CEREAL QUALITY MEASUREMENT

X-Ray and Photomicrographic Examination of Rice

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X-ray techniques have been used successfully for the rapid examination of rough (unhulled) rice for cracks, checks, insect damage, and immature seed. Radiographic evidence of cracks (or checks) in grains of rough rice was confirmed by photomicrographs of halves of the same kernels. Improved techniques were used in the preparation of thin sections of rice kernels for study of the detail of the inner structure of the kernels. Quality factors, such as yields of head rice, color, stability to oxidation, and nutritive value, are related to the structural elements of the rice kernel that remain in the milled rice.

THE MONETARY RETURN that a rice miller receives from a given lot of rice depends on the yields of head rice (whole kernels), second heads, screenings, and brewer's rice. The values per pound of these products decrease in the order named. Any breakage or weakening of kernels that develops prior to or during milling consequently assumes an economic importance because of its bearing on milling yields and monetary return. Proper control of the handling, drying, and processing operations is necessary in order to minimize the degradation of the product through formation of cracks and checks in the endosperm (white, starchy part of the kernel).

The terminology used in the technical literature to describe seed damage is somewhat indefinite. In this paper, "breakage" signifies separation of a kernel into two or more parts, even though the parts remain enclosed within an intact hull. The terms, "checking" (used extensively in the rice industry), "fissuring" (used most commonly in regard to wheat damage), and "cracking" indicate the presence of cracks which weaken kernels but do not extend entirely through them.

In the rice field prior to harvesting the seed suffers some damage which is commonly called sun-checking, even though it is now believed (*4*) that this checking is due to changes in the moisture content of the seed and not to sunshine. Rough treatment of the seeds during combining adds to the damage. It has been estimated (*1*) that about 5% of the rice arriving at rice mills is checked or broken before it reaches the drying plant. Improper methods of drying are one of the most important causes of checks and cracks. Cracks result from stresses induced in the kernel by excessive moisture gradients and possibly from thermal changes.

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In spite of the economic significance of checking and breakage of kernels, there has been no adequate method for measuring them. The only practical test that has been adopted in the rice industry for revealing checking and breakage is the standard milling test (*10*), in which 1 kg. of rice, dried to a moisture content of about 12%, is hulled in a McGill sheller and milled in a McGill mill under controlled conditions. The yield of head rice is determined and used in assessing the value of the sample. This test is of limited value in determining the condition of rice in its early stages of processing, because the rice must be dried before testing and kernels may be damaged during drying. This milling test does not distinguish between kernels broken within the hulls and checked kernels that break during hulling or milling.

Kik (*4*) examined cracks in rice kernels by placing them on the stage of a stereoscopic microscope and observing them by transmitted light. Such examination of carefully hulled kernels gives some indication of the condition of the kernels, but it is too time-consuming to permit adequate sampling of a lot.

X-Ray Examination of Grains

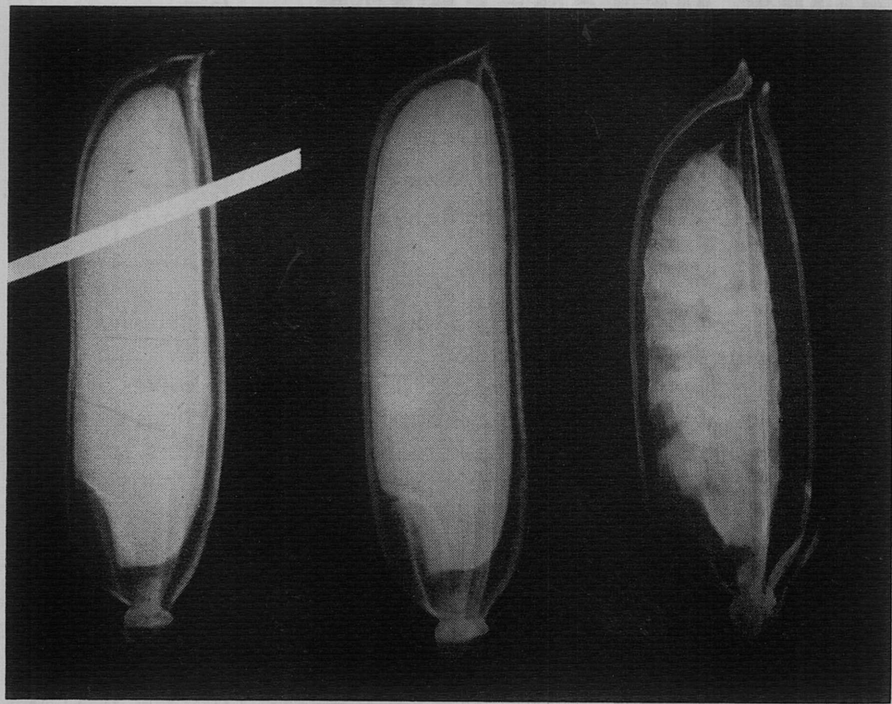
X-ray techniques were applied by Milner, Lee, and Katz (*6*) to the determination of insect infestation in wheat and other grains. These techniques have been improved and are now used routinely for detection and measurement of internal infestation of grains. Research studies have indicated that there is a correlation between the amount of insect infestation shown by x-ray tests and that shown by the cracking-flotation method (*2*), which is used by numerous control laboratories.

Milner and coworkers (*5, 8*) have demonstrated the usefulness of the radiographic technique for the detection of other physical anomalies in grains. Practical application of this sort included the detection of cracks or broken kernels in rough rice (unhulled) prior to milling as well as the internal fissuring of certain grains such as maize due to uneven stresses arising from severe drying conditions. Utility of the technique was illustrated with cuts made from the original radiographs.

Milner and Shellenberger (*7*) found radiological evidence of the existence of internal fissures in weathered wheat. They also found that rewetting dry wheat and then redrying it sometimes caused reductions in both the density of the wheat and the power required for grinding it. Both of the latter changes correlated with radiographic evidence of fissuring.

A knowledge of the structure of a rice kernel would appear to be basic to an understanding of the factors which contribute to breakage, checking, and impairment of cooking quality. Too little is known about the internal fine structure of normal rice kernels and, except for the work of Milner *et al.* (*5, 8*) and more recently that of Henderson (*3*), no reports including photomicrographs have been published that contribute to an understanding of the conditions present in damaged (checked) rice kernels.

There exists the possibility that x-ray and photomicrographic methods can lead to a better knowledge of the damage that occurs in rice kernels at every stage in their handling and processing. Accordingly, a cooperative program for this type of study on rice was established. The work was planned so that both x-ray and photomicrographic measurements were made on the same selected grains of rice. The x-ray work was done by personnel of the Southern Utilization Research Laboratory and the sectioning and photomicrographic studies were made at the Northern Utilization Research Branch. Use of the latest refinements in seed sectioning made it possible to obtain photomicrographs



A B C

Figure 1. X-ray photographs of rice kernels

A. Cracked or checked grain (same grain as Figure 3, *B*). White bar is image of portion of wire grid crossing seed holder
B. Normal grain
C. Incompletely developed grain

which reveal in new and finer detail the inner structure of the rice grain.

Materials

The radiographic studies and most of the sectioning tests were carried out on Bluebonnet rice, a long grain variety, grown in the vicinity of Crowley, La., having a moisture content of approximately 22% when harvested on September 9, 1952. This rice was air-dried to 10 to 11% moisture content and stored at 25–26° C. until tested. The rice used for the sections shown in Figures 5 to 8 was from a sample of the Zenith variety harvested and air-dried in October 1951, near Crowley, La.

Equipment

Radiographs were obtained with the General Electric x-ray inspection unit, which employs a beryllium-window x-ray tube operated at 25-kv. peak and 5 ma. Exposures were made on Kodak Type M film, 14 × 17 inches.

Experimental Procedures

X-Ray Photography. A 100-gram sample of the rice under test was spread uniformly on the surface of a plastic tray in the top of the x-ray unit. The cover, containing the unexposed film, was put in place and the controls were set for voltage, amperage, and exposure time. After the exposure was made, the film was developed and dried before examination. Approximately six samples could be radiographed per hour.

Sectioning. The method used for preparing a median longitudinal section, which would show histological structure of a rice kernel, was a modification of that which had been developed for preparing fresh-frozen sections of corn and wheat kernels. Selected kernels from the lot of radiographed Bluebonnet kernels were steeped 4 or 5 days in 1% sodium carbonate solution at room temperature, and sections either 30 or 60 microns thick were prepared. Use of the sodium carbonate solution increased the rapidity of water penetration and minimized fragmentation of the endosperm during sectioning. Large cracks could be observed in the previously intact kernels. When these kernels were air-dried at room temperature, the cracks were still easily observable. The cracks were most obvious when a beam of light was focused at right angles to the side of the kernel under observation. It was tentatively concluded that either the steeping procedure caused the cracks or the cracks were initially present but were too small to be observed under the conditions used.

The most satisfactory method used for revealing cracks in kernels involved cementing some of the rice kernels to microscope slides with Pliobond 20, manufactured by Goodyear Tire and Rubber Co., Akron, Ohio. Subsequently, the kernel was rubbed back and forth carefully against a ground-glass plate until approximately half of the kernel had been removed.

Photomicrography. Photographs of sections were taken in the customary way, using transmitted light. The halves of kernels were photographed by using light reflected from the ground surface of the kernel (oblique illumination).

Results and Discussion

Comparison of X-Ray Photographs and Photomicrographs. X-ray photographs of samples of rough rice showed most of the kernels as solid white areas (endosperm) surrounded by a veillike sheath (hull) (Figure 1, *B*). The embryo was too transparent to the x-rays to appear in the pictures. Across the endosperm of a few of the kernels appeared thin dark lines, which were shown by subsequent photomicrographic work to be due to cracks or checks in the endosperm (Figure 1, *A*).

Kernels that had been sound and had given x-ray pictures like that in Figure 1, *B*, were found to be badly checked after being steeped for 5 days in 1% sodium carbonate. The cracks or checks were visible before sectioning, but they could be photographed more readily when half of the kernel had been cut away, a section at a time, on the freezing microtome (Figure 2). Other sound kernels, which had not been steeped in any solution but had been ground down against ground glass, gave photomicrographs that confirmed the absence of cracks in these kernels (Figure 3, *A*).

Some rice grains, like the one presented in Figure 1, *A*, show a pattern of dark lines after being ground down by the dry technique and photographed under oblique lighting (Figure 3, *B*). All the dark lines in the x-ray picture appear as cracks in the photomicrograph. Two small additional cracks not visible in the x-ray picture also appear in Figure 3, *B*. These two cracks were either too small to be visible in the x-ray film or were formed during the grinding away of the upper half of the kernel. Repetition of this study on other kernels confirmed the observation that all of the cracks that showed up as dark lines in x-ray films were found in the photomicrographs, as well as a few small additional ones.

Some abnormal and damaged kernels were discovered. One grain of rough rice, which appeared normal on visual examination prior to hulling, gave an abnormal x-ray picture (Figure 1, *C*), in which the endosperm appeared mottled instead of being solid white like normal kernels. On hulling, that kernel was found to be incompletely developed (Figure 4). A few other kernels which had given normal x-ray pictures were found, when ground down against ground glass, to possess soft starchy centers resembling the inner part of a kernel of corn. An example of this type of defect is shown in Figure 3, *C*. Several kernels showed evidence of damage attributed to insects.

Potential Uses for X-Ray Techniques. A series of tests was made to ascertain whether counting of whole and damaged kernels in an x-ray film can be used to predict milling yields. On five commercial lots of good quality rice (four different varieties) the percentages of complete seeds showing radiographic evidence of breaks ranged from 2.5 to 5.3, while the percentages of complete, uncracked kernels ranged from 92.3 to 94.4. Yields of head rice, obtained commercially, on these same lots of rice ranged from 55.5% to 67.9%. Correlation between breakage counts and commercial mill yields for individual lots was poor. This lack of correlation and the fact that the milling yields were much smaller than the maximum yields indicated to be possible by radiographic tests suggest that either the kernels in the rough rice contain radiographically invisible checks or the yields are affected by other uncontrolled factors.

X-ray examination of the grain before and after harvesting may prove to be useful in research on harvesting procedures and harvesting equipment. Similarly, serious breakage of rice during various stages in the drying process can be revealed quickly by x-ray examination. If desired, a quick x-ray test can provide a permanent record of breakage and insect infestation of a rice sample at the time of testing. Although the x-ray method reveals breaks and large cracks, it probably does not reveal the small cracks and checks.

Internal Structure of Rice Grain. After kernels of Zenith rice had been soaked for 5 days in 1% sodium carbonate solution, it was possible to remove some good 30-micron sections on a freezing microtome and to prepare photomicrographs from them. A nearly perfect longitudinal section of a complete kernel is shown in Figure 5, and enlargements of portions of this section are shown in Figures 7 and 8.

Certain properties of rough rice, such as its tendency toward microbial contamination within the hull, can be understood better when its development and structural factors are considered. At one stage in the development of the flower, the style (Figures 5 and 6) is open to permit the pollen to enter and fertilize the ovule. During subsequent development of the seed, the long hulls close around the developing rice kernel. Until the seeds mature, these hulls protect whatever microorganisms entered the ovule with the pollen. In Figure 6 are shown the style and stigma of a mature rice kernel with pollen grains still clinging to the stigma. It appears possible that mold spores (and possibly those of bacteria and yeasts) may enter the flower together with the pollen and remain in viable form, ready to grow whenever the rice is stored under conditions favorable for their growth. Microorganisms may also enter the seed at the point of kernel attachment (*KA*, Figure 5). This lack of kernel sterility is a pertinent factor whenever the preservation of quality during storage is considered.

Examination of the photomicrograph (Figure 5) of the complete rice kernel and a knowledge of the composition of the various kernel components indicate how milling of brown (dehulled) rice to

Figure 2. Rice grain (× 13)
Showing cracks formed when grain was steeped for 5 days in 1% sodium carbonate. Half of kernel was cut away on a freezing microtome

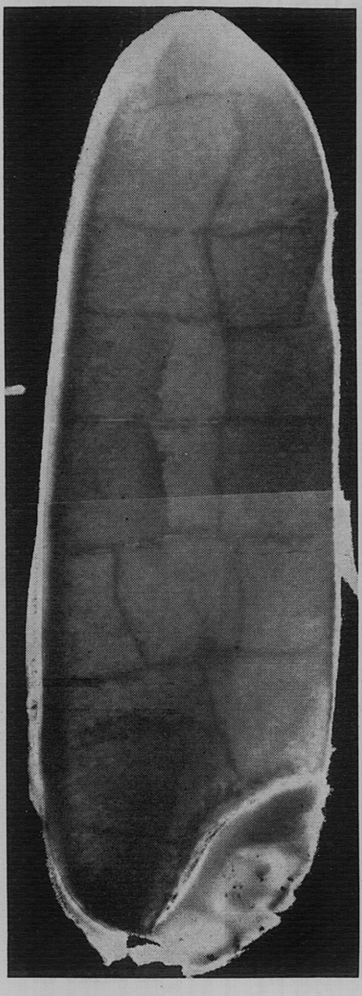
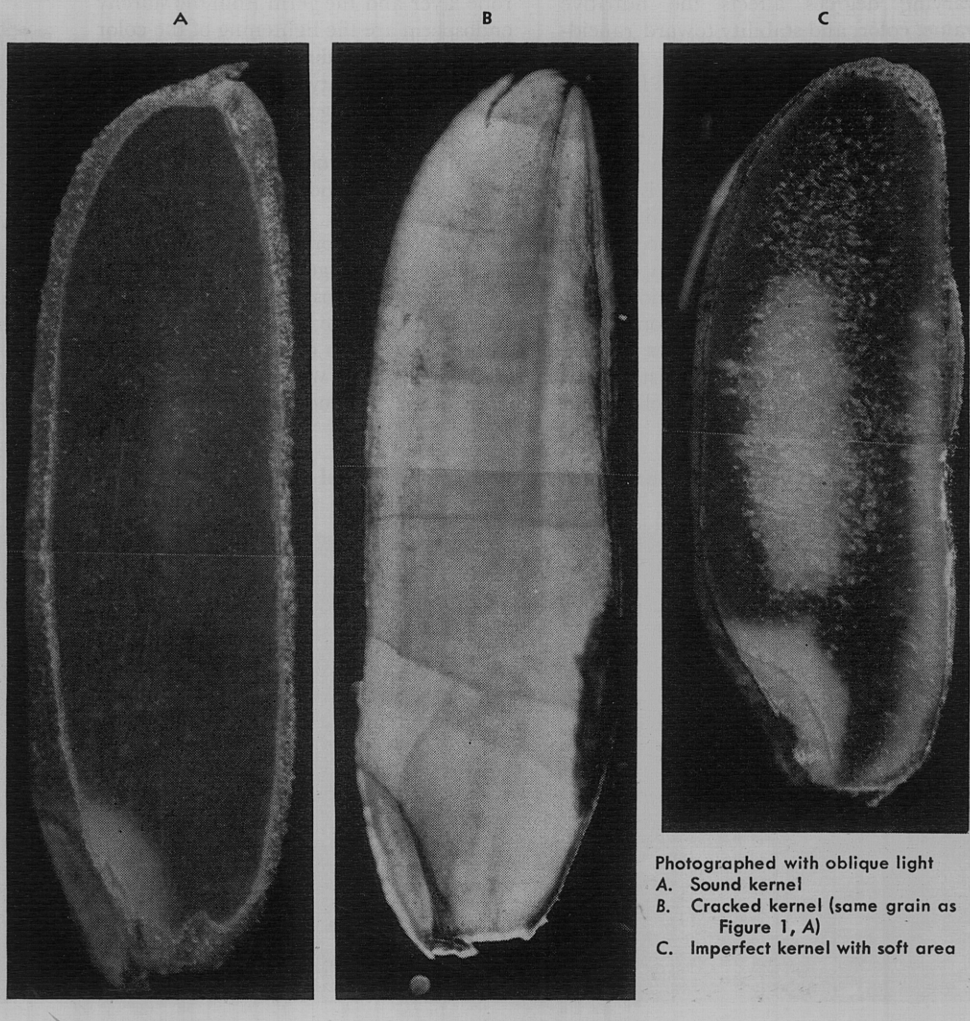


Figure 3. Rice kernels after one half had been ground away against ground-glass plate × 13)



Photographed with oblique light
A. Sound kernel
B. Cracked kernel (same grain as Figure 1, *A*)
C. Imperfect kernel with soft area

Figure 4. Wrinkled kernel found defective in x-ray radiograph prior to removal of hull (×10)

Photographed under oblique light

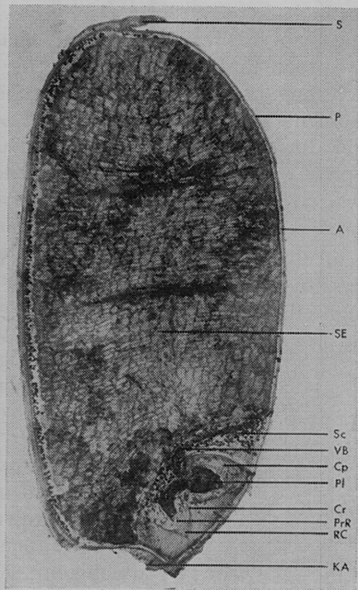


Figure 5. Median longisection of Zenith variety rice kernel

A. Aleurone
Cp. Coleoptile
Cr. Coleorhiza
KA. Kernel attachment
P. Pericarp
Pl. Plumule
PrR. Primary root
RC. Root cap
S. Style
Sc. Scutellum
SE. Starchy endosperm
VB. Vascular bundle

varying degrees affects the nutritive value, color, and stability toward rancidity of the milled product.

At the start of the milling the first portion to be removed is the pericarp, *P*, which forms a fibrous covering about the endosperm and germ. The pericarp should be removed because it is fibrous and low in food value, and contains much phytin that is undesirable because it combines with calcium and iron, making them unavailable for absorption from the intestine. Also removed completely in commercial milling processes are the aleurone layer, *A*, which consists of the outermost one or two layers of cells of the endosperm, and the germ (at lower right side of section). Unlike the starch-bearing cells, *SE*, which constitute most of the endosperm, the aleurone layer and the germ are rich in protein, oil, and vitamins. Nichols (*9*) has stated that the oriental practice of pounding rice by hand removes all of the pericarp but only part of the aleurone layer and only part of the scutellum (*Sc*, portion of germ). It is claimed that parboiling improves the nutritive value of rice by causing a migration of oil, minerals, and vitamins from the aleurone layer and germ into the starchy endosperm, *SE*. The only advantages derived from the removal of the light brown aleurone layer and the germ from the starchy endosperm are the lightening of the color of the milled product and the improvement of its stability through removal of the oil-containing portions (aleurone layer and germ). Thus, quality from a nutritional point of view is inconsistent with the color and stability aspects of quality.

The physical properties of the rice kernel depend largely on the properties of the starchy endosperm cells, which are large and bricklike in shape (Figure 7), with the exception of those in the center of the endosperm, which are smaller and more nearly round (Figure 8). The radial arrangement of the endosperm cells is undoubtedly responsible for the fact that most checks and breaks occur directly across the kernel.

Summary

X-ray photographs of rough (unhulled) rice show dark lines caused by cracks or checks crossing some of the kernels. The cause of these dark lines was established by grinding away half of each selected kernel and photographing the remaining half under oblique lighting.

Seed-sectioning techniques developed for use on other grains were modified and used in the preparation of photomicrographs which show in excellent detail the internal anatomy of the rice grain.

The structure of rice kernels, as re-

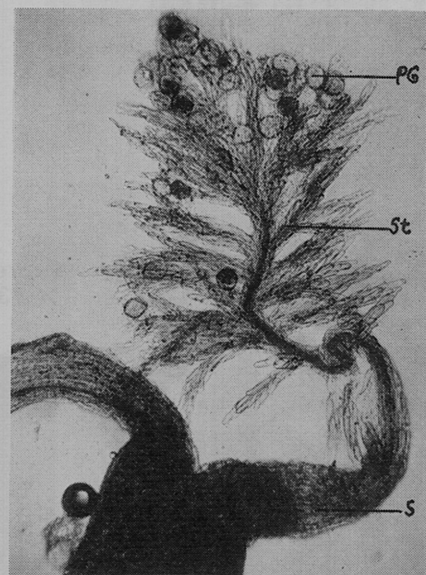


Figure 6. Mature kernel of Zenith variety rice (×67)

PG. Pollen grains still on stigma
S. Style
St. Stigma

Figure 7. Endosperm cells near aleurone layer of rice kernel (×100)

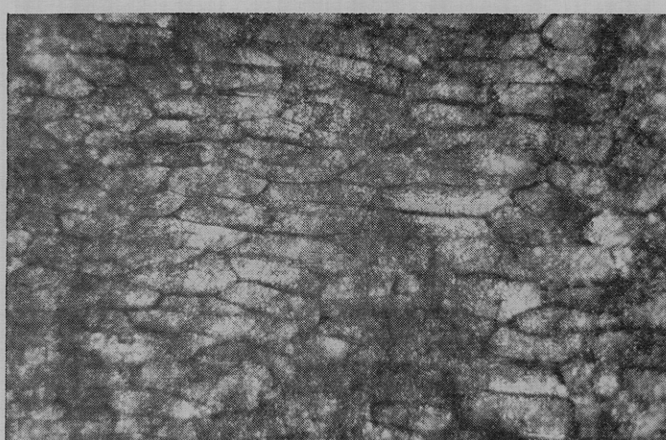
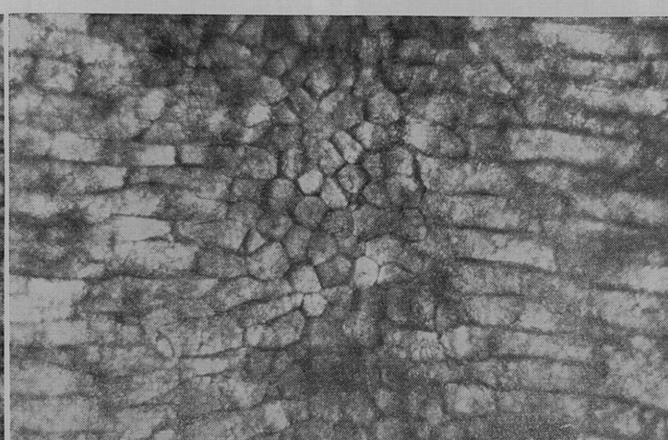


Figure 8. Endosperm cells in center of rice kernel (×100)



vealed by x-ray and photomicrographic techniques, is related to yields of head rice, color, stability to oxidation, and nutritive value.

Acknowledgment

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Determination of Moisture in Chocolate

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A RAPID, precise, and accurate method was needed for determining moisture in chocolate. Oven-drying methods require too much elapsed time, and distillation methods are not satisfactory at moisture levels normally encountered in chocolate. Therefore, the the Karl Fischer titration was investigated.

Kentie and Barreveld concluded that Karl Fischer titration is the fastest and best method for following water content during processing (*1*), but presented no data on its accuracy.

The Karl Fischer reagent used in this laboratory is that suggested by Seaman *et al.* (*3*). The conventional Fischer reagent is split into two parts: Reagent A containing methanol, sulfur dioxide, and pyridine, and Reagent B containing iodine and methanol. Sodium tartrate dihydrate is used to standardize the reagent (*2*). In the work reported, both the Fisher Titrimeter and the Beckman Aquameter were used for the titration.

Water is extracted from the chocolate sample by heating with methanol just to boiling, cooling, adding Reagent A, and after 10 minutes titrating with Reagent B. When samples were extracted at room temperature with methanol or Reagent A, the values were low. A longer boiling time did not increase the amount of water extracted.

To show that added water could be recovered, several experiments were performed in which water was added to chocolate and determined by Karl Fischer titration. Some difficulty was encountered in quantitatively adding water to the chocolate. Melting the chocolate at 70° C., so that the water might be beaten into it, caused the loss of some water, even in a closed (though not hermetically sealed) system. However, gravimetric determinations before and after addition of water gave a good measure of the amount of water actually retained in the sample.

Five experiments using the gravimetric measure of water added showed that the recovery ranged from 90 to 117% of the total water present in the sample, when the sample was analyzed on the same day as prepared or the day following (Table I). The standard deviation for a single determination, calculated from the duplicate determinations represented in Table I, is ±4% of the moisture value.

Table I. Recovery of Added Moisture in Chocolate

Expt.	H_2O in Original, %	Change in H_2O Found Grav., %	Total H_2O, % Calcd.[a]	Total H_2O, % Found K.F.	Recovery Based on Total H_2O in Sample, %
A	0.98	+0.33	1.31	1.40	107
		+0.76	1.74	2.04	117
B	0.65	+0.43	1.08	1.00	93
		+0.80	1.45	1.31	90
C	0.82	+0.40	1.22	1.22	100
		+0.86	1.68	1.71	102
D	0.52	+0.37	0.89	0.91	102
		+1.18	1.70	1.68	99
E	0.60	+0.41	1.01	1.02	101
		+0.94	1.54	1.53	99

[a] Calculated from original H_2O content by Karl Fischer and gravimetric change. All values are duplicate determinations obtained 1 day after moisture addition.

Proper sampling of chocolate is also a consideration in determining moisture. One experiment showed a moisture gradient as follows in a bar 1 inch thick, but additional data are needed to establish the normal moisture gradient in bar chocolate.

	Water, %
Skin, 1/8 inch thick	1.13
Inner layer, 1/4 inch down	0.55
Center 1/4 inch	0.62

In order to eliminate the effect of surface moisture, it is advisable to remove the outer skin and chip the remainder of the bar to get a homogeneous sample. The best method of obtaining a representative sample is to melt the sample in an oven at 50° C., stir, and then sample, but this takes longer than shaving and chipping. No moisture was lost after several hours at 50° C. in a closed container.

As the results found in two laboratories in different sections of the country were in agreement, no correction for the effect of humidity seems necessary.

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