A Bowl of Rice and SEM

Miloslav Kaláb

Scanning Electron Microscopy (SEM) was used to take a closer look at some of the most important components of rice grains such as tightly packed starch granules and minute protein bodies. Also examined was the microstructure of natural and processed grains such as parboiled (converted), instant, and puffed rice, rice flour, and a bran by-product. Natural "monacolin" statin present in red yeast rice was found in the form of minute crystals on the grain surfaces whereas the endosperm was filled with partially to severely degraded starch granules and a mass of dry *Monascus purpureus* hyphae.

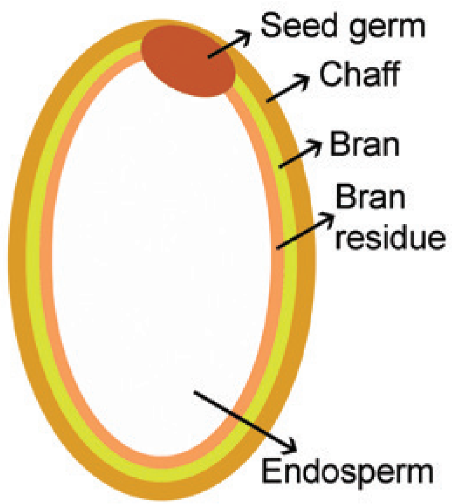


Figure 1. Diagram of a rice grain.

Rice is the seed of the grass species *Oryza sativa* (Asian rice) [Internet 1] or *Oryza glaberrima* (African rice) [Internet 2]. It was the third largest worldwide agricultural commodity (741.5×10^6 tonnes) in 2014, surpassed only by sugarcane (1.9×10^9 tonnes) and maize (1.0×10^9 tonnes) but unlike the other crops, rice is grown almost exclusively only for human nutrition. However, all parts of the plant are utilised [Internet 3]. As a cereal crop of about 2500 varieties, it grows in the form of small starchy grains of various sizes, several different colours, and many different cooking properties.

Other cereals grown globally in very large quantities include wheat, corn, oats and rye. Rice provides staple foods to the populations of most countries. Globally, only 5% of starchy staple food come from root crops such as cassava, potatoes, and yams.

Rice (*Oryza sativa*) is native of the Indian subcontinent. It consumes more water than any other cereal crop during its growth. However, the water in the rice paddies is increasingly being utilised to also produce shrimp – they feed on the paddy crops while the waste from the shrimp culture fertilises the rice. Paddy rice is highly productive and can give two harvests in a single season (McGee 1984). Because of higher yield, Asian rice has been replacing African rice. Wild species in

Figure 2. Brown rice. Bar: 5 mm.

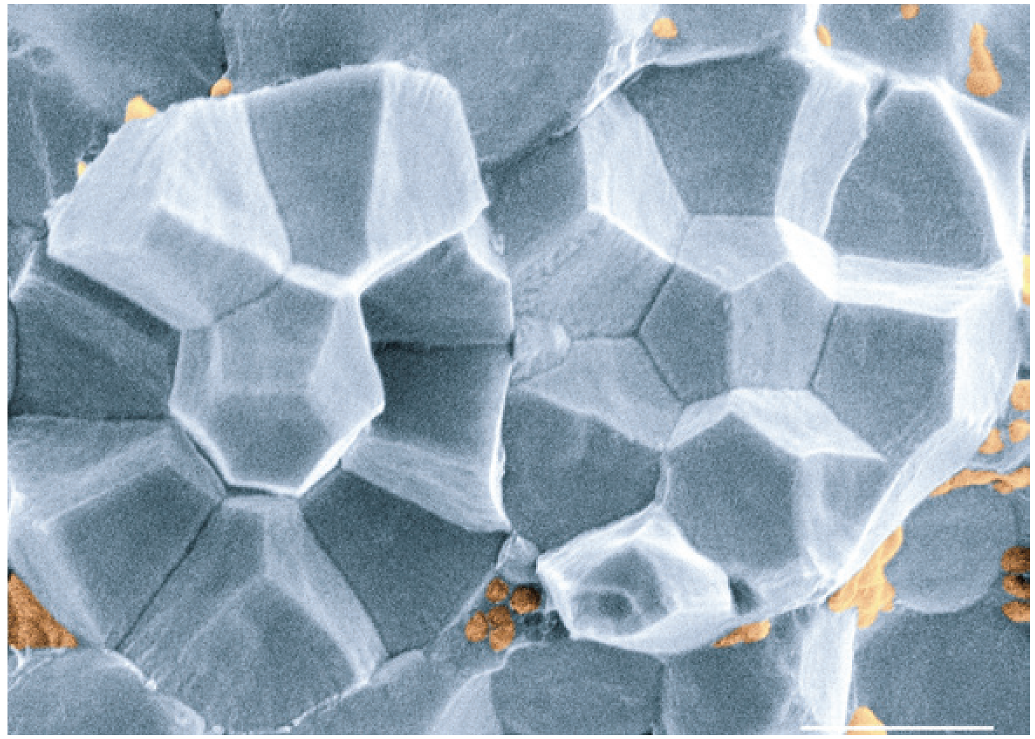


Figure 3. Rice starch granules (polyhedrons) appear tightly packed in two cells of an Arborio rice grain; minute protein bodies are seen in yellow. Bar: 5 μm.

the genus *Oryza* such as *Orufipogon* are perennial [Internet 4], spreading vegetatively by above-ground stems, and producing 3 or even 4 crops per annum under favourable conditions.

In spite of the very high number of different varieties of rice [Internet 5], there is only one commercially significant difference: Indian rice (*O. indica*) is long-grained and tends to be dry, flaky, and easily separated when cooked. Japanese rice (*O. japonica* or *O. sinica*, *Oryza sativa var. glutinosa*) is short-grained, moist, firm, and sticky when cooked. The difference is caused by a higher proportion of amylopectin [Internet 6] in the starch granules of this rice.

Materials and Methods

Rice grains and products were of commercial origin. Rice starch was obtained from the SPI Group in San Leandro, California, USA. A Pentax K2000 (10 megapixels) digital camera was used with a 50 mm macro lens and RAW format images were converted to JPG files. For scanning electron microscopy (SEM), samples mounted on aluminum stubs were sputter-coated with ~9 nm of gold and examined in a Philips XL30 ESEM microscope operated at 3.5 or 5 kV accelerating voltage and recorded at a resolution of 2420 lines/frame. The grayscale images were coloured using Adobe Photoshop CS software.

Images of rice and its products

The rice grain consists of starchy endosperm coated with bran and chaff. It also contains a seed germ (Figure 1) [Internet 1]. Removal of the inedible outer husk produces "brown rice" (Figure 2). White rice [Internet 7] is a rice grain with the husk, bran layer and cereal germ removed [Internet 8]. A simple method to study whole rice grains was described by Ogawa et al. (2003B).

Rice is consumed directly in the form of cooked or steamed rice alone or with other meals and is also processed into various food products [Internet 9] such as noodles, cakes, puddings, baby cereals,

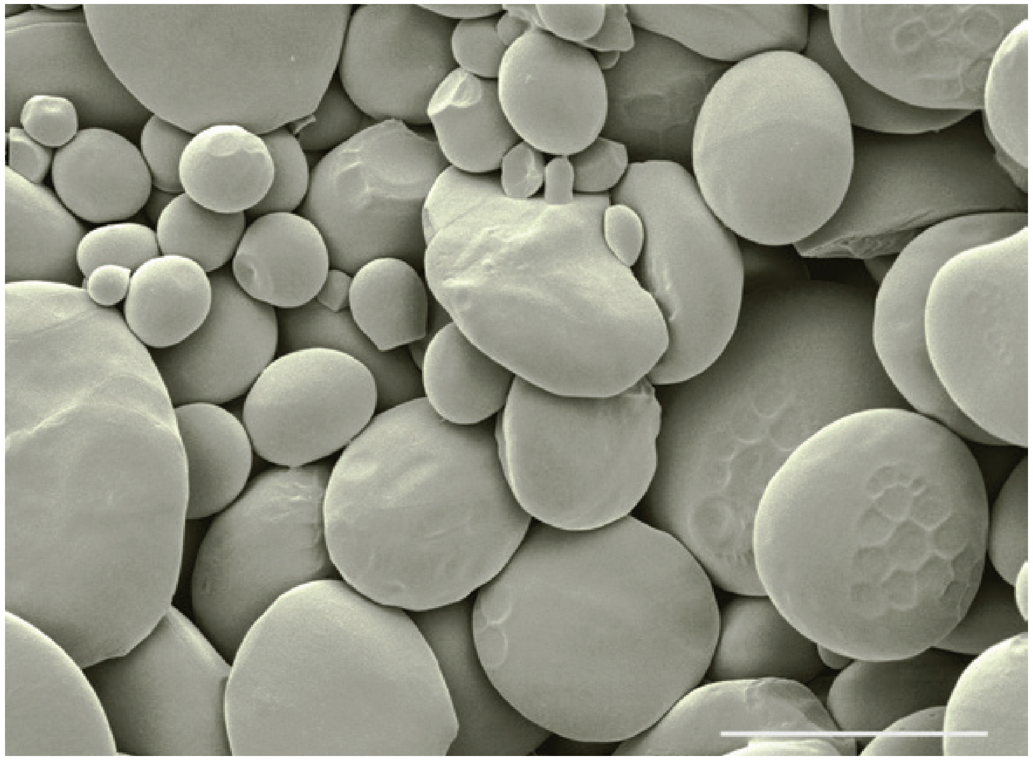


Figure 4. Oval starch granules from a wheat grain are of two size groups. 15-20% are small (2-15 µm in diameter) and 80-85% are large (20-35 µm in diameter). Bar: 10 µm.

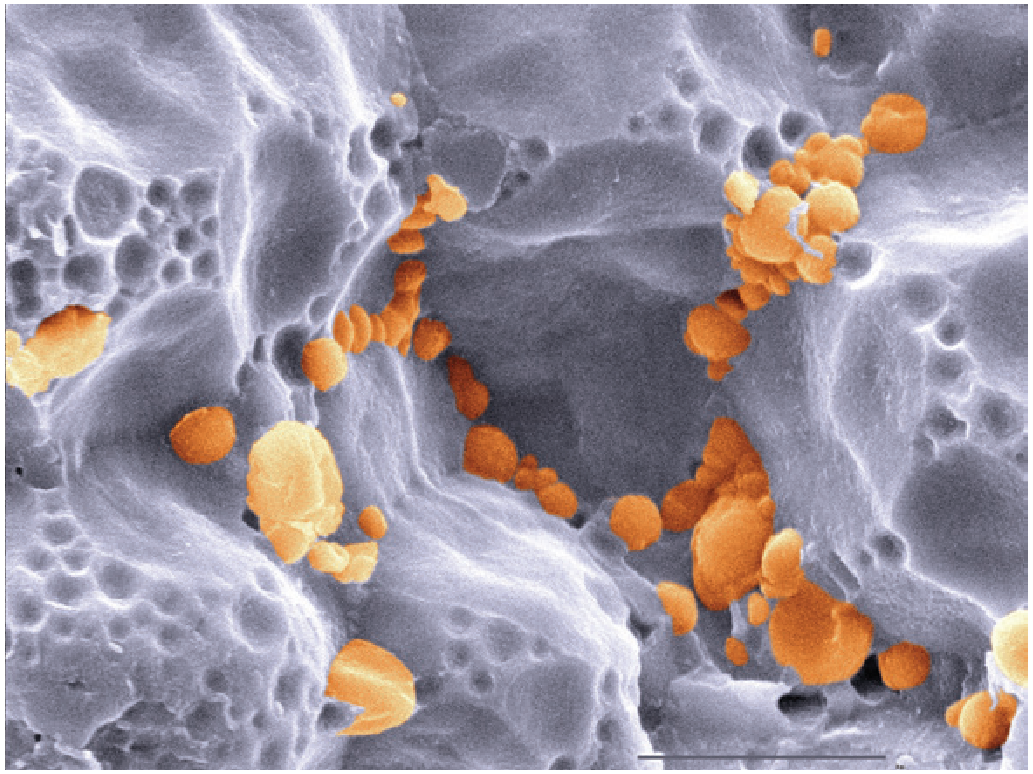


Figure 5. Protein bodies (beige) between starch granules (blue) in black glutinous rice. Bar: 5 µm.

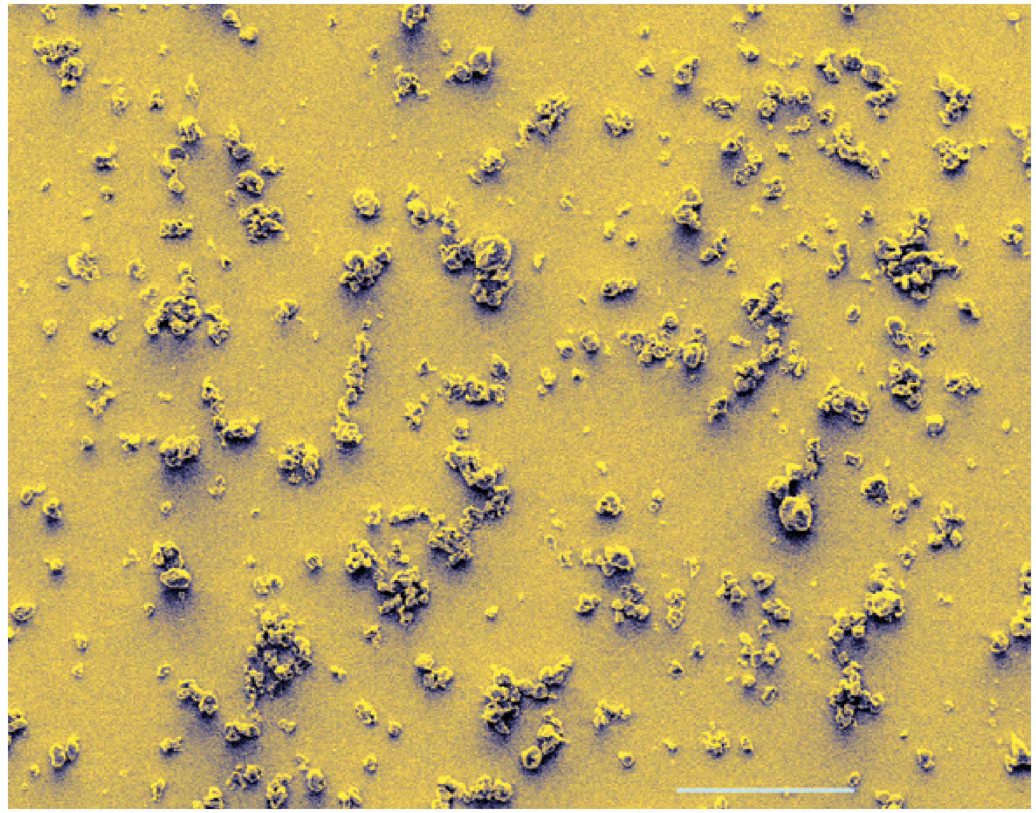


Figure 6. A thin layer of milled rice dust particles. Bar: 50 µm.

rice wine, and rice vinegar. This study presents the following subjects on rice:

1. Rice grain structure.
2. Rice for cooking.
3. Rice flour and starch.
4. Puffed rice.
5. Rice bran.
6. Red yeast rice.

1. Rice grain structure

In a cross section, rice grains markedly differ from the grains of other cereals such as wheat (Heneen & Brismar, 1987) or barley (Black, 2001). The starch granules in the rice grains are packed very tightly in the form of polyhedrons (Figure 3) whereas wheat starch granules are oval (Figure 4).

Protein bodies, 1.8 to 8.0 µm in diameter, are present in the rice grains in three regions: the aleurone, the subaleurone portions of the endosperm, and the embryo (Bechtel & Pomeranz, 1978). In the aleurone, protein bodies are tightly packed between the polyhedron starch granules (Figure 5). Separation of the starch granules from each other by fracturing reveals imprints of the protein bodies in the form of minute dimples. Barber et al. (1991) reported that tetrabromofluorescein may be used to distinguish rice protein bodies from those of other seeds using fluorescence microscopy.

Experiments aimed at separating protein bodies from starch granules in dry-milled rice (Horsman et al., 2009, unpublished results) have pointed to difficulties posed by the tight packing of rice starch granules in the grains and, after milling, by the similarities in the sizes and densities of both kinds of organelles and by the presence of cell wall fragments. Pure starch is not as easily prepared from rice as from wheat (Guraya 2002). Conventional hammer milling produced approximately 90 µm wide aggregates with smaller particles jutting from their surfaces. Subsequent jet milling produced a powder with a bimodal particle distribution with peaks at 1 µm and 5 µm, i.e., at dimensions similar to those of the protein bodies and the starch granules, respectively. The author of this presentation has adapted a preparatory technique which would facilitate the examination of the very fine rice powders using SEM. Glass cover slips (13 mm in diameter) had been first sputter-coated with gold (~8 nm) and then a minute amount of the powder was applied. Although no mechanical procedure would spread the powder particles evenly, a drop of 95% ethanol applied to the powder spread it rapidly in a single-particle layer over the gold surface. Following ethanol evaporation, the samples were sputter-coated and examined. This procedure ensured an uninterrupted conductive path over the sample and prevented so-called charging artifacts. It has been found useful particularly in SEM of particles 1 to 10 µm in diameter [Internet 10] (Figure 6).

2. Rice for cooking

From the cooking point of view [Internet 11], there are many major rice varieties such as:

Long grain rice: American long-grain white rice – often cooked in a covered pot with a measured amount of water, which gets completely absorbed by the rice, produces "steamed rice". – American long-grain brown rice has its bran and germ layers left in place, giving the rice a nutty, grainy flavor and a chewy bite. Brown rice may be cooked with an excess water which is then drained. – Basmati rice with extra-long grains and a nutty flavour is the predominant variety in the Indian and Pakistani cuisine. It needs to be soaked for half an hour or more before cooking. – **Jasmine rice**, from Thailand, has a slightly floral aroma caused by aromatic compounds such as 2-acetyl-1-pyrroline. The name "jasmine" refers to the colour of the rice, which is as white as the jasmine flower.

Medium grain rice: Japanese-style rice [Internet 12] is used for sushi and is also served plain as a finish to a typical meal. It's slightly translucent when raw, and firm but somewhat sticky when cooked (it should not be confused with Japanese sticky rice, used to make Japanese rice cakes called "mochi"). – Bomba is the rice of choice for the Spanish classic paella. It absorbs up to twice as much water as long-grain rice but does not become sticky.

Short grain rice: Arborio rice [Internet 13] (Figure 8) is the most widely available variety of Italian "superfine" rice, used to make risotto (the other rice varieties include "carnaroli" and "vialone nano"). All of them have plump grains and a high proportion of amylopectin, a type of sticky starch that is responsible for the trademark creamy texture of risotto. – Short-grain brown rice, like other short-grained varieties, has a higher level of amylopectin, making it slightly sticky. The intact bran gives it more chew than white short-grain rice.

Figure 7. Long grain rice. Bar: 5 mm.

Figure 8. Arborio short grain rice. Bar: 5 mm.

Specialty rice: Chinese black rice [Internet 14] (Figure 9), also known as forbidden rice, because it had been reserved in China for the royalty, has the anthocyanin content higher - 740 mg/kg in a *Chakhao Poireiton* rice cultivar (Asem et al., 2015)

Figure 9. Black rice. Bar: 5 mm.

Figure 10. Red rice. Bar: 5 mm.

Figure 11. Photograph of parboiled (converted) rice. Bar: 5 mm.

- than any other grain, including brown rice, red rice (Figure 10), red quinoa, or other coloured whole grain varieties. Most cook books advise that irrespective of the kind of rice, all grains should be washed in water, drained in a colander, and allowed to sit for at least 30 minutes before cooking. This allows the rice to swell and results in a tender cooked rice, the histological structure, compression deformations, and structural relationships of which were studied by Ogawa et al. (2003A, 2006). The question is, however, what is the goal of rinsing or washing. Uncooked grains contain starch granules on their surfaces. If the boiled rice should be in the form of individual grains, then washing suits that

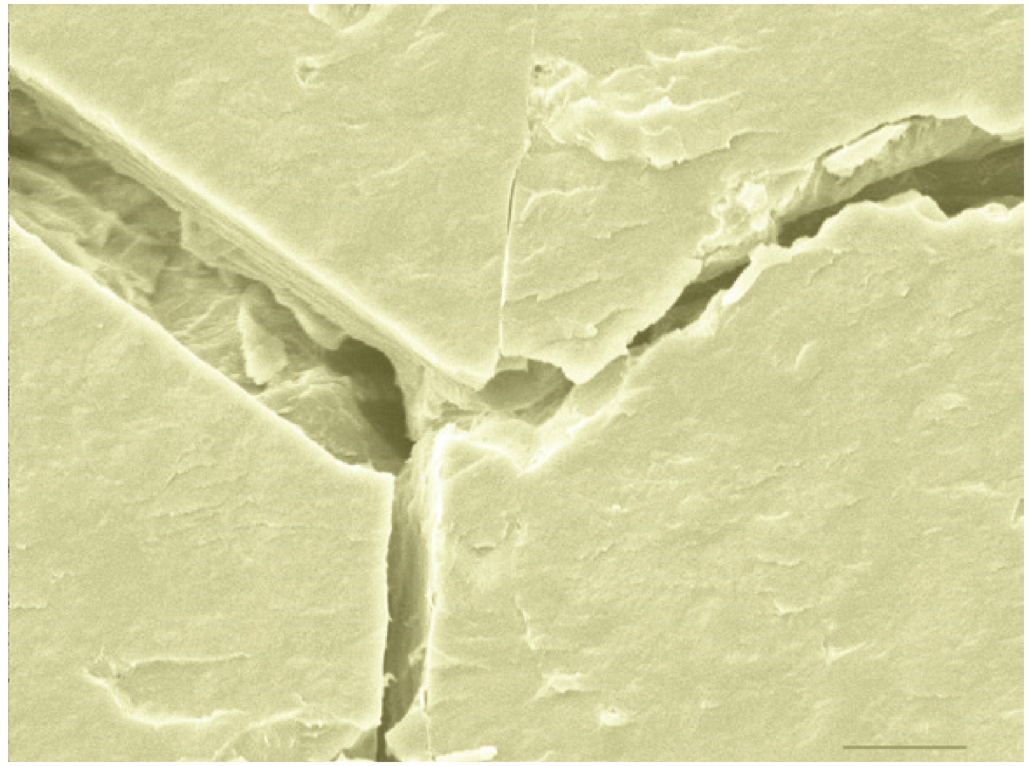


Figure 12. A cross fracture through a parboiled rice grain shows a glassy, non-corpuscular microstructure. Bar: 5 μm.

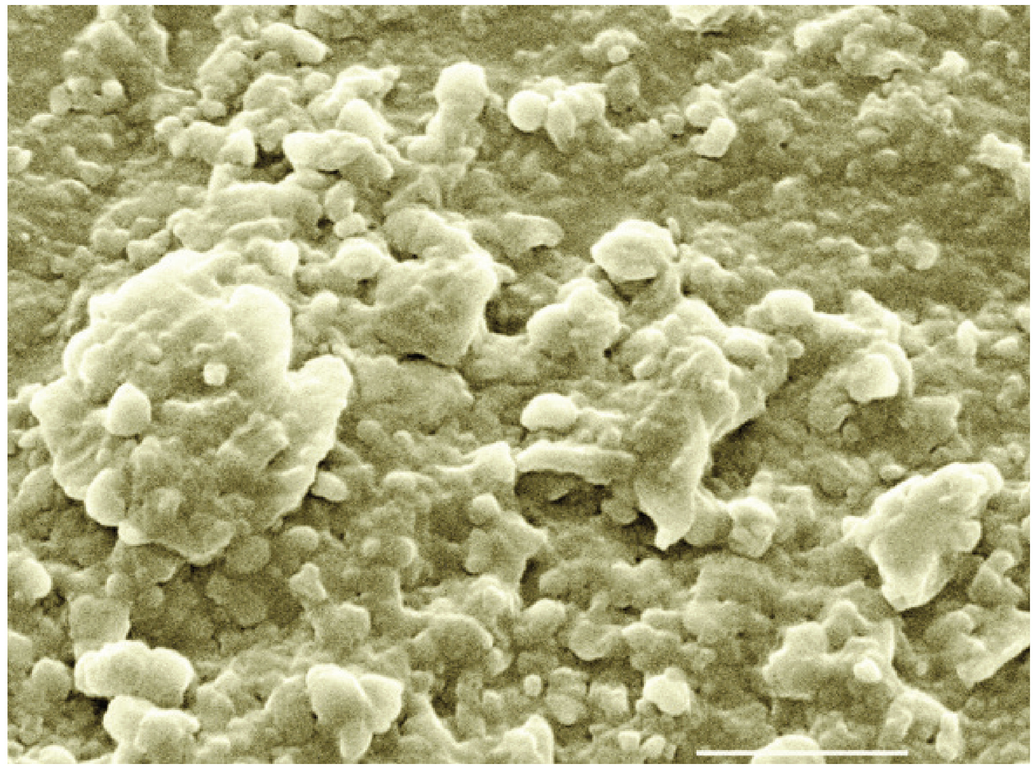


Figure 13. Corpuscular surface of a parboiled rice grain by SEM. Bar: 5 μm.

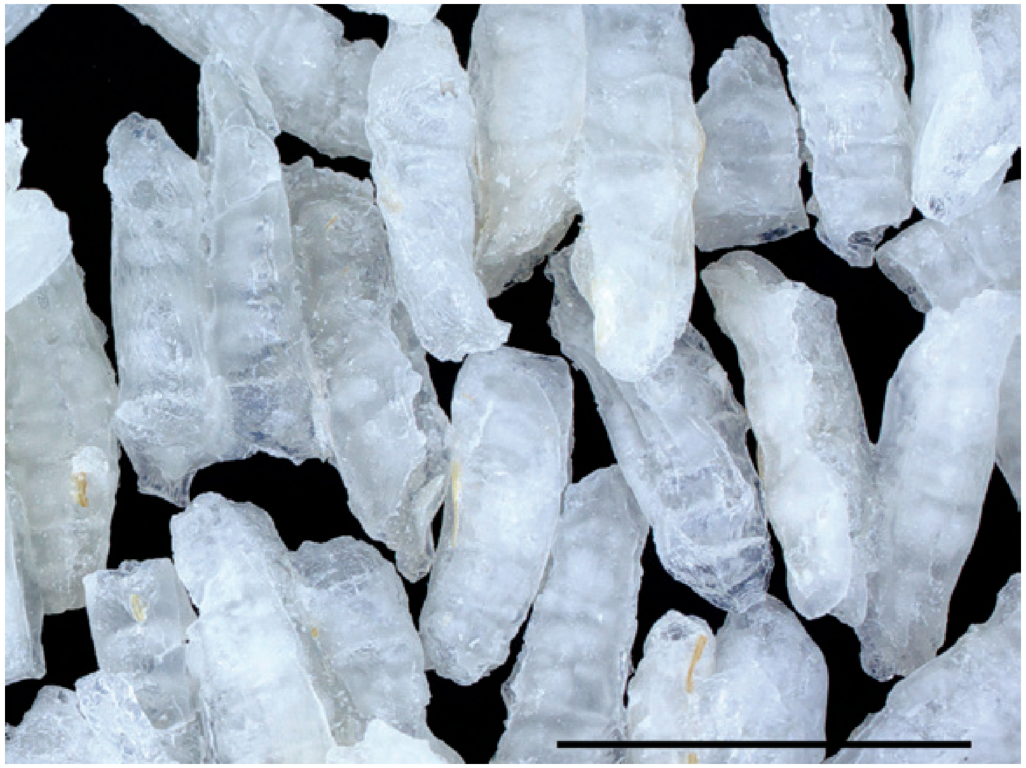


Figure 14. Dry 5-minute instant rice grains have a glassy appearance and a high proportion of them are fused in small clusters (photograph). Bar: 5 mm.

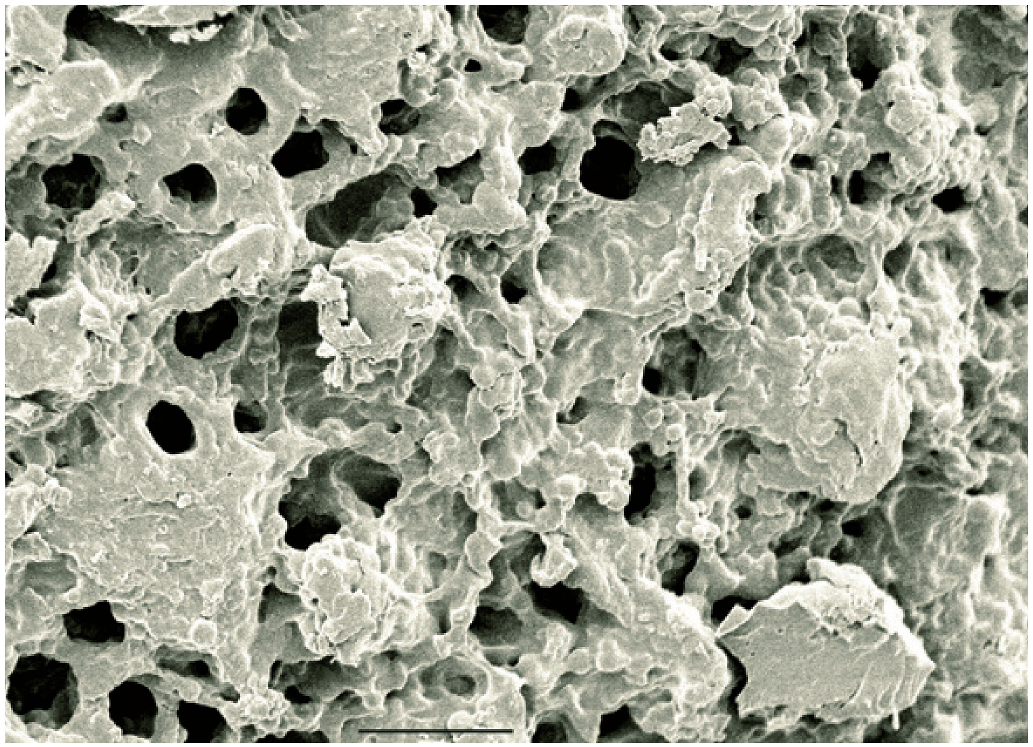
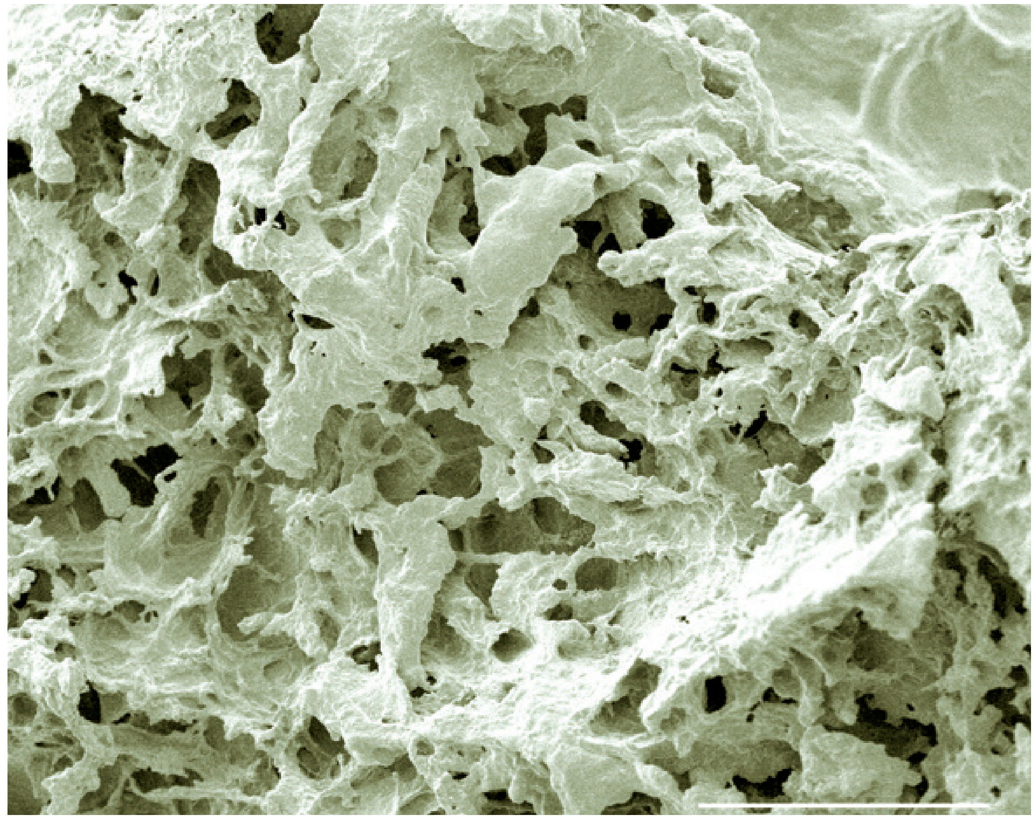
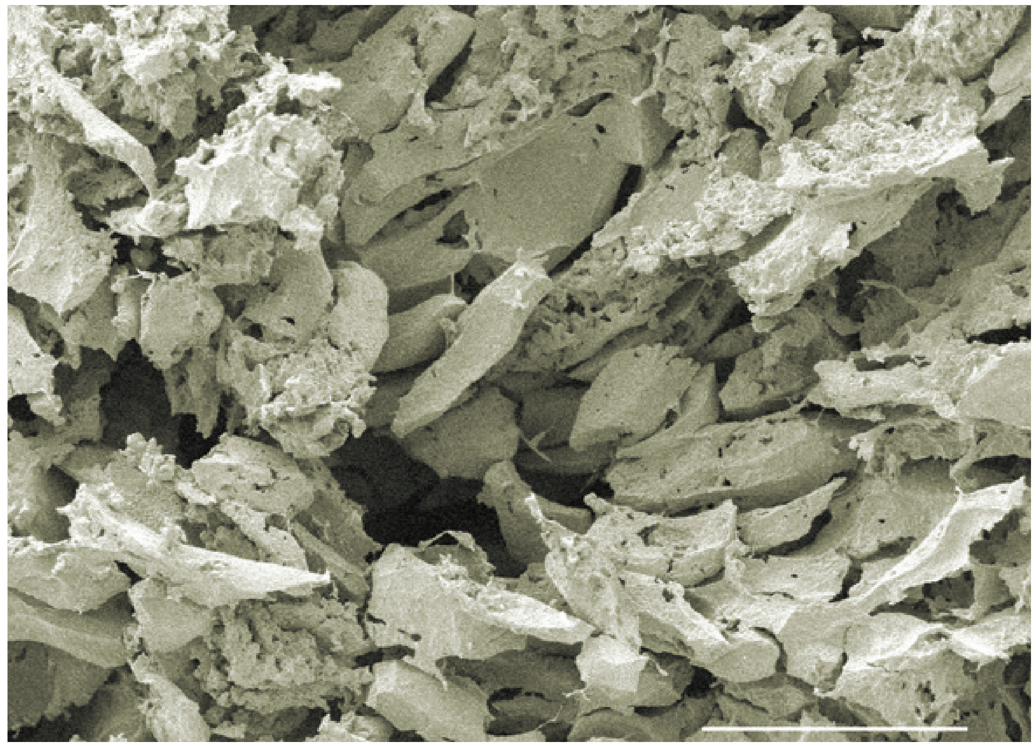


Figure 15. Under SEM, instant rice grain surface appears porous and has a corpuscular microstructure. Bar: 20 µm.





Figures 16 & 17. Steamed, frozen, and freeze-dried rice appears porous at a low magnification both on the surface (top, Figure 16) and in the interior (above, Figure 17). Bars. 200 µm.

Figure 18. Steamed long grain rice. Bar: 5 mm.

Figure 19. Bacillus cereus bacteria. Bar: 2 µm.

purpose, but not if risotto is being made, when the free starch contributes to the stickiness of the cooked rice. In the USA, white rice must be supplemented with vitamins, in which case washing negates the effect of that supplementation.

Rice in many Asian countries is not consumed as "white rice" but as "parboiled" (also called "converted") rice [Internet 15]. Harvested rice is partially boiled in the husk in three basic steps, i.e., soaking, steaming and drying, which transfer some nutrients, especially thiamin, from the bran into the endosperm. Parboiled white rice is, thus, 80% nutritionally similar [Internet 16] to brown rice. Because of this nutritional advantage, parboiling has been adopted by North American rice growers since the early 20th century. About 50% of the world's paddy production (i.e., raw rice grains) is parboiled. The treatment gelatinizes the starch granules and, thus, changes the appearance of the rice grains making them partially translucent and yellowish (Figure 11). The interior has a glassy, featureless structure (Figure 12) but the grain surface is not smooth under the SEM (Figure 13).

Rice takes relatively a long time of 20 minutes (white rice) to 55 minutes (brown rice) to cook, but several kinds of pre-cooked – so called "instant rice" – are commercially available for quicker cooking. The technology of instant rice consists of cooking – cooling – freezing – thawing – drying – and packaging of the finished product. It had

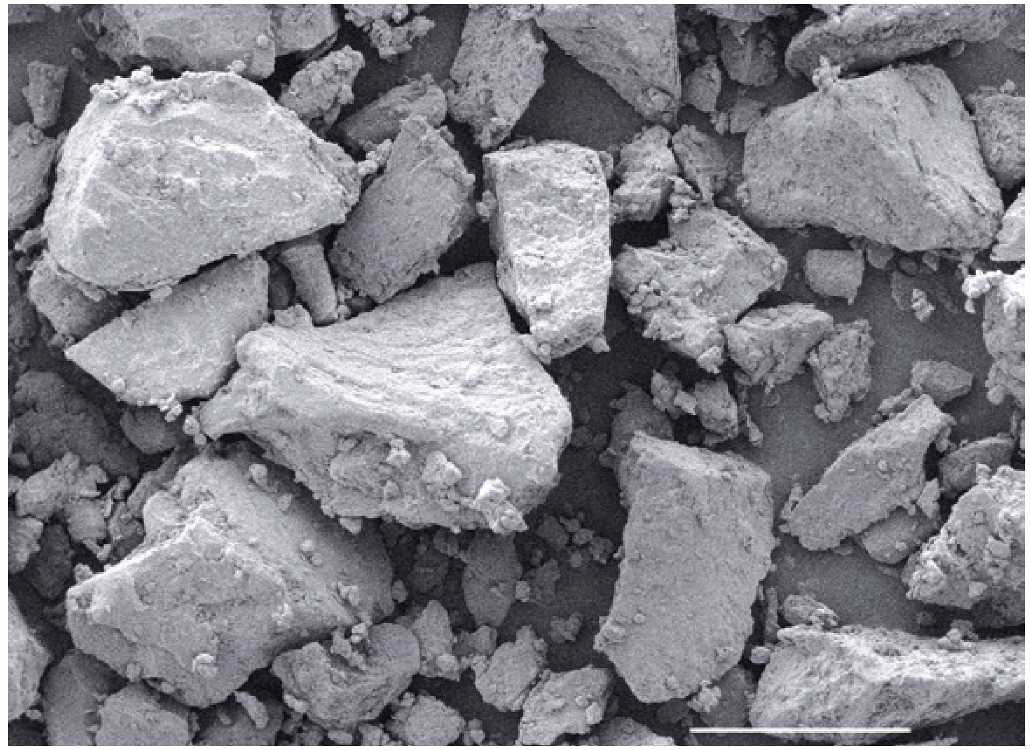


Figure 20. Rice flour. Bar: 200 µm.

been invented by Ataullah Durrani, a researcher of Afghan origin, who lived in New York before World War 2. Unable to find work in petroleum research – his expertise – he started experimenting with rice, which was not very popular in North America at that time because it was difficult to store and prepare. With support from the Arkansas Rice Growers' Cooperative Association, Durrani succeeded with his "instant rice". His invention was purchased by General Foods in the 1940s and all the instant rice produced was used to supply the armed forces, but as early as 1946, instant rice was introduced to the civilian food market.

The manufacturing process retains the external appearance of steamed rice in instant rice (Figure 14). The cooking, freezing, and drying, however, has several effects on the microstructure of the rice grain such as gelatinization of the starch granules, formation of ice crystals, their subsequent thawing and evaporation leading to a porous microstructure (Figure 15), which quickly absorbs water and facilitates cooking.

Experimental freezing and subsequent freeze drying in a freezer extended that process farther and has confirmed that ice crystal formation contributes to porosity of the final product (Figures 16 & 17).

Cooked – steamed – rice (Figure 18) should be consumed soon after it is made. If it is kept warm for many hours, which may happen with unconsumed steamed rice obtained as part of a take-out order and not placed quickly into a refrigerator, it may pose a health risk [Internet 18].

The culprit is a sporulating bacterium, *Bacillus cereus* (Figure 19), which may have been present in the raw rice. Its spores survive the cooking and germinate when the rice is kept warm for several hours. The risk may be prevented by refrigerating leftover rice as soon as possible.

3. Rice starch – rice flour and starch

Rice flour (also **rice powder**) is produced mainly in two forms – glutinous and non-glutinous

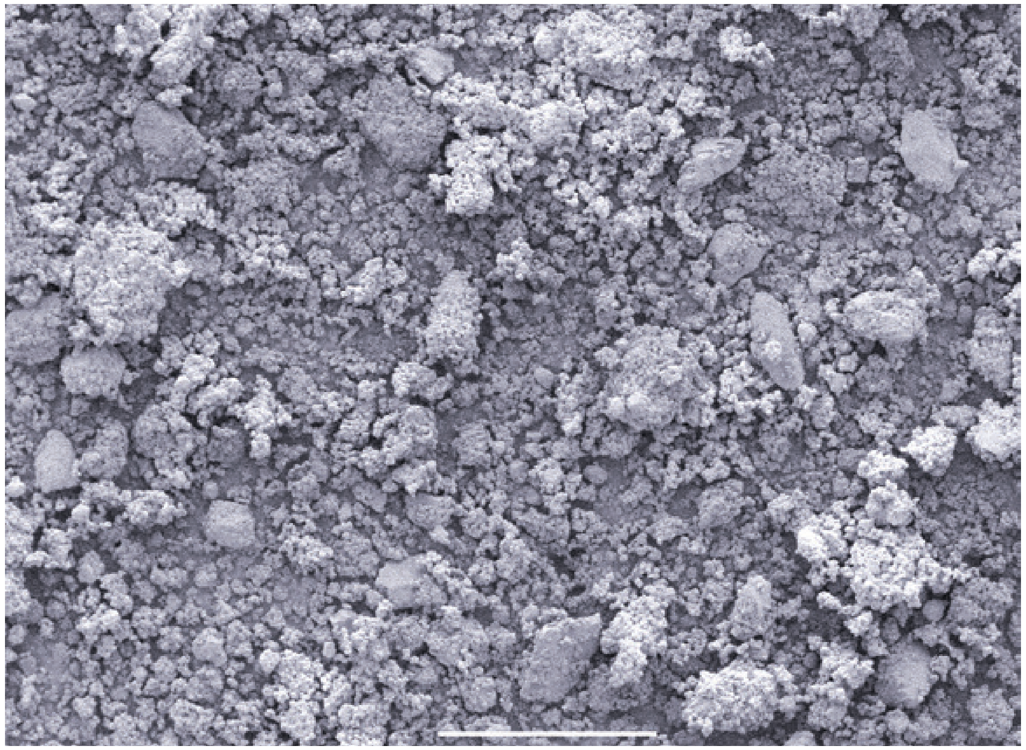


Figure 21. Glutinous rice flour. Bar: 200 μm.

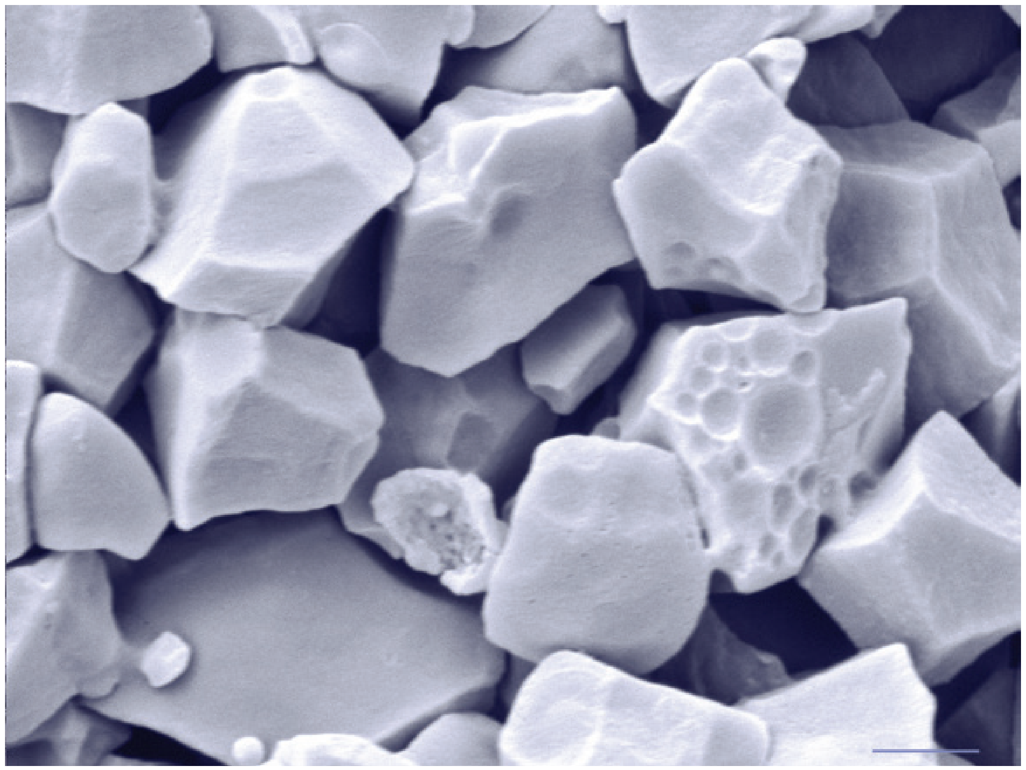


Figure 22. Rice starch granules. Bar: 2 μm.

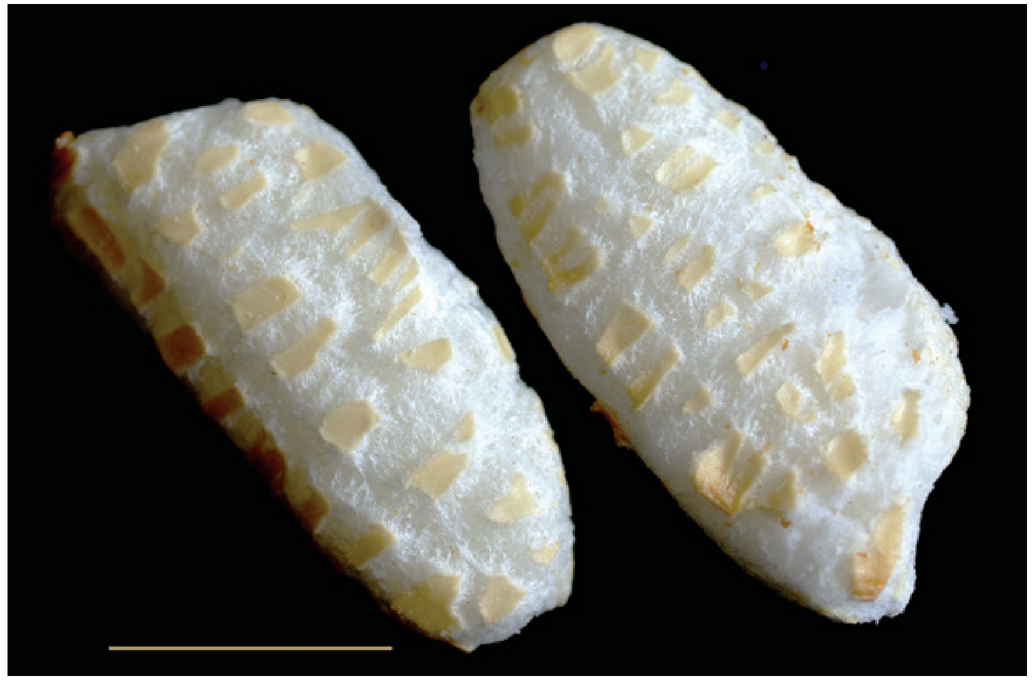


Figure 23. Photograph of 2 puffed rice grains. Bar: 5 mm.

(Figures 20 & 21, respectively). The latter flour is called "sweet" but its taste does not differ from the other flour. When viewed at the same magnification, the regular commercial rice flour consists of particles markedly larger that the glutinous flour. The term "glutinous" means its stickiness when it is cooked and is not related to "gluten" which rice does not contain. All rice flour is made from finely milled rice and is distinct from rice starch, which – in the form of polyhedron granules (Figures 3 & 22) – is the major component of the flour.

In spite of its high starch content, rice is a minor source of it [Internet 20]. Only about 1% of starch on the global scale is made from rice, whereas maize is the source of 70% of starch (produced in North America), potatoes are the source of 12% (Europe), followed by tapioca (9%) and wheat (8%). A widespread use of rice starch is limited by its higher price [Internet 21] relative to the other kinds of starch. Rice starch (Figure 22) used to be produced (Juliano 1984) by steeping rice in lye (NaOH) to slowly dissolve rice protein and to release the tightly packed starch granules. New technology developed by Guraya and James (2002) physically splits apart the starch-protein agglomerates using a high-pressure microfluidizer. Figure 22 was obtained using rice starch made for scientific purposes because pure starch could not be obtained from any food store. The absence of protein body imprints in the pure starch preparation, with a few exceptions, may thus be explained by the harsh conditions needed to separate the individual starch granules.

In addition to starch, rice flour contains cell wall fragments and protein bodies but it does not contain gluten and is, thus, often used as a substitute for wheat flour by people unable to digest wheat gluten. Rice flour may serve as a thickening agent in recipes of foods that are refrigerated or frozen since it inhibits liquid separation after thawing.

4. Puffed rice

Puffed rice (Figure 23) is a convenient form of rice [Internet 22] ready to eat either alone or with salt for added taste, or in a combination with milk (e.g., as Rice Krispies produced since 1927 by the Kellog Company). It may be processed into a variety of

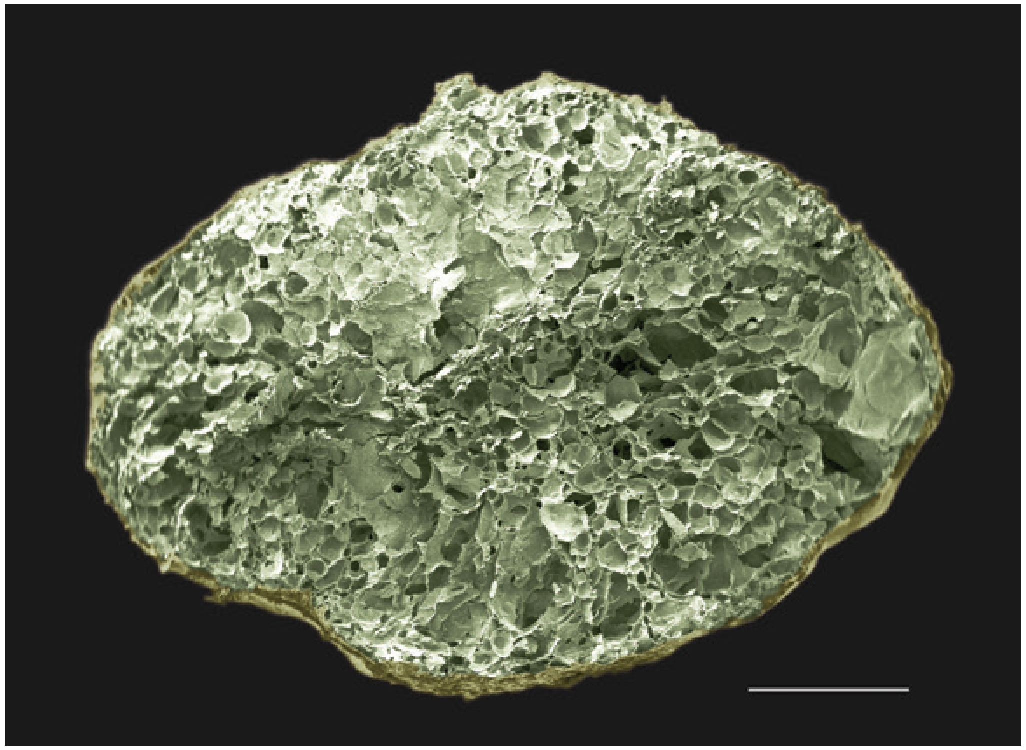


Figure 24. Cross section of a puffed rice grain showing air cells and a compact grain surface. SEM. Bar: 1 mm.

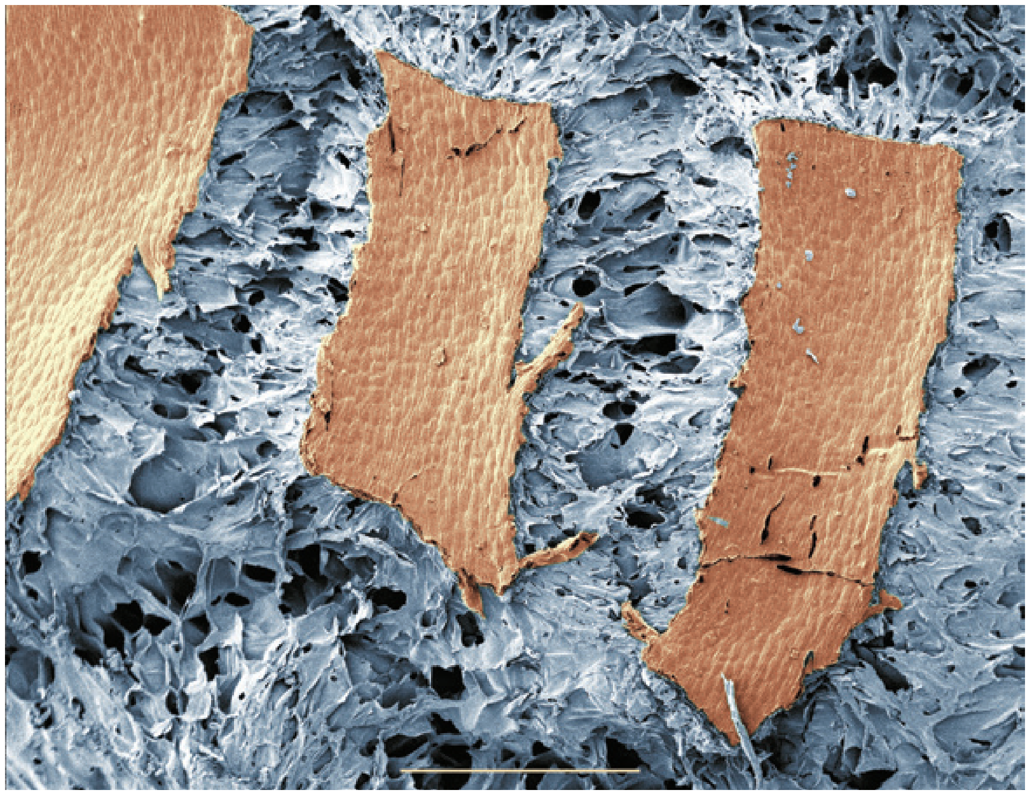


Figure 25. SEM detail of a puffed rice grain surface. (Bar: 500 µm).

food products such as rice cakes in combination with honey or chocolate, coated with sugar as coloured puffed rice candies, as an ingredient in other foods.

Puffed rice consists of grains expanded quickly by vapour formed in the grain from the inherent water. There are two terms, "popping" and "puffing", used for grains forming products such as corn (popcorn), or puffed rice or wheat (Mishra et al., 2014). The rice grains do not contain enough water to accomplish the explosive expansion unlike corn, the "popping" of which may be done at home on the stove or in a microwave oven.

Whole rice grains are heated in an industrial setting at high pressure with steam in a containment vessel. When the pressure is quickly released, the steam bloats the endosperm of the kernel and the volume increases to several times its original size. Chandrasekhar & Chattopadhyay (1990) studied this process using SEM. A proper control ensures that the grains do not disintegrate but become "fluffy" or "foamy" from the formation of numerous air cells as shown in Figure 24. Unlike in corn, the rice grain surface does not disintegrate but it expands like an inflated balloon. The seed coat is broken into "islands" which remain attached to the expanded grain (Figure 23). At a higher magnification (Figure 25), the seed coat fragments are seen attached to the puffed endosperm. Although recipes may be found to make puffed rice at home, the results of industrial manufacture cannot be duplicated and the domestic products are inferior.

5. Rice bran

Rice bran (Gul et al., 2015) is a valuable commodity in Asia and is used for many daily needs. It is a by-product obtained from the rice milling processes, particularly of the conversion of brown rice to white rice. It is a moist, oily inner layer of the grain cover (about 12% oil) which also contains vitamins B_1, B_6 and E and has beneficial effects on human lipid metabolism. There is a high proportion of dietary fibre (beta-glucan, pectin, and gums) but also an elevated level of arsenic (Ruangwises et al., 2012). Black rice bran [Internet 23] has a very high content of antioxidants, considerable higher than other foods including blueberries.

Figure 26. Macro-photos of rice bran (left) and oat bran (right). Bar: 5 mm.

Figure 27. Rice bran at a higher magnification (photo). Bar: 2 mm.

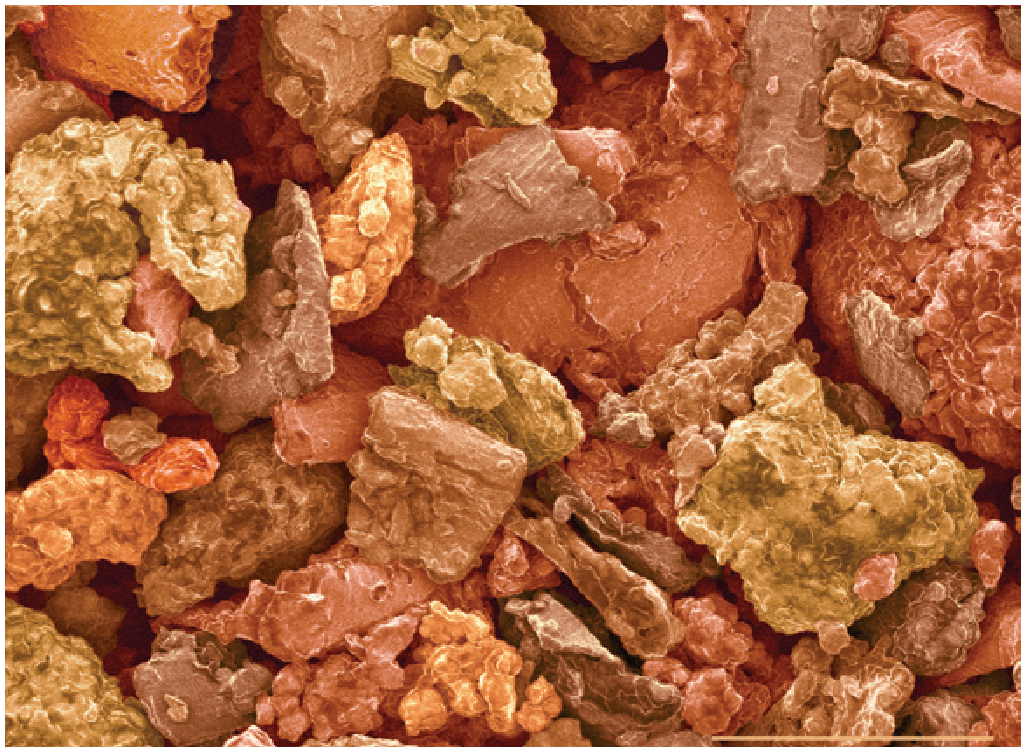


Figure 28. SEM micrograph of individual rice bran particles. Bar: 200 µm.

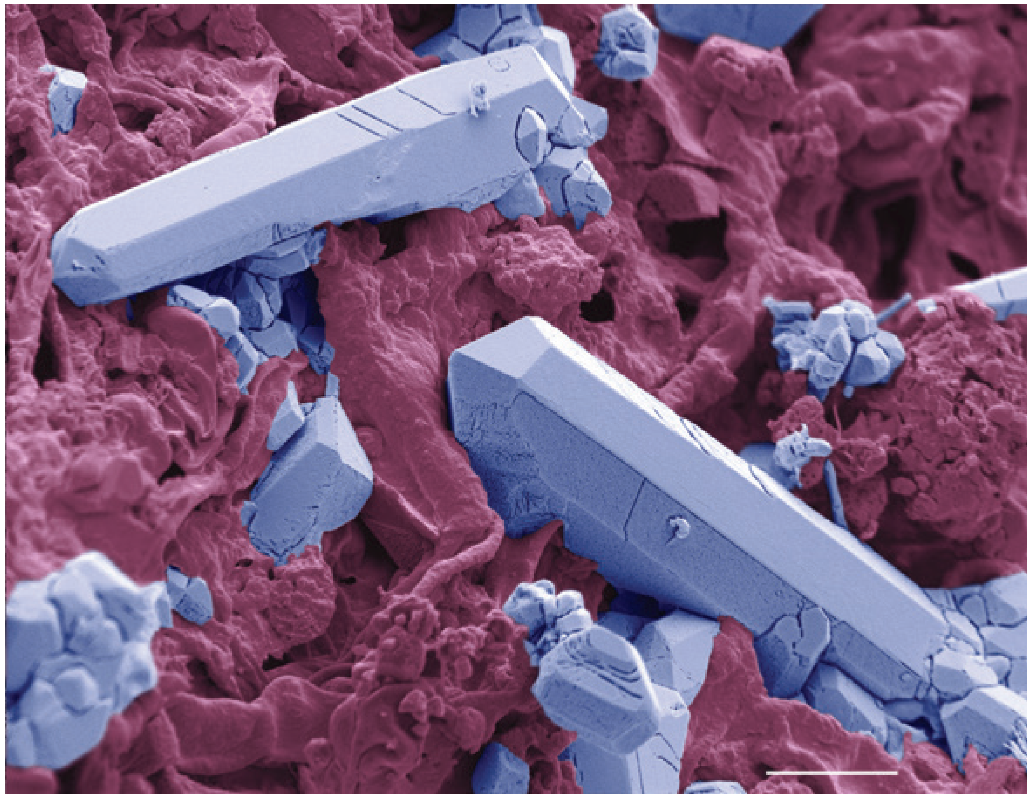
In spite of its high nutritional value (Saunders, 1990), rice bran is not easy to buy as it cannot be found in most food stores including health food stores, apparently for its lack of stability at room temperature. Figures 26 - 28 show the product of Bob's Red Mill in the USA that had been purchased online from *i*Herb. It was in the form of a fine powder with particles (Figures 26 & 27) occasionally up to 1.5 mm in diameter, i.e., considerably finer than other cereal brans, available in bulk food stores, which may be up to several mm in diameter and may contain a considerable amount of starch granules like the oat bran shown for comparison. Rice bran is also available in the form of straws [Internet 24].

6. Red yeast rice

Fermenting cooked rice with *Monascus purpureus* fungus (yeast) turns the rice purple (Figure 29). If added to foods, it imparts a pleasant flavor. It is called red yeast rice [Internet 25] or Went Yeast (after the Went strain of the yeast) in China, red rice koji in Japan, ang-khak in the Philippines, and ang-quac in Indonesia (Hu, 2005). Actually, red are the fungal hyphae but not the rice itself until the pigment consisting of red monascorubin and yellow monascoflavin, diffuses out. (The earliest reports on this discovery appeared in 1890). The product has been used in China and Japan mainly as a food colouring for a variety of foods such as pickled tofu, red rice vinegar, many Chinese pastries, wines, and even the Peking Duck.

The fermented rice has been used for centuries in China as both food and medicine to lower cholesterol and improve blood circulation (Klimek et al., 2009) and digestion. A great turmoil originated around red yeast rice in the late 1970s, when researchers in the USA and Japan studied the production of lovastatin [Internet 26] by the *Aspergillus* fungus and the production of monacolins in the *Monascus*. In the United States, dietary supplements containing red yeast rice were marketed to lower blood levels of cholesterol and related lipids. Then it was found that the red yeast rice products may not

Figure 29. Photograph of red yeast rice grains. Bar: 5 mm.



Figures 30. Red yeast rice grain surface showing monacolin statin crystals (blue) and dry M. purpureus hyphae (purple). Bars. 10 μm.

be safe [Internet 27], because some may contain a potentially harmful mycotoxin citrinin [Internet 28]. The Food and Drug Administration (FDA) has declared that red yeast rice products, which contain monacolin K, i.e., lovastatin, are identical to a drug and, thus, are subject to regulations as a drug. It is available online as a nutraceutical (Nguyen & Santini, 2017) in the form of tablets, caplets and capsules. In the traditional form, red yeast rice is available in Chinatown grocery stores in various countries.

Red yeast rice viewed by SEM shows the grain surfaces to be covered with dried fungal hyphae interspersed with monacolin crystals (Figure 30), which vary in size and may be up to 0.1 mm long.

The dry hyphae are abundant in the entire grain but the innermost part of some grains may still be white (Figure 29) with a few hyphae and the endosperm cells detached and the starch granules partially disintegrated to a different extent (Figures 31 & 32).

Conclusions

Scanning electron microscopy was used to examine the grains of rice as one of the major cereal crops. Rice shows many unique features such as the grain size and composition and a tight arrangement of polyhedral starch granules with minute protein bodies compressed at their joints. Transfer of important nutrients from the seed coat during the production of parboiled rice severely alters the grain structure as does any other kind of heating such as cooking, steaming or puffing. The grain structure also changes when white rice is fermented by *M. purpureus* to produce red yeast rice.

Acknowledgments

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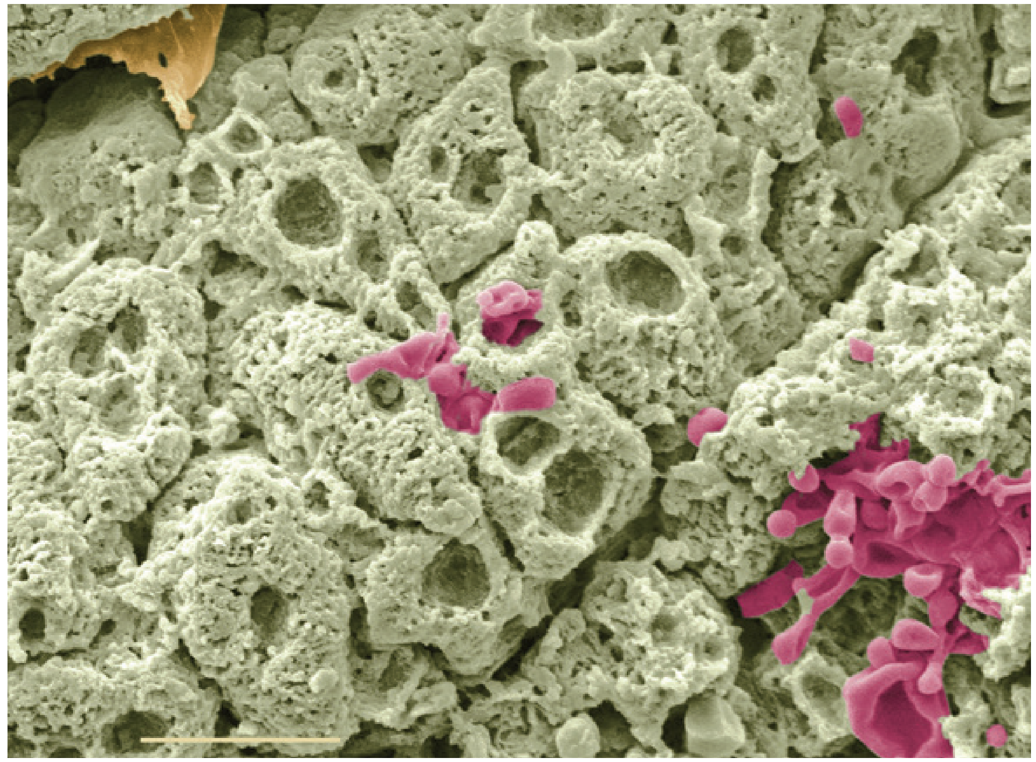

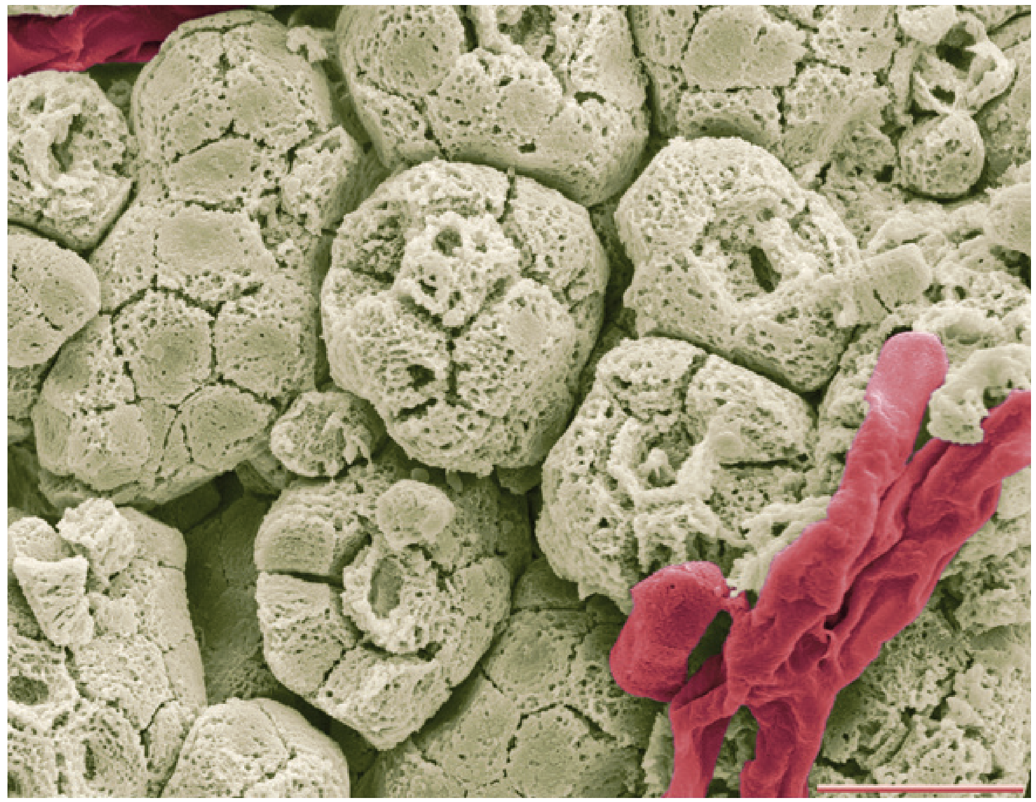

Figures 31 & 32. Red yeast rice grain interior. Starch granules are off-white unless they had been invaded with the red Monascus purpureus hyphae. Bars: 10 μm.

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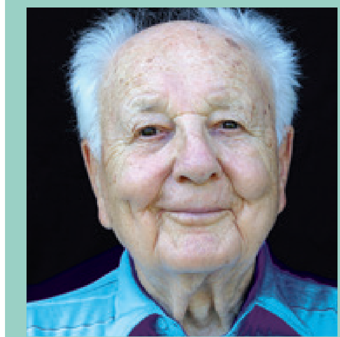

The article on rice has been preceded by 4 other articles in the Infocus magazine on SEM of bacteria (issue 10, 42-61, 2008), the beauty of milk at high magnification (issue 18, 4-37, 2010), microscopy and hygiene (issue 23, 4-32, 2011), and on colourful vegetables (issue 40, 54-71, 2015). Miloš was born in 1929, graduated in 1952 in Czechoslovakia as a sugar industry chemical engineer (MEng.), and obtained his PhD degree for research of apple pectin in 1957 from the Slovak Academy of Sciences in Bratislava. Several years later, he joined the Faculty of Medicine at Palacký University in his home town of Olomouc as an Assistant Professor. By defending a thesis on the enzyme arginase he was promoted to Associate Professor (Docent) and Chairperson of the Department of Organic and Biological Chemistry at the Faculty of Science.

From 1966 to 1968, Miloš studied lipoproteins as a post-doctoral fellow at the National Research Council of Canada in Ottawa. When his fellowship ended in August 1968, he had the luck to be hired as a research scientist at the Canadian Federal Department of Agriculture. Appointed to develop a wiener imitation from skimmed milk powder he had not been successful but he converted that failure into his lifetime electron microscopy studies of milk products. The American Dairy Science Association conferred the Pfizer Award for his research of cultured milk products on him in 1982, in the same year when the new Food Structure journal was established in Chicago. In 1994 he decided not to retire. As an Honorary Research Associate, he still does electron microscopy. In addition to agricultural projects such as this study on rice, he collaborates with the Canadian Blood Services. He has authored or co-authored 197 scientific and technical publications which he shares with the scientific community through ResearchGate.

On June 6 2018, the Food Structure and Functionality Forum conferred the "Dr. Isaac Heertje Distinguished Scientist Award" on Miloš at their congress in Montreal.

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Part 2

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