STUDIES ON THIAMINE IN CEREALS BY A PHOTOGRAPHIC METHOD

I. THE DISTRIBUTION OF THIAMINE IN DOMESTIC RICE AT DIFFERENT STAGES OF MATURITY

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(Received July 3, 1956)

A method to determine the localization of thiamine microscopically was used by Somers *et al.* (1), Pollock and Geddes (2), and Simpson (3) mainly in cereals. As to the reliability of this method, Araki (4), based on the study using animal and human tissues, found good reasons to believe that the induced fluorescence derived from thiochrome. The present investigation, employing the photographic method of thiochrome fluorescence, was undertaken to ascertain the distribution of thiamine in rice kernels at different stages of maturation.

EXPERIMENTAL

Methods

1. Preparation of Kernels and Photographing the Fluorescence.

Each kernel was held for sectioning by inserting it between the two halves of a pith stalk. Sections were carefully made with a sharp razor blade using a cylinder microtome, followed by mounting on glass slides. The sections were lightly treated with a freshly prepared mixture of one volume of 0.5 per cent potassium ferricyanide and 5 volumes of 5 per cent sodium hydroxide. The fluorescence of the sections was photographed one hour after treatment. As far as possible, the photographic method described by Simpson was followed, using Zeiss Super Semi-ikonta (*f* 3.5). In place of Corning filters, the following Wratten filters were used with satisfactory results: No. 18A in front of the mercury vapor lamp and Nos. 8 and 39 on the camera. Kodak Super XX was used for recording the fluorescence. The film was developed with a Kodak D 76 developer. According to Simpson, the method does not distinguish cocarboxylase from thiamine. Therefore, no distinction was made in this work between these two substances.

2. Collection and Preparation of Rice Kernels.

Each sample of known maturity was supplied from the Agricultural Experimental Field of Tokyo University. Sampling of the heads was begun 5

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days after blossoming and continued at successive 5 day intervals until maturity. The kernels taken from the central sections of the head were treated and photographed. The varieties, "Norin No. 8" and "Rikuu No. 132", were used in this experiment.

Results

1. Development of Kernels.

The moisture content as well as the fresh and dry weights per thousand kernels were calculated to serve as an index of maturity. Thiamine contents were measured by diazo method (5). The mean values both for moisture content, and for the fresh and dry weights per thousand kernels at successive stages of development are shown graphically in Fig. 1.

Mos., Moisture. G.W., Green weight. D.W., Dry weight. B₁, Thiamine.

FIG. 1 Progressive Changes in Moisture Content, Green Weight, Dry Weight and Thiamine Content of Rice Kernels during the Course of Maturity.

2. Distribution of Thiamine at Different Stages of Maturity.

Photographs of both longitudinal and cross sections of rice kernels taken 5 and 15 days, respectively, after blossoming as well as at maturity (35 days) are given in Fig. 2. No significant difference was observed between Norin No. 8 and Rikuu No. 132 concerning the thiamine distribution at different stages of maturity. The vitamin in rice was concentrated remarkably during the rapid development of the tissue. At an extremely early stage, the fluorescence of the vitamin appeared intensively in the palea. Until 5 days after blossoming the fluorescence was distributed mainly in the ovary wall surrounding the kernel. In 10 days, it became more concentrated both in the embryo and in the tissue surrounding the kernels as illustrated in Fig. 3. In 15 days the ovary wall became pericarp, very rich in the vitamin. Thereafter the distribution of the vitamin showed no marked change till the end of the maturation period. The distribution of the vitamin in rice during maturation

FIG. 2 (1) *Photographs of a Rice Kernel at Different Stages of Maturity.*

was different from that in wheat or barley. In the case of rice, the distribution of the vitamin is completed in a half period compared with that of wheat or barley.

3. Thiamine in Mature Rice.

The fluorescence in the section of mature rice are shown in Fig. 4. As seen from the photographs, very rich in the vitamin is both the embryo and aleurone layer, considerably rich both the pericarp and testa, poor the endosperm of the rice kernel. The longitudinal section of the embryo, as shown in Fig. 4, photographed by Ultropak, shows the very high concentration of the vitamin in the door tissue, epiblast and epithelium of the rice embryo. According to Simpson, the highest concentration of the vitamin occur in the scutellum and in the cells of the endosperm adjacent to the embryo, a finding which is not in accord with our photographs.

4. Difference in Thiamine Distribution between Natural and Artificial Drying.

The rice kernels at the late maturation period (25 days after blossoming) were dried naturally and by dryer (at 60°) respectively but little difference

Cross section of a whole kernel, 5 days after blossoming.

Palea, 5 days after blossoming.

Longitudinal section of a kernel with embryo, 10 days after blossoming.

Cross section of pericarp and aleurone layer, 20 days after blossoming.

FIG. 3 Photographs of a Rice Kernel (Norin No. 8) Taken with Ultropak.

Longitudinal section of an embryo.

Longitudinal section.

Cross section.

- a, scutellum.
- b, epithelium.
- c, foliage leaves. (primary leaf).
- d, primary root.
- e, door tissue.
- f, epiblast.
- g, pericarp.

FIG. 4 Photographs of a Mature Rice Kernel and Embryo. (Rikuu No. 132)

was studied by photographing thiochrome fluorescence, and the moisture content, weight per thousand kernels and thiamine content were also measured. The distribution of thiamine in mature rice was studied in detail.

ACKNOWLEDGEMENT

The authors wish to express their heartfelt thanks to Professor Masaya Araki and Dr. Sueo Sakurai for their encouragement, to Dr. Yakichi Noguchi for samples, and to Mr. Toshiaki Otaka for his performance of the analysis.

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