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Water Imbibition by Normal and Hard Soybeans[1]

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ABSTRACT

By observing the imbibition of dyed water, soybeans were classified into damaged (break in seed coat), normal and hard beans. Hard soybeans were unique in having a long, variable lag time before starting imbibition, but once water uptake was started, the rate was similar to that of normal beans. Soaking hard beans in methanol or ethanol for 24 hr at 20 C made them permeable to water. The cuticle was the most likely site of the water barrier in the seed coat of soybeans.

INTRODUCTION

For production of soymilk, soybeans usually are soaked in water, and after imbibing water, the soaked soybeans are heated to inactivate lipoxygenase and trypsin inhibitors. If, upon soaking, the soybean does not imbibe water, the subsequent heating may not inactivate antinutritional factors which could cause poor quality in the final product.

Soybeans that do not imbibe water (hard beans) have been studied (1-4), but there is no agreement as to the cause of failure to imbibe water, nor is there any extensive study of conditions that would cause hard beans to imbibe.

Smith and Nash (4) observed that the seed coat was the principal barrier to water imbibition and defined hard beans as those that do not absorb enough water in 16 hr to soften normally when cooked in steam. They also observed that hard beans usually were smaller and drier than soybeans that imbibe normally.

Saio (3) studied the seed coat of hard and normal soybeans and concluded that hard beans had more fiber and Ca in their seed coats than normal soybeans. Also, she observed that the micropyle seemed to be closed (when observed by scanning electron microscopy) in hard beans, and this fact may account for the failure to imbibe water.

Growing conditions are known to affect water imbibition by soybeans. Baciu-Miclaus (1) found that soybeans grown under conditions of low relative humidity tended to produce more hard beans than when grown at high relative humidities. Also, Smith and Nash (4) indicated that development of hardness is partly the result of hot dry weather during ripening.

Duangpatra (2) studied some of the beneficial aspects of hard soybeans and found hard seed (after mechanical scarification) had better total germination than normal seed. He hypothesized that the seed coat, except for the hilar region, was water-impermeable and that a suberin layer under the hilum prevented water from entering in hard beans.

In a study of textural differences in legumes, Sefa-Dedeh and Stanley (5) observed that normal soybeans imbibe water faster and to a greater extent than other legumes studied (cowpea, white beans, pinto beans, adzuki beans and U.S. black-eyed peas). They had no explanation based on structural differences in seed coats that would cause different rates of water imbibition.

To learn about the cause of hardness in soybeans and how to control it, we studied the imbibition of water by normal and hard soybeans under a variety of conditions.

MATERIALS AND METHODS

The soybeans used in these experiments were Amsoy 71 and were of seed quality. For soaking in dyed water, a blue food dye (FD and C blue No. 2, indigotine) was used at 0.075%.

Weight increase was calculated on an "as is" basis, with the moisture content of "as is" soybeans ranging from 10 to 13%. The individual beans were weighed, immersed in distilled water for the time indicated, removed from the water, blotted, weighed and returned to the water.

Hard beans were selected by sieving seed beans with a No. 16 round mesh sieve. About 1 kg of small beans passed the screen for every 28-kg bag. The small beans were soaked in distilled water for 24 hr at 5 C. All beans that did not take up water during this time were labeled hard beans and saved for further experiments.

For soaking in organic solvents, 10 hard beans were placed individually in small vials and covered with each of the 5 solvents: hexane, chloroform, acetone, ethanol (95%), and methanol. There were 2 beans exposed to one solvent

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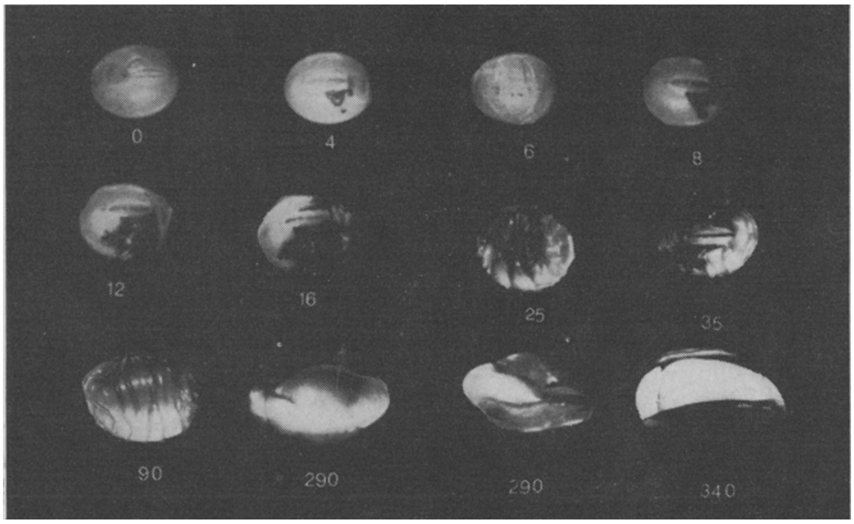

FIG. 1. Sequence of imbibition of dyed water by a single damaged soybean. Numbers are time in min.

(but in separate vials) for each experiment. The vials were slowly moved on an inclined rotor at room temperature for 24 hr. Thus, the hard soybeans were constantly exposed to a renewed layer of solvent.

Moisture determination of seed coats was done by drying in an air oven at 100 C. Moisture analysis of the cotyledons also was done at 100 C after the beans were ground to a 60-mesh flour.

RESULTS AND DISCUSSION

To better understand the mechanism by which soybeans imbibe or fail to imbibe water, we soaked them in 18-23 C water containing a blue food dye. Careful observation led us to classify soybeans into 3 categories based on their imbibition of the dyed water.

Soybeans that had a break in their seed coats through which the dyed water moved immediately upon immersion were termed damaged beans. Figure 1 shows a sequence for water imbibition by a single damaged bean. Note the break in the seed coat near the hilum at 0 min and the dye penetrating the break at 4 min. The view at 6 min shows the side of the same soybean opposite the hilum and shows very little penetration of dye, but moisture was penetrating this area as indicated by the wrinkled seed coat. Successive photos show the progression as dyed water continued to penetrate the seed coat and as the cotyledons imbibed the water. At 340 min, the seed coat was split intentionally to show that the cotyledons had imbibed water but not dye.

By examining the seed coats carefully with a magnifying glass, it was possible to select soybeans with no obvious damage. We called these soybeans normal, and when soaked in dyed water, they imbibed as shown in Figure 2. The seed coats of normal soybeans and of damaged soybeans usually were wrinkled in the area opposite the hilum (0 time in Fig. 2). Invariably in normal soybeans, the water imbibition started in that wrinkled area (6 min, Fig. 2). The dye did not readily penetrate the seed coat in normal beans, but it was not totally excluded, either. At 12 min and at 25 min in Figure 2, dye can be seen filling the center portion of the hilum, yet water had not penetrated to the cotyledons, as can be seen by the lack of wrinkling in the hilum area at 12 min.

Most of the soybeans that we examined were either damaged or normal and imbibed water as shown by Figures 1 and 2. Saio (3), in an analysis of hard beans, suggested that water may enter through the micropyle and that the explanation for hard beans is that the micropyle is plugged. Our results indicated that water normally penetrated the seed coat opposite the hilum or through a break in the seed coat but not through the micropyle.

If normal beans were placed in shallow water so that only the hilum area was under water, they still imbibed. Hence, all the seed coat area of normal soybeans was permeable to water, but the area opposite the hilum (where wrinkling existed) was the area through which water penetrated first.

When hard beans (selected by no imbibition at 5 C) were immersed in water at 20 C, they resisted imbibition for varying times. However, once a hard bean started to imbibe water, the sequence and the time for imbibition was like that for normal beans. Some hard beans imbibed water first through the hilum area with the resulting swelling and wrinkling in that area, but imbibition through the hilum was not invariably true for hard beans.

We observed that hard beans had smooth seed coats, whereas normal soybeans usually were wrinkled in the area opposite the hilum.

We next investigated the rates of water imbibition at different temperatures for 3 types of soybeans. Figure 3 shows the data for damaged soybeans at 5, 15, 25 and 40 C. The damaged soybeans had greater variability in water imbibition rates than normal soybeans at all temperatures. We attributed this variability to differences in the sizes of the breaks in the seed coat.

Data for normal soybeans are shown in Figure 4. At 5 C, it took about 4-9 hr for the soybeans to imbibe 50% of their weights. At 15 C, the time range was 2-6 hr for 50% imbibition (the soybean imbibing water much earlier probably was damaged). At 25 C, the range was 2-4 hr and, at 40 C, 1-2 hr for 50% imbibition. These data show that, at any one temperature, the rates of imbibition were roughly equal, but the starting times caused variation in the time required for 50% imbibition. Variability in water imbibition due to different amounts of damage, as in Figure 3, was understandable, but it was more difficult to understand the variability in normal soybeans. One possible source of the variability was a difference in soybean size. Because percentage increase in weight was used as a measure of water imbibition, a small soybean would have a larger per-

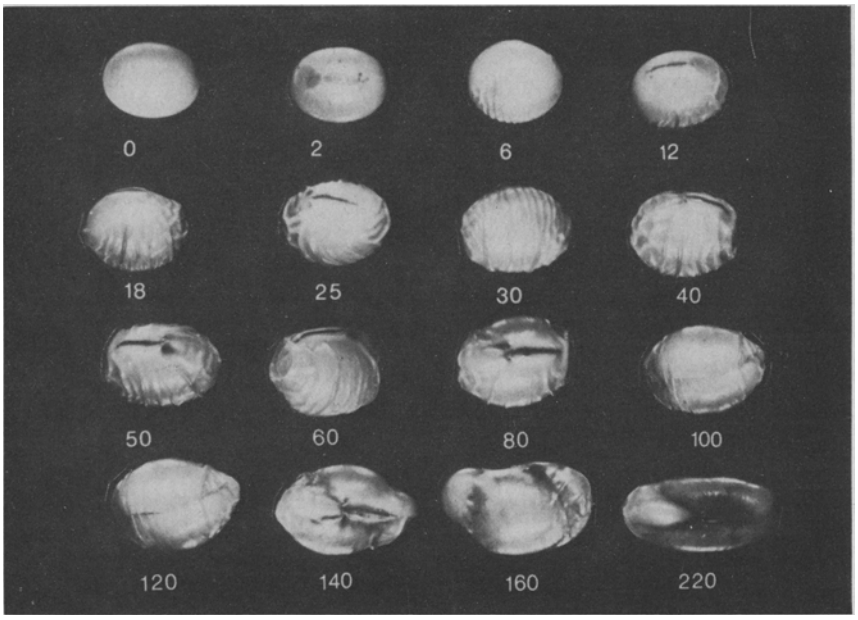

FIG. 2. Sequence of imbibition of dyed water by a single normal soybean. Numbers are time in min.

centage increase than a large soybean if both were imbibing the same amount of water/hr. The difference in size could account for different rates of water imbibition but would not be an explanation for different starting times of imbibition.

Figure 5 shows data for water imbibition by hard soybeans at the 4 temperatures. The large differences in water imbibition at any one temperature were obviously due to the time for starting imbibition. Once the process was started, the rate was essentially the same as for normal soybeans.

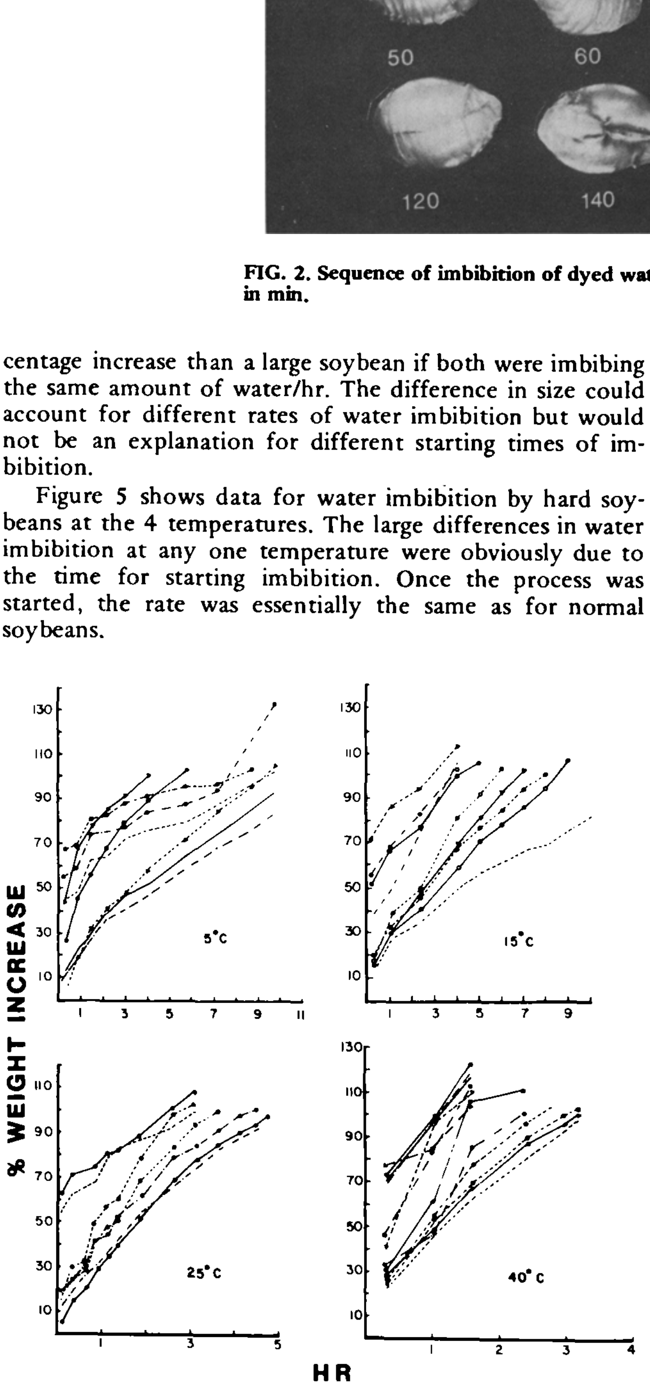

FIG. 3. Rates of water imbibition of single damaged soybeans at 5, 15, 25 and 40 C. Each plotted line is the weight increase for a single bean.

We did a few experiments with normal and hard beans at 80 and 100 C. The start of imbibition of water was obvious by wrinkling of the seed coat, and there was no difference between normal and hard beans in the time required to start imbibition at 100 C. At 80 C, some hard beans started to imbibe as soon as normal beans, but other hard beans remained hard after 30 min. Hence, at 80 C, we saw the variability for hard beans in time to start imbibition that is evident at lower temperatures.

Thus, if soybeans were soaked at 80 C or below, some hard beans may not have imbibed water, and a subsequent steaming to inactivate antinutritional factors and lipoxygenase would not be effective. However, if soybeans were heated at 100 C, all soybeans would start imbibition, and completion of imbibition at a lower temperature followed by steaming or continued imbibition at 100 C should be sufficient to inactivate antinutritional factors and lipoxygenase.

Data of Figure 5 show that, with increasing temperature, the lag time for hard beans to begin imbibition shortens. Hence, whatever was happening to allow hard beans to start imbibition was speeded up by increasing temperature. The variability in lag time was still very evident at 40 C.

To investigate further the nature of the variability in water imbibition by soybeans, we removed the seed coats from both hard and normal soybeans and measured imbibition at 20 C. Since cotyledons from hard beans are smaller than cotyledons from normal beans, hard beans appeared to imbibe water faster than normal cotyledons. But there was very little variation in water imbibition by the cotyledons, and amounts of imbibition were greater for both types of cotyledons at 20 C than for normal soybeans with seed coats at 25 C. This experiment showed that the seed coat is the cause of the lack of water imbibition by hard beans and also the cause of most of the variability in water imbibition in normal and in hard beans.

One of the prevailing ideas about hard beans is that they result from a lowered moisture content (4). We measured the moisture content of the hard and normal soybeans with these results: hard soybean cotyledons had 7.4% moisture, and their seed coats had 8.3%. Normal soybean cotyledons had 13.1% moisture, and their seed coats had 9.8%. The

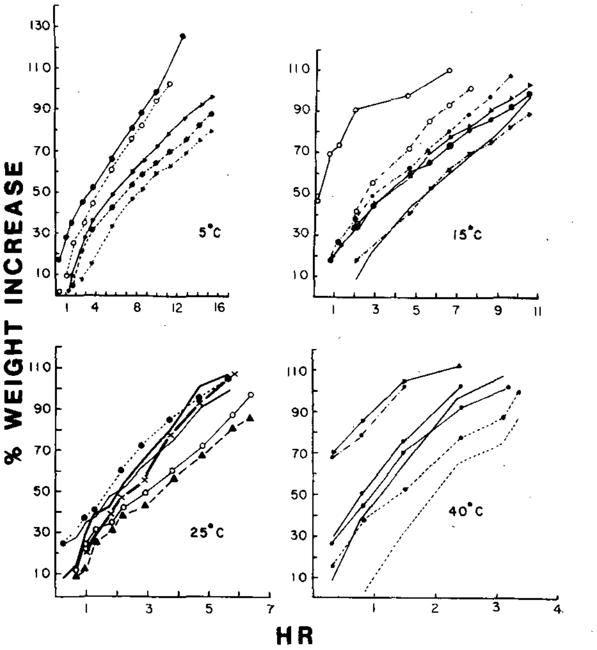

FIG. 4. Rates of water imbibition for single normal soybeans at 5, 15, 25 and 40 C. Each plotted line is the weight increase for a single bean.

differences in moisture content between hard and normal soybeans for the cotyledons and for the seed coats were statistically significant at the 1% level. Until separated by sieving, the hard and normal soybeans had been stored together at 5 C. It is reasonable to think that the differences in moisture content between normal and hard soybeans may be a consequence of the size difference. As soybeans dry in the pod, the smaller beans with the larger surface-to-volume ratio would tend to lose more moisture/ unit weight than larger beans.

Although it is obvious that small beans may dry out more than large beans during maturation, it is not at all obvious what property would maintain hard beans at a lower moisture content than normal beans when the 2 types are stored together. We hypothesized that the cuticular layer of the soybean may be the site of the moisture barrier, and if this is true, removal or modification of the cuticle may change the lack of imbibition in hard beans.

As a mean of modifying the cuticular layer, we soaked hard soybeans in hexane, chloroform, acetone, methanol, or ethanol for 24 hr at room temperature. Upon subsequent soaking of these treated hard soybeans in water, we observed essentially no change in behavior of the hexane-, chloroform-, or acetone-soaked soybeans. However, soybeans soaked in either methanol or ethanol had a dramatic change in that they behaved as normal beans with no extended lag in water imbibition.

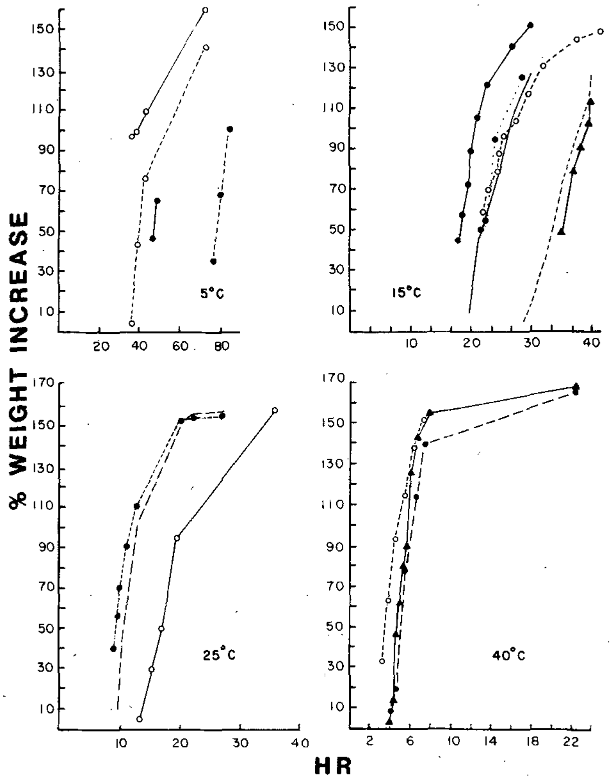

FIG. 5. Rates of water imbibition for single hard soybeans at 5, 15, 25 and 40 C. Each plotted line is the weight increase for a single bean.

When hard soybeans were modified mechanically by scraping a small area of the cuticle with a razor blade and being careful not to damage the seed coat, we observed water imbition immediately in that scraped area. The water imbibition was evident from a wrinkling of the seed coat.

We conclude from these experiments that the cuticle is the site of water impermeability in soybeans. Why normal soybeans imbibe water and hard beans do not (when both have virtually identical cuticles by microscopic observation) is an unanswered question, but water permeability undoubtedly is influenced by the growing conditions at the time the cuticular layer is deposited.

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