which we weighed the water liberated every hour or oftener that moisture continued to be given off for over 4 hrs. To give an example: It was found that on heating Swedish filter paper for 1 hr. at 184° C., 4.79 per cent water was obtained, while on continuing the heating for 4 hrs. more an additional 0.11 per cent moisture was liberated. Similar results were obtained in other cases.

SUMMARY

The method of determining the moisture contents of cereals and other colloidal organic substances outlined above consists essentially of heating the material in a very high vacuum for definite periods of time. The amount of moisture liberated is condensed quantitatively in a small tube surrounded by solid carbon dioxide from which it is accurately weighed.

Since the smallest amount of decomposition can be accurately determined, this method permits us to determine to what temperature and for what length of time a substance may be heated without encountering an appreciable amount of water from decomposition. Observations on the evolution of gases and moisture where measurable decomposition was taking place gave us an idea of the rate of decomposition at these temperatures.

LABORATORY EXPERIMENTS ON THE MANUFACTURE OF CHINESE ANG-KHAK IN THE UNITED STATES

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Chinese red rice, or ang-khak (ang-quac),[1] is produced by means of a noteworthy fungus, *Monascus purpureus* Went. Red rice evidently originated in one of the provinces of China, and even to-day may be procured only in certain localities of that country. It is well adapted to its special use, the coloring of food products, such as Chinese cheese, because of its property of breaking into fine particles when rubbed or brought into contact with watery solutions. The Chinese have been very secretive concerning the preparation of red rice, and the literature contains only the following facts on the subject. Ordinary rice is moistened and somehow infected or inoculated with a fungus which under humid conditions produces cottony mycelium, eventually binding the rice grains together. The rice grains, at the end of a brief period, are thoroughly impregnated with mold hyphae which produce the red color. When ready for the market, the red rice, the grains of which may be readily powdered between the fingers, imparts a clear carmine color to any moist food substances to which it is added. This same fungus is, according to Went,[2] responsible for the red rice of the Malay Islands, and is also employed in Formosa in making anchu, a rice drink.

Monascus purpureus has been reported from silage in America by Buchanan.[3] We have also obtained it repeatedly from silage where it grew in a substratum 6 in. below the surface, and also from spoiling cornmeal samples. Notwithstanding the competing organisms, *Monascus purpureus* has always been successfully isolated from Chinese red cheeses, which are colored with red rice. A culture of the same or a closely related mold has also been obtained from moldy carob beans. The most interesting source of all, however, is "freckled" codfish. We have not completed experiments to prove that the "freckling" is due to *Monascus purpureus*, but we have observed curious spore-like bodies, which in part compose the freckles, develop into the fungus in question when transferred to highly salted agar plates. The medium used was Czapek solution agar, slightly acidulated with normal lactic acid, and modified by the addition of 15 per cent sodium chloride. The spore-like bodies, transferred from the codfish "freckles,"[1] remained apparently unchanged on the agar surface of the plate for weeks, during which time the medium evaporated and the salt appeared as crystals in the agar and on its surface. Careful microscopic examination of these plates showed that the spore-like bodies developed in this concentrated material. The mold growth continued microscopic in area long enough for the plates to be overlooked. On reëxamination this microscopic growth had developed into a local, living, white fluffiness of mold hyphae, bearing the monilia-like spores of *Monascus purpureus*, and showing under the microscope the characteristic ascus-like bodies.

The chief interest of this investigation is the production of the red color. Lafar claims for red rice a clear carmine to gray-red color on pulverization. Buchanan's *Monascus purpureus* from silage grew on silage agar and broth, and on gelatin solutions, with the production of white to red to deep carmine mold growth, according to the age and the food given. On rice flour medium the surface mold growth was gray, with the medium a brilliant carmine.

Two strains of *Monascus purpureus* from silage, sent in by A. R. Lamb, of Iowa State College, were retained and studied[2] in the Microbiological Laboratory. One, A, is a more or less floccose fungus of a white, soiled white, or gray color; the other, B, is similar to Buchanan's organism in its color reactions, and it grows in a submerged manner. Four strains of *Monascus purpureus* were secured from Chinese products, three from the superficial red coloring on soy bean cheeses and one from red rice. Each shows some variation from the others in the intensity of the color reaction and rate of growth under the same conditions. Nevertheless all are apparently morphologically alike, and duplicate the description of Buchanan, Went, Ikene,[3] Uyeda,[4] and Harz.[5]

The laboratory experiments with the pure culture manufacture of red rice were begun in December 1917.

[1] F. Lafar, "Handbuch der technischen Mykologie," **2**, 265–269, Jena, 1906.

[2] *Ann. Sci. Nat. Bot.*, **1** (1895), 1–16, plates 1 and 2.

[3] "*Monascus purpureus* in Silage," *Mycologia*, **2** (1910), 99–106, plates 22 and 23.

[1] A. W. Bitting, "Preparation of the Cod and Other Salt Fish for the Market," U. S. Dept. of Agr., Bureau of Chem., *Bulletin* **133** (1911), 1–63.

[2] Czapek solution agar with 3 per cent sucrose was used as a standard medium.

[3] "Über die Sporenbildung und Systematische Stellung von *Monascus purpureus* Went," *Ber. botan. Ges.*, **21** (1903), 259.

[4] *The Botanical Magazine, Tokyo*, **15** (1902), 160.

[5] "Physomyces heterosporus," *Botan. Centr.*, **41** (1890), 378, 405.

Water was added to unpolished, uncooked rice, and small amounts of this material were sterilized in Erlenmeyer flasks. Strain A from silage, strain C from red rice, and strains D and E isolated from cheeses, were used.

The silage organism gave a pink color to rice grains, and caused some of the grains to split into small portions, while others could be crumbled into smaller, hard particles. Strains C and D produced very deep, almost red-black color throughout the rice, but they broke down the grains so completely that the mass became slushy. In the belief that the softening might proceed still further with no definite results, the reddened rice grains were spread out on a paper and dried. On drying they became a brilliant to black-red, crumpled and shrunken, with grains adhering in small masses, and tough in texture. The mold discoloration penetrated almost completely. The use of strain E, also from Chinese soy cheese, resulted in more promising material. After being dried out from a somewhat slushy consistency, each grain of rice stood intact, although not plump or regularly defined. The superficial deep red layers crumbled in what was considered the approved fashion, but below these the rice was hard and pinkish as in the case of strain A. The material, however, was considered worthy of exhibition.

These laboratory products were compared with a sample of red rice collected in China by Dr. Yamei Kin, of the Bureau of Chemistry. In this typical sample each rice grain is a regular, clear-cut particle, of a deep but dull carmine color. On rubbing between the fingers, these red rice grains crumble into a tissue-like envelop formed by the exterior surface of the grain, and a very fine, soft red powder, the particles of which go into suspension in water and into solution in ethyl alcohol or chloroform as well as in other solvents.

A second experiment was laid out with exactness, replacing the rough, preliminary methods. Uncoated and unpolished rice was sterilized in Erlenmeyer flasks. After cooling, 25 per cent additional sterile water was added to some samples and 30 per cent additional sterile water to others.

The results obtained with strains A, C, and D are worthy of note. On rice to which 25 per cent of water was added, strain A grew with a most delicate rosy color, and caused a somewhat more complete disintegration of the rice grains than in the preliminary experiment. It did not grow at all on the rice to which 30 per cent of water was added. It is evident, then, that less than 25 per cent of water should be added to obtain red rice. Strain C produced a dull, orange-red color on rice to which the 25 per cent of water had been added. The grains adhered occasionally, and when an attempt was made to crush them they merely split into smaller hard portions. The same results were obtained when 30 per cent additional water was used, except that a rather noticeable amount of close, felty mycelium, cementing the grains together, was obtained. It is believed that too much water is an inhibiting factor here. On rice to which 25 per cent of water was added, strain D actually produced reddened rice kernels, which may without hesitation be called American-made Chinese ang-khak. The rice kernels, which were well shaped, each intact in itself, and of a rich carmine-red, crumbled to powder between the fingers, and the outer layers resembled a tissue-like coating. Thirty per cent additional water caused this strain to act like strain C, the material, however, being tougher, more floccose, and entirely unsatisfactory.

Evidently rice which contains an addition of 25 per cent of water, when held at room temperature (22–24° C.), is at a critical point, so far as the various strains of *Monascus purpureus* Went are concerned. This fungus, although employed commercially only in the Orient, is more cosmopolitan in its habits than its applied use would indicate. Apparently, *Monascus purpureus* Went is a specific name applicable to a group of strains having a rather consistent morphology, but varying greatly in their quantitative production of the same substances.

SUMMARY

I—The characteristics of red rice, a common Chinese vegetable color used in food products, are due to a mold.

II—This mold, known as *Monascus purpureus* Went, although apparently found outside the Orient, is utilized in this way only in China.

III—All strains of *Monascus purpureus* are not adapted to the production of red rice, since each varies quantitatively in its physiological activity. Only those strains which produce a rich dark red growth throughout rice with a water content low enough to permit well-appearing grains in the finished product are acceptable for this purpose.

THE EFFECT OF MOLD UPON THE OIL IN CORN

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Under certain conditions of storage, corn undergoes spoilage induced largely by an excess of moisture and usually manifested by a moldy appearance. A common mold which attacks corn under these conditions is *Penicillium*, the growth of which necessarily causes deterioration, the extent and nature of which depends upon the length of the growing period of the mold and the particular constituents of the corn which are subject to decomposition. From the general appearance of corn attacked by this mold it is evident that changes are taking place in the grain and it was for the purpose of studying the effect of the mold upon the fatty oil in the corn that this investigation was undertaken. It is natural to assume that as the time period during which the mold is allowed to grow, increases, certain constituents of the corn are contributing to the growth and are therefore undergoing change, while certain other compounds are possibly being metabolized by the mold. The fatty oil belonging to