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Post-Harvest Stability of 5-Hydroxytryptophan in Griffonia Simplicifolia Seeds

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ABSTRACT: This study examines the stability of 5-Hydroxytryptophan (5-HTP) in harvested *Griffonia simplicifolia* seeds and attempts to establish the best temperature regime for storing harvested seeds. High Performance Liquid Chromatography (HPLC) was used to determine 5-HTP levels of seeds kept under different storage condition. The study revealed that 5-HTP is not stable in harvested seeds. The content fell at a rate of 4.62% w/w per month of storage under tropical atmospheric conditions. Further studies conducted showed that refrigeration could not preserve 5-HTP in harvested seeds. The rate of decomposition was found to be 6.77% w/w per month. However, the rate of decomposition was drastically reduced in seeds heated at 60°C for 30 min. The average rate of fall in 5-HTP content was 1.79% w/w per month.

Key words: Griffonia simplicifolia, 5-Hydroxytryptophan, Antidepressant, Serotonin, High Performance Liquid Chromatography

Introduction

Griffonia simplicifolia is a medicinal plant found principally in the West African Countries of Ghana, Ivory Coast and Togo. It is particularly common in Ghana [1]. Scientific investigations around the world show that the seeds contain high concentration of 5-Hydroxytryptophan (5-HTP). This discovery may possibly explain the medicinal and physiological properties attributed to the plant by the native Africans [1, 2]. Research has shown 5-HTP to possess antidepressant activity [3, 4]. The overwhelming advantages of 5-HTP over synthetic antidepressants in alleviating numerous effects of serotonin deficiency syndrome such as depression, anxiety, insomnia, and migraine headaches among others, [5, 6] have attracted commercialization of the seeds and are commonly exported abroad.

Effective commercialization requires that the active drug substance, 5-HTP, is present at high level and stable in harvested seeds. This would raise the level of confidence that exist between traders as poor quality of consignment may seriously affect further trade with the supplier. Unfortunately, much of the published research on 5-HTP has to do with its use in the treatment of depression with no attention given to its stability in harvested *Griffonia simplicifolia* seeds.

The average rate of decomposition of 5-HTP content in harvested *Griffonia simplicifolia* seeds stored under tropical atmospheric conditions is reported to be 4.86% w/w per month of storage. This may be due to continued enzymatic activity in harvested seeds. Storage conditions that would arrest or reduce drastically enzymatic action in harvested seeds could preserve 5-HTP levels in seeds. The optimal temperature for most enzymes falls in the range of 20–40°C, but the majority of enzymes are inactivated at temperatures above 50°C. A number of enzymes are inactivated on cooling to temperatures close to 0°C [7]. Thermal behaviours of enzymes were exploited in an attempt to establish the best temperature regime for storing seeds. Sampled seeds were kept under various conditions of temperature for varying duration and their change in 5-HTP content determined with time using HPLC.

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Experimental

Sample treatment

Seed samples were heated separately at 60°C and 75°C at 30 minutes intervals for two hours. Another seed sample was refrigerated whilst a portion of the seeds was kept under normal room temperature (28°C) to serve as control. Samples were taken at two months intervals and 5-HTP content analysed.

Preparations

100 mg of crushed seeds were extracted with 100 ml 50% methanol and filtered under suction. 0.01% w/v of pure 5-HTP was prepared from pure 5-HTP (product from Sigma) sample provided for the work by Bio-Resource International Inc. (BRI), USA.

HPLC of Crude Extract and Pure 5-HTP

The complete chromatography system consisted of Gilson model 302-pump, a rheodyne fitted with 20 µl loop and Spectra 100 variable wavelength Detector connected to a Potentiometric Recorder. A 25 cm x 4.6 mm Hichrom S10 ODS 2 analytical column was used. The mobile phase used was 3% methanol in 0.005 M NaH_2PO_4 buffer at pH 4.8. The solvent was filtered through a membrane filter, porosity 0.3 mm. A 20 µl volume of the pure 5-HTP and the seed extract were loaded and injected in turn onto the column. The eluent was monitored at 275 nm at 0.2 a.u.f.s. Chromatography was carried out at room temperature (28°C) and at a flow rate of 1.5 ml/min. Peak areas were estimated from chromatogram using integrator and used as a measure of concentration.

Results And Discussions

Quantitative Assay of 5-HTP

The levels of 5-HTP in all samples analysed were estimated from the peak areas of extracts and that of standard pure 5-HTP run alongside it (Table 1).

Table 1
Content of 5-HTP in Seeds Refrigerated (≤0°C) and those kept at Normal Room Temperature (28°C)

Sample	*Condition*	*Average % w/w 5-HTP in Seed*		
		March	*May*	*July*
T_0	≤ 0°C	18.20	11.44	10.81
T_{28}	28°C	18.24	15.66	13.18

Apparently, enzymes responsible for decomposition of 5-HTP in seeds were activated during the first two months of cold storage as evidence by the sharp change in 5-HTP content. The average rate of fall in 5-HTP content was 6.77% w/w. However, at normal laboratory temperature (28°C), the 5-HTP content changed in a manner typical of first order reactions from an average value of 18.24% w/w in March to 13.18% w/w in July. The average rate of fall in 5-HTP content was found to be 4.62% w/w of the initial 5-HTP determined in seeds.

From Table 2, the content of 5-HTP decreased for all durations of heating at 60°C over the study period. However, seeds heated for 120 min maintained the highest 5-HTP content at the end of the study. Analysis of variance indicated that the duration of heating at 60°C had no effect on the content of 5-HTP in seeds. The critical F value was 6.591 (0.05) and the calculated F values were respectively 5.496, 5.702 and 3.931 for March, May and July. The average rate of decomposition was found to be 1.79% w/w per month of storage under tropical atmospheric conditions.

Table 2
Content of 5-HTP in Seeds Heated at 60°C for Varying Durations

Sample	*Heating Duration/ Min.*	*Average % w/w 5-HTP in Seed*		
		March	*May*	*July*
A_1	30	16.63	15.50	15.14
B_1	60	16.37	15.76	14.80
C_1	90	16.46	16.11	13.80
D_1	120	16.90	16.06	15.33

It is possible that heating at 75°C for longer periods has an effect of breaking down 5-HTP in seeds as could be seen from Table 3. However the level of 5-HTP is maintained better in such seeds when stored under tropical atmospheric conditions. Although, seeds heated at 75°C for 120 min gave low 5-HTP content in March the change in content was comparatively negligible at the end of the study period.

Table 3
Content of 5-HTP in Seeds Heated at 75°C for varying Durations

Sample	*Heating Duration / min.*	*Average % w/w 5-HTP in Seed*		
		March	*May*	*July*
A_2	30	16.86	15.81	13.42
B_2	60	16.69	15.48	12.61
C_2	90	16.03	15.69	14.75
D_2	120	16.23	16.21	15.47

The differences in 5-HTP content recorded among samples in March could not be accounted for by random errors. The calculated F value 21.96 was greater than the critical F value 6.59 (0.05). This implies that at 75°C the duration of heating had effect on the level of 5-HTP in seeds. Least Significant Difference (LSD) test showed that the exposure to heat at 75°C for longer duration affected the 5-HTP content in seeds. However, in May and July the duration of heating at 75°C had no effect on the levels of 5-HTP in seeds. The calculated F values were 0.24 and 5.65 respectively.

The 5-HTP content decreased at an average rate of 2.69% w/w per month of storage under tropical atmospheric conditions for seeds heated for less than one hour whilst those heated for greater than one hour gave 2.14% w/w change in 5-HTP content.

Effect of Temperature on 5-HTP Content of Harvested Griffonia Simplicifolia Seeds

In March, the differences in 5-HTP contents determined in refrigerated seeds and those kept under normal laboratory condition (28°C) were negligible whilst seeds treated at 60°C and 75°C showed a significant decrease in 5-HTP contents. The results suggest that 5-HTP content in seeds falls at higher temperatures with the effect becoming more pronounced when seeds are heated beyond 60°C for longer durations. However, it was observed that seeds treated at higher temperatures showed a comparatively slight decrease in 5-HTP content on storage.

In general, it is possible that heating seeds at higher temperatures inactivates a significant enzymatic action in harvested seeds whilst cold storage rather activates the enzymes. Notwithstanding this inactivation of enzymes at higher temperatures, some amount of 5-HTP in seeds may be decomposed at the same time. There is therefore the need for a compromise between amount of 5-HTP that may be lost on treatment at higher temperatures and the content that may be preserved on storage.

Conclusion

The level of 5-HTP in harvested seeds of *Griffonia simplicifolia* was found to decrease at an average rate of 4.62% w/w per month of storage under tropical atmospheric conditions. Stability studies showed that refrigeration which is based on the principle of maintaining moisture while arresting enzyme activity, and usually used when no biochemical changes are required in harvested plant tissues could not preserve 5-HTP in seeds. Interestingly, results obtained showed that refrigeration probably activated enzymes responsible for decomposition of 5-HTP is seeds. The seeds were found to decrease at an average rate of 6.77% w/w per month of cold storage.

On the other hand, heating *Griffonia simplicifolia* seeds to temperatures between 60°C and 75°C was found to generally preserve 5-HTP content in seeds on storage. Seeds heated at 60°C for 30–120 min gave the lowest average rate of decrease in 5-HTP content (1.79% w/w) per month of storage compared to all other storage conditions used. It could be concluded that heating seeds at 60°C for 30 min is enough to inactivate enzymes and hence preserve 5-HTP content in harvested *Griffonia simplicifolia* seeds.

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