal and non-crystal Reb-A at conventional storage conditions.

**Influence of heating temperature on purity of crystal and non-crystal Reb-A:** Weighed 2.00 g crystal and non-crystal Reb-A powder to clean weighing bottle and heated for 2 h at 100, 120, 150 and 180°C separately. Determined the content of Reb-A by HPLC. Inspected the influence of heating temperature on purity of crystal and non-crystal Reb-A by the variations of content.

**Influence of heating time on purity of crystal and non-crystal Reb-A:** Weighed 2.00 g crystal and non-crystal Reb-A powder to clean weighing bottle and heated for 1 h, 2 h, 3 h, 4 h and 5 h at 150°C separately.

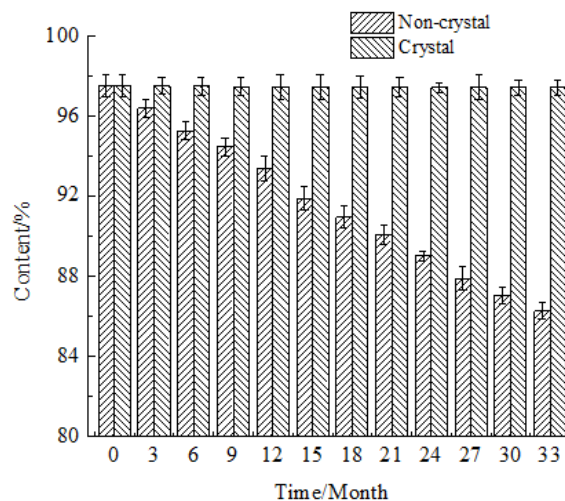


Fig. 1: Influence of storage time on purity of crystal and non-crystal Reb-A

Determined the content of Reb-A by HPLC. Inspected the influence of heating time on purity of crystal and non-crystal Reb-A by the variations of content.

**Influence of oxygen on purity of crystal and non-crystal Reb-A:** Weighed 2.00 g crystal and non-crystal Reb-A powder to closed container and filled with oxygen (aerobic) and nitrogen (anaerobic) separately and heated for 4 h at 150°C. Determined the content of Reb-A by HPLC. Inspected the influence of oxygen on purity of crystal and non-crystal Reb-A by the variations of content.

**Influence of light on purity of crystal and non-crystal Reb-A:** Weighed 2.00 g crystal and non-crystal Reb-A powder to clean weighing bottle and heated for 4 h at 150°C in highlight, sunlight and dark conditions separately. Determined the content of Reb-A by HPLC. Inspected the influence of light on purity of crystal and non-crystal Reb-A by the variations of content.

**Influence of UV on purity of crystal and non-crystal Reb-A:** Weighed 2.00 g crystal and non-crystal Reb-A powder to clean pan paper and exposed them in UV for 6, 19, 27, 91 and 115 h, respectively with the 1500 μw/cm<sup>2</sup> UV intensity separately. Determined the content of Reb-A by HPLC. Inspected the influence of UV on purity of crystal and non-crystal Reb-A by the variations of content.

**Influence of irradiation on purity of crystal and non-crystal Reb-A:** Weighed 2.00 g crystal and non-crystal Reb-A powder to closed foil bags and exposed in Co60 for 6, 12, 24, 48 and 72 h, respectively with the 8 KGy/h Co60 intensity separately. Determined the content of Reb-A by HPLC. Inspected the influence of irradiation on purity of crystal and non-crystal Reb-A by the variations of content (Fig. 1).

## RESULTS AND DISCUSSION

**Influence of storage time on purity of crystal and non-crystal Reb-A:** Content of Reb-A in non-crystal Reb-A decreased along with time and the change was significantly by one-way analysis of variance. Content of Reb-A in non-crystal Reb-A decreased from 97.73 to 86.25% along with time and the change was significantly by one-way Analysis of Variance (ANOVA). Content of Reb-A in crystal Reb-A kept almost the same. This indicated that non-crystal Reb-A was poorer in stability than crystal Reb-A.

**Influence of heating temperature on purity of crystal and non-crystal Reb-A:** Influence of heating temperature on purity of crystal and non-crystal Reb-A was as shown in Fig. 2. Both the crystal and non-crystal Reb-A decreased in different degrees after heating for 2 h at 100, 120, 150 and 180°C, respectively. ANOVA indicated that the decrease of crystal Reb-A was very little and it was non-significant. But non-crystal Reb-A was on the contrary. The purity of non-crystal Reb-A decreased significantly with the rise of heating temperature. And it became 65.58% from 99.15% after heating for 2 h at 180°C and the color became yellow. This stated that Reb-A would be destructed greatly at 180°C. 180°C was not fit to simulate the destruction of Reb-A at room temperature. So 150°C was selected to study the influence of heating on the purity of crystal and non-crystal Reb-A.

**Influence of heating time on purity of crystal and non-crystal Reb-A:** Influence of heating time on purity of crystal and non-crystal Reb-A was as shown in Fig. 3. Both the purity of crystal and non-crystal Reb-A decreased after heated for 1 to 5 h at 150°C. ANOVA indicated that the decrease of non-crystal Reb-A was significantly while the crystal Reb-A was not with the purity decreased less than 2% just at the preliminary stage. This maybe caused by the existence of a small number of imperfect crystals. These imperfect crystals degraded after heating.

**Influence of oxygen on purity of crystal and non-crystal Reb-A:** Figure 4 showed the influence of oxygen on purity of crystal and non-crystal Reb-A. The existence of oxygen had no effect on purity of Reb-A. ANOVA explained that the purity of non-crystal Reb-A was 69.15 and 69.55% after heating for 4 h both aerobic and anaerobic conditions, but it decreased almost 30% compared with original samples which was 99.15%. While the purity of crystal Reb-A kept almost the same which decreased just 4%. This indicated that oxygen was not the key factor caused the degradation of non-crystal Reb-A. And no matter what the conditions were, crystal Reb-A showed better stability than non-crystal Reb-A.

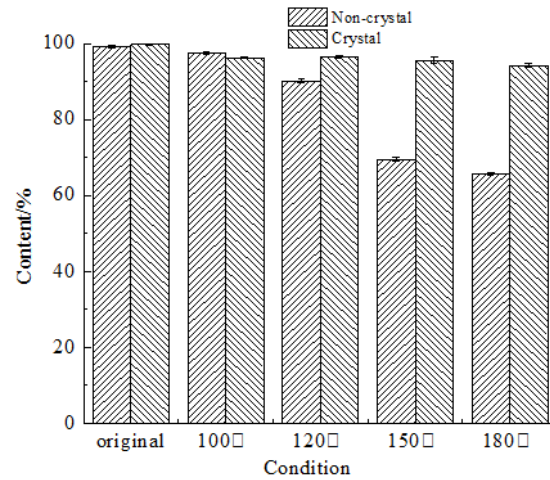


Fig. 2: Influence of heating temperature on purity of crystal and non-crystal Reb-A

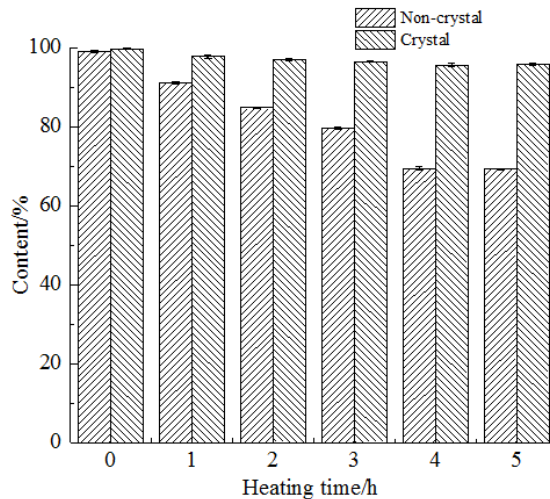


Fig. 3: Influence of heating time on purity of crystal and non-crystal Reb-A

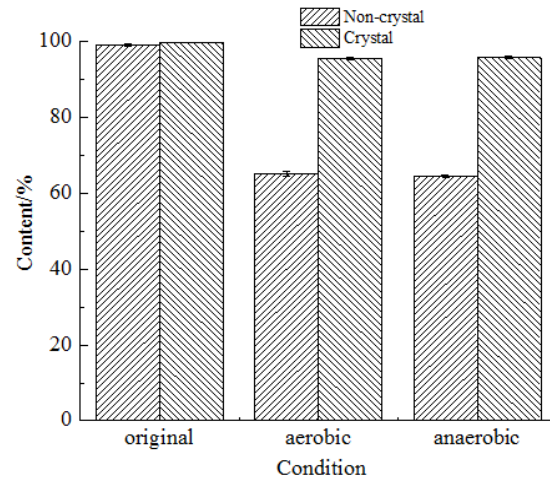


Fig. 4: Influence of oxygen on purity of crystal and non-crystal Reb-A

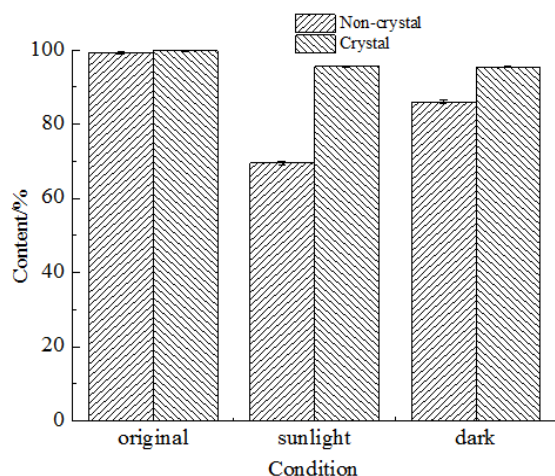


Fig. 5: Influence of light on purity of crystal and non-crystal Reb-A

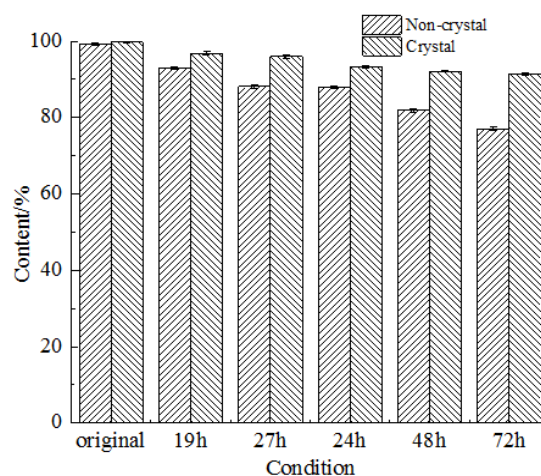


Fig. 7: Influence of irradiation on purity of crystal and non-crystal Reb-A

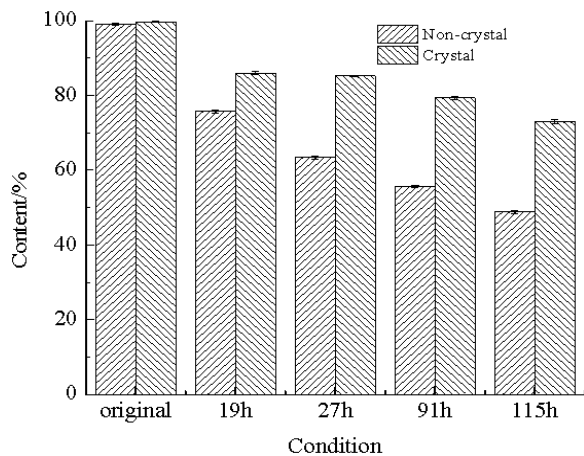


Fig. 6: Influence of UV on purity of crystal and non-crystal Reb-A

**Influence of light on purity of crystal and non-crystal Reb-A:** Influence of light on purity of crystal and non-crystal Reb-A was shown in Fig. 5. It has a great difference between the samples. ANOVA revealed that purity of non-crystal Reb-A decreased to 69.54% after heating for 4 h in sunlight conditions and to 85.95% in dark conditions. The distinction was significant. For crystal Reb-A, it decreased to about 95% and was no significant both in sunlight and dark conditions. The purity decreased just 4% compared with original samples. This maybe caused by the existence of a small number of imperfect crystals. So light had no significant effect on the purity of crystal Reb-A.

**Influence of UV on purity of crystal and non-crystal Reb-A:** UV has a stronger damage capability for the molecular structure of organic materials in sunlight, especially for materials with double bond and benzene ring. There were different quantities of double bonds in

each compound of steviosides. So UV should have great influence to steviosides. The results were shown in Fig. 6. The purity of both crystal and non-crystal Reb-A decreased greatly along with the exposed time in UV, especially for the non-crystal Reb-A which decreased more than 60% after exposed for 96 h. At the same time, crystal Reb-A decreased more than 26% and was less than non-crystal Reb-A. Compared with the factors above, UV had significant influence on stability of both crystal and non-crystal Reb-A.

**Influence of irradiation on purity of crystal and non-crystal Reb-A:** As one kind of high-energy ray, Co60 could release  $\gamma$  ray and had stronger penetrating power which was used to sterilize. It was found that the purity of both crystal and non-crystal Reb-A decreased after irradiated by different dose of Co60, as was shown in Fig. 7. ANOVA indicated that the decrease of purity of non-crystal Reb-A was very significant along with the increase of Co60 dose. While for crystal Reb-A, it was not. After irradiated for 72 h, the purity of non-crystal Reb-A decreased 22% which was almost three times of crystal Reb-A.

## CONCLUSION

The arrangement of Reb-A molecular was the critical factor which affects the stability of Reb-A samples. The arrangement of crystal Reb-A molecular was tight and with some regulation, while it was disorderly and unsystematic for non-crystal Reb-A. Because of the Van der Waals and hydrogen bond forces among molecules, crystal Reb-A was more steady than non-crystal Reb-A.

The results explained that both heating, light, UV and  $\gamma$  ray could affect the stability of Reb-A powder. Heating by oven belongs to infra red radiant heating. Molecules and atoms of the material would shake and rotate severely because of the resonance effect with red

radiant and made the temperature of the material increased. So the stability of the material could be affected by heating. Heating time should be short and temperature should be low in production of Reb-A. And vacuum drying was better than spray drying which could destroy the integrity of crystal structure. The stability could be ensured and the expiration date could be extended.

Oxygen played an important role in changes of material, especially the redox reaction. Many materials with reductibility would become other materials because of oxygen and the purity decreased. It was illustrated that oxygen had no effect on the stability of both crystal and non-crystal Reb-A by filled with oxygen and nitrogen. So packed Reb-A products with vacuum packaging could only have the effect of moisture and no influence on the stability.

99.9% energy of sunlight concentrates in the infrared, visible light and ultraviolet area. So the factor which could promote the degradation of Reb-A powder in sunlight maybe the UV. And this could be proved by the following UV experiment. UV could destroy double bond, triple bond and such unsaturated bond. There were unsaturated bonds in both the parent structure and side chains of Reb-A structure. Therefore, UV was not suitable to do sterilization in actual production. And it was very necessary to keep away from light for the storage of Reb-A.

As one kind of high-energy, short-wavelength ray,  $\gamma$  ray was penetrating. When Reb-A samples were irradiated by  $\gamma$  ray with 8 KGy/h illumination intensity at different doses, the purity of both crystal and non-crystal Reb-A decreased. Compared with the UV,  $\gamma$  ray was more powerful but the decrease was not as significant. These maybe result from the difference of functional mechanism. Illuminated for 4 h at 8 KGy/h intensity could reach the goal of sterilization in general.

On this premise, it was inappropriate to illuminate for too long time.

In conclusion, the crystal Reb-A was more stability than non-crystal Reb-A in all these conditions.

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