Quality Deterioration of Winged Bean during Storage

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Summary Seeds of winged bean (Psophocarpus tetragonolobus) were stored under four different sets of conditions; 65% relative humidity (RH) at 5°C, 35% RH at 37°C, 65% RH at 37°C, and 85% RH at 37°C. After storage for three and five months, samples were taken out to explore quality changes during storage. The germination capacity decreased during storage at high temperatures. Although little change was found in content in extractable oil, a decrease in the iodine value and an increase in the acid value and in the TBA value were significant during storage at high temperature and high moisture, indicating deterioration of oil. Aldehyde in defatted bean extract increased under all conditions for storage. Protein extractability was greatly reduced at 36°C and 85% RH, while no reduction was observed at other conditions.

Key Words winged bean, Psophocarpus tetragonolobus, storage, germination capacity, oil, aldehyde, protein

Winged bean (Psophocarpus tetragonolobus), a leguminous plant grown in the tropics, is of great promise as a tropical food crop equivalent to the soybean. The winged bean seeds are rich in protein and oil. Furthermore, flowers, leaves, tubers and immature pods are all edible and have higher protein contents than do the counterparts of other plants. A report from the National Academy of Sciences (USA) has called attention to the need for research on this promising legume (1). Identification of major globulins in winged bean seeds has been done, and amino acid compositions of the proteins, fatty acids, and carbohydrates have been studied (2-5). Our objective in the study presented here is to explore quality changes in winged bean seeds during storage and also to find the most desirable conditions for storage. The results will be useful when the seeds are stored for cultivation as well as for food purposes.

MATERIALS AND METHODS

Winged bean seeds. Winged bean seeds were obtained from Ryuku University
Storage conditions. The sterilized seeds were stored in a dark place under four different sets of conditions; 65% relative humidity (RH) at 5°C, 35% RH at 37°C, 65% RH at 37°C, 85% RH at 37°C. The samples were placed in single layers in petri dishes, which were then placed in airtight vessels which had been conditioned to their relative humidities by putting dishes containing salt-saturated solutions at the bottom; 35% RH was made with calcium chloride, 65% RH with sodium nitrite, and 85% RH with magnesium chloride. Storage was started by putting the vessels in incubators adjusted to the required temperatures. After storage for three months, some of the vessels were taken out and samples were transferred into a freezer to keep at −80°C until use. Other vessels were stored for another two months. The control sample (sample for zero time storage) was kept in a freezer at −80°C for five months.

Assay of whole beans. Germination capacity: Forty beans of a stored sample were immersed in water for one night and then placed in single layers on fully moistened cotton wool in a plastic vessel. After incubation in a dark place at 20°C and 60% RH for 96 hr, germinating beans were counted.

Moisture content: Beans were ground in a cyclone mill in the presence of solid CO₂ and the flour (about 5 g) was weighed accurately. Then the flour was subjected to drying at 130°C for 3 hr and weighed. The moisture content was calculated from the difference in weight.

Analyses of oil. Oil was obtained by extracting a ground sample (10–14 g) with ether in a Soxhlet incubator. The extracted oil sample was weighed after complete removal of ether with an evaporator. Oil content was calculated on a moisture-free basis.

The TBA value of an extracted oil was determined according to the method reported by Ottolenghi (6). Malonaldehyde was used as a standard. The acid value and iodine value were determined according to the methods described by Tsuchiya (7).

Preparation of defatted winged bean extract. Winged bean seeds were ground in a Retsch cyclone mill with a 0.5-mm sieve. The flour was defatted twice with a sufficient volume of cold hexane and then air-dried. The defatted flour was suspended in 10-fold (v/w) 35 mM KP₄ buffer, pH 7.6, containing 0.4 M NaCl. After suspension was stirred at 25°C for 1 hr, it was centrifuged at 12,000 × g for 20 min. The supernatant was referred to as defatted winged bean extract or, simply, extract.

Analyses of defatted winged bean extract. Protein in an extract was determined according to the method reported by Bensadoun and Weinstein (8), using bovine serum albumin as a standard.

Electrophoretic analysis of proteins in the extracts was performed on sodium dodecyl sulfate-polyacrylamide gels in the absence of mercaptoethanol, according to Laemmli (9). The diluted extract of 15 μl containing 15 μg of protein was subjected to electrophoresis on a 4–25% linear gradient gel and the gel was stained.

with Coomassie brilliant blue.

Ultracentrifugal analysis of protein in the extracts was performed with a Beckman 50.1 rotor. Extracts containing about 2 mg of protein were layered on a 5–20% sucrose linear gradient in 35 mM KP₁ buffer, pH 7.6, containing 0.4 M NaCl, and centrifuged at 35,000 rpm for 16 hr at 20°C. After centrifugation, the gradients were fractionated. Protein content in each fraction (0.2 ml) was determined with the method reported by Bensadoun and Weinstein (8). Ovalbumin (3.7 S) and rabbit muscle aldolase (7.8 S) were used as markers.

Aldehyde in an extract was determined enzymatically with bovine mitochondrial aldehyde dehydrogenase (10). The broad substrate specificity and high affinity for substrates of this enzyme make it possible to measure rapidly a large variety of aldehydes.

RESULTS AND DISCUSSION

Germination

Figure 1 shows a decrease in germination capacity of winged bean seeds during storage. The germination capacity was completely abolished during storage for 3 months at 37°C and high moisture (65% and 85% RH), while it remained appreciable in the sample stored at low moisture (35% RH). After storage for 5 months at 37°C, germination was undetectable for all samples. The seeds stored for 5 months at low temperature (5°C), however, had 80% of the germination capacity of the control sample which was kept at −80°C for 5 months. The capacity of the control sample was unchanged.

![Fig. 1. Germination capacity. Storage conditions are: (○) 5°C, 65% RH; (△) 37°C, 35% RH; (●) 37°C, 65% RH; (▲) 37°C, 85% RH.](image-url)
Moisture content

Moisture contents of the beans were measured before storage (8.1%) and after storage for 3 and 5 months and it was found that the contents reached almost

Fig. 2. Properties of oil. A, oil content; B, iodine value; C, acid value; D, TBA value. Storage conditions are: (○) 5°C, 65% RH; (∆) 37°C, 35% RH; (●) 37°C, 65% RH; (▲) 37°C, 85% RH. Aldehyde quantity is expressed as μM in defatted flour extracts.

Fig. 3. Aldehyde in defatted flour extract. Storage conditions are: (○) 5°C, 65% RH; (∆) 37°C, 35% RH; (●) 37°C, 65% RH; (▲) 37°C, 85% RH.
constant levels after storage for 3 months. The moisture contents after 5 month storage were 7.6% for the sample stored at 5°C and 65% RH, 8.6% for one at 37°C and 35%, 9.9% for one at 37°C and 65% RH, and 14.7 for one at 37°C and 85% RH.

**Oil**

In Fig. 2, oil content and analytical data of the extracted oil are shown. Oil content shows little change during storage (Fig. 2A). A decrease in the iodine value of the samples stored at 37°C and high moisture (65% and 85% RH) indicates oxidation of unsaturated fatty acids (Fig. 2B). The acid value significantly increased in the sample stored at 37°C and 85% RH, indicating that fatty acids were released from lipids (Fig. 2C). An increase in the TBA value was found in the sample stored at 37°C and 85% RH (Fig. 2D). This is due to breakdown of unsaturated fatty acids.

**Defatted extract**

It has been shown that not only free aldehydes but also protein-bound aldehydes in defatted soybean extracts can be measured by aldehyde dehydrogenase (10–12). Aldehyde levels in buffer-extracts of defatted flour samples, which were prepared from winged bean seeds stored under the various conditions, were measured (Fig. 3). In all conditions of storage, an increase in aldehyde was observed after storage for 5 months. The increase was notable in the samples stored at high temperature (37°C) and high moisture (65% and 85% RH). Aldehyde was

![Graph showing protein extractability from defatted flour](image)

**Fig. 4.** Protein extractability from defatted flour. One hundred percent was defined as the protein extractability of the control sample (200 mg/g of flour). Storage conditions are: (○) 5°C, 65% RH; (△) 37°C, 35% RH; (●) 37°C, 65% RH; (▲) 37°C, 85% RH.
probably derived from unsaturated fatty acids and a remarkable increase in free fatty acids was seen after storage at high temperature and high moisture (see Fig. 2C). However, further experiments are needed to answer the question whether aldehyde was produced by the action of lipoxygenase on released unsaturated fatty acids or through their autooxidation.

Protein extractability from defatted winged bean flour is shown in Fig. 4. The extractable protein was greatly reduced in the sample stored at 37°C and 85% RH, but no change was found for other samples. Figure 5 shows SDS-PAGE patterns of extracted proteins. It is apparent that bands 3, 4, 5, and two bands which move more slowly than does band 7, have disappeared completely after storage for 5 months at 37°C and 85% RH. It has been reported that winged bean seeds contain three major proteins; psophocarpin A, an 8S protein fraction, which is disaggregated by SDS to a subunit of molecular weight near 40,000, psophocarpin B, a 2S protein fraction, which gives a major band of molecular weight of 20,000, and psophocarpin C, a 6S fraction, which is dissociated by SDS to give a number of subunits with molecular weights ranging from 15,000 to 80,000 (4). The components which have become unextractable during storage seem to be psophocarpin C. Indeed, ultracentrifugal analyses of extract in sucrose density gradients indicated that proteins with 6–7S decreased to a great extent in the sample stored for 5 months at 37°C and 85% RH (figure not shown).

The results in this paper indicate that humidity rather than temperature is crucial for storage of winged bean seeds. The moisture contents of beans varied...
depending on the humidity for storage at 37°C; the higher humidity, the higher moisture content. Thus it appears that the moisture content affects processes of quality change of the beans and it is possible to store the seeds without quality change in the tropics if one can keep the humidity low. Likewise, studies on soybean \((13, 14)\) and soybean flour \((14)\) have shown the importance of low humidity.

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REFERENCES