# Colourful vegetables - colourful micrographs

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Bright-field optical microscopy, confocal laser scanning microscopy and scanning electron microscopy were used to show the details of physical structure, distribution of some micronutrients and the beauty of ten of the most common vegetables grown underground as well as above the ground.

## Introduction

Humans are omnivorous, which means that they eat food of plant as well as animal origin, while only a minority (vegetarians and vegans) prefer to eat plants mostly or exclusively, respectively. High meat consumption has not been to people's benefit, as current research is showing. On the contrary, there is growing evidence (Campbell & Campbell, 2006) that diets rich in vegetables and fruits are associated with a lower risk of so-called diseases of the Western civilisation [Internet 1] such as coronary heart disease, diabetes, and various kinds of malignancies. The American Institute of Cancer Research estimates that a simple change in human diet such as increasing the consumption of fruits and vegetables to 5 servings per day would decrease the incidence of cancer by 20% (Keck & Finley, 2004). Plants contain all three major food components groups, i.e., proteins, lipids, and carbohydrates such as starch and inulin, and dietary fibre. Some of the edible plants are consumed as vegetables either as side dishes complementing other foods such as meat, fish, eggs, cheeses, or as cereals. A gradual departure from foods of animal origin increases the role of vegetables. In fact, "wild" plants [Internet 2] not considered edible some time ago such as dandelions, chickweed, nettle, ground ivy, green pea shoots, etc. are now included as vegetables in salads, soups, and stir-fried dishes.

# Vegetables

Vegetables are plants with a variety of shapes, structures, colours, compositions, and flavours, that are used partly or wholly for food. They grow either underground as roots, tubers, and rhizomes or above the ground as stems, stalks, leaves, flowers or flower buds, and seeds. They can be consumed fresh, cooked, or preserved.

The term 'vegetables' encompasses a wide variety of plant parts and thus a wide variety of plant tissues [Internet 3]. Different vegetables contain various substances, many of which have beneficial nutritional

properties [Internet 4]. In addition to macronutrients (proteins, carbohydrates, lipids), they also contain micronutrients such as minerals (Cu, I, Fe, Mg, Mn, K, panthotenic acid), antioxidants, flavours, and other constituents. Dietary fibre is another component of vegetables important for human wellbeing. Breeding has improved most vegetables which existed a century ago and has developed many new varieties and cultivars [Internet 5]. Advances in agriculture and marketing have made it possible for urban consumers to have access to most vegetables all year long even if they have to be brought to the market over long distances. Some scientists, e.g., Dr. Kirsten Brandt [Internet 6], suggest that it would be more reasonable to advise consumers that they eat particular types of vegetables in certain quantities rather than "five portions of fruit and vegetables per day". Further research will lead to new growing techniques for vegetables which will emphasize phytochemical composition and keeping quality rather than mere quantity.

# Microscopy

As one of the most colourful groups of foods of different structures with many beneficial properties for human health, vegetables evoked our interest how they would appear at high magnification using different imaging methods. Kesseler (2006, 2008) has already shown the beauty of plants including fruits at higher magnifications. Microscopy reveals the microstructure of cells and cellular structures and the distribution of substances either directly ( $\beta$ -carotene, lycopene) or from their reactions with stains (Baker & Horson, 1952), e.g., for proteins (Nigrosin, Coomassie Blue) and lipids (Sudan Black, Oil Red O), by their natural autofluorescence (chlorophyll) and fluorescence induced after staining, e.g., for starch (Safranin O), cell walls (Calcofluor White), DNA (Acridine Orange, DAPI), etc. (Aguilera & Stanley, 1999; Flint, 1994). A corn grain (Figure 1) is shown as an example of the latter case.

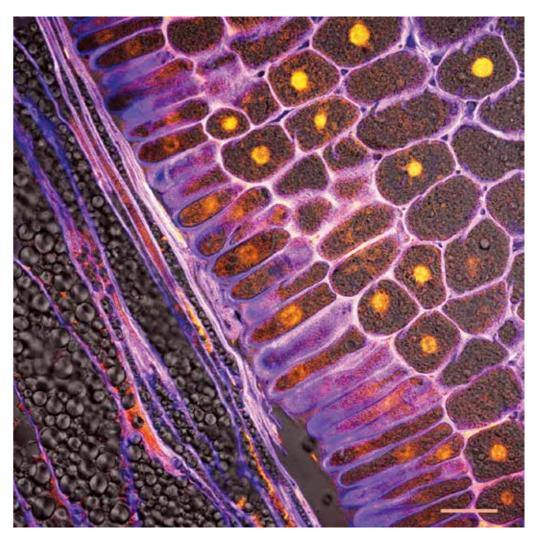


Fig 1: Corn seed section stained for CLSM using Acridine Orange (for cell nuclei) and Calcofluor White (for cell walls) to induce fluorescence of the seed constituents. Bar: 25 µm.

**Bright-field microscopy** is one of the easiest optical methods, whereby the sample is illuminated with white light from one side and the image created by the transmitted light is captured and enlarged by the microscope on the other side. Dark images appear on a bright background.

**Scanning electron microscopy** (SEM) provides images of sample surfaces at magnifications considerably higher than optical microscopy. The images are in grayscale and may by digitally coloured for emphasis of specific features (Kaláb et al., 2008). Environmental SEM makes it possible to examine

hydrated specimens without prior coating of the sample.

**Confocal laser scanning microscopy** (CLSM) uses light of various wavelengths from one or several lasers depending upon the component of interest in the specimen studied. Fluorescence occurs when a molecule absorbs light energy and then instantaneously emits energy as light of a longer wavelength. Plant structures and substances in the specimen (Hepler & Gunning, 1998) may autofluoresce, i.e., emit light when excited by a particular wavelength without prior staining;

components which do not naturally fluoresce may be induced to do so by the application of various fluorochromes, or dyes. CLSM images are typically pseudo-coloured, that is, the operator assigns different colours as related to the different exciting wavelengths [Internet 7].

The different kinds of microscopy complement each other: SEM provides images of the specimen surfaces and CLSM may capture features under the physical surface of the specimen as deep as several micrometers. This kind of microscopy has already been used to study the uptake of herbicides, plant growth regulators and systemic fungicides by apples and green peppers, and by the leaves of beans, wheat and cabbage. It has been used to examine various foods (Dürrenberger et al., 2001)) and it may help to explain why cooking improves (Turkmen et al., 2005) the health benefits of some vegetables.

# Vegetables growing underground

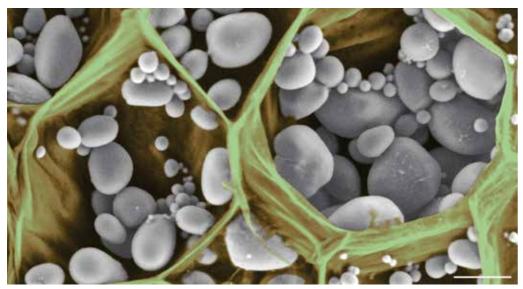
Vegetables may be classified by various criteria. The vegetables featured here were simply divided between those which grow underground [Internet 8] (potatoes, carrots, and radishes) and those which grow above the ground (lettuce, spinach, celery stalks, nasturtium petals, broccoli, cauliflower) and also tomatoes, which botanically speaking are a fruit.

## **Potatoes** (Solanum tuberosum)

Potatoes originated in Peru and nowadays they are one of the most important staple foods for many inhabitants of Europe and North and South America. China and India produce a large proportion of the world's potato crops.

Potatoes are classified as tubers, that is a swollen part of a stem or root for energy storage. Potatoes store energy in the form of starch, but they are also rich in several micronutrients, especially vitamin C which promotes iron absorption. Vitamin C concentration decreases during storage of the tubers and also with cooking. In addition, potatoes provide vitamins  $B_1$ ,  $B_3$  and  $B_6$ , folate, pantothenic acid and riboflavin and minerals such as K, P, Mg and Fe. In freshly harvested potato tubers, the protein content on a dry weight basis is similar to that of cereals and is very high in comparison with other roots and tubers.

A part of potato starch is considered to be resistant [Internet 9] to digestive enzymes [Sievert & Pomeranz, 1989] in the upper digestive tract of humans and thus proceeds as far as the large intestine. In this respect it behaves as a dietary fibre. Starch grains are the most prominent feature when



**Fig 2:** Raw potato with starch grains in the tuber cells. Colour-enhanced ESEM. Bar: 50 μm.

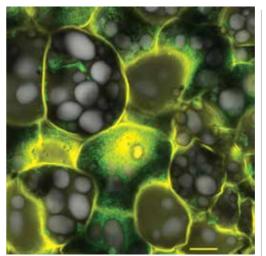
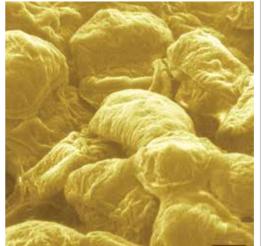


Fig 3: Raw potato by CLSM. Starch grains gray, cell walls yellow. Bar: 50 μm.



**Fig 5:** Boiled potato by ESEM. On fracturing, whole cells separated from each other making it impossible to view their contents. The samples were sticky from gelatinized starch. Bar: 50  $\mu$ m.

a raw potato is examined by microscopy such as ESEM (Figure 2) or CLSM (Figures 3 and 4). In this latter kind of microscopy, the starch grains were stained with Safranin and the cell walls were stained with Calcoflour White. The resulting images may differ depending on the sample preparation, excitation wavelength, and image manipulation.

Figure 3 is showing the cell walls in yellow, the starch in grey and unidentified substances in green. Figure 4 is a 3D representation of a stack of images like figure 3, showing cell walls in blue and starch in green.

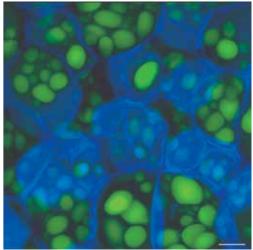


Fig 4: Three-dimensional representation of raw potato by CLSM. Bar: 50  $\mu$ m.

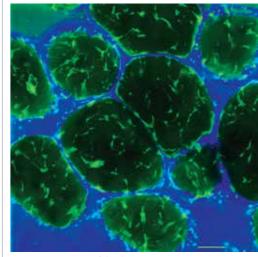


Fig 6: Boiled potato by CLSM. Starch grains are gelatinized in boiled potato and are no more visible in the cells whereas cell walls are shown in blue. Bar: 50  $\mu$ m.

Unlike many other vegetables, potatoes are not consumed raw. They are boiled, baked, or fried. During boiling, pectin in the cell walls is solubilized and the starch granules inside the cells absorb water, swell, and gelatinize. The softened cells are not cut during fracturing for ESEM, but separate whole from each other along the cell walls (Figure 5). The laser beam of CLSM penetrates the gel (Figure 6) and demonstrates the cell walls. The fused starch granules appear as a dark mass, with no individual granules visible.

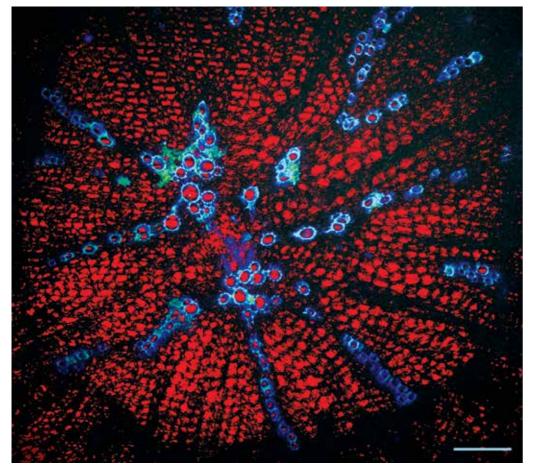


Fig 7: Detail of a carrot cross-section by CLSM. Autofluorescence and reflected laser light. Bar: 200 µm.

#### **Carrots** (Daucus carota)

Carrots are one of the most popular root vegetables. European carrots are almost cylindrical taproots of bright orange colour with both ends rounded, while saffron coloured carrots with long tapering tail-like lower ends are grown in Asia. The green leaves are also edible and attract various herbivores, as many gardeners know. Figures 7 and 8 show the same carrot section by CLSM and are examples of different illumination techniques and deliberate image manipulation by the microscopist using autofluorescence in conjunction with reflected laser light or differential interference contrast (DIC), respectively.

Carrots are nutritionally important for their contents of  $\beta$ -carotene (~8 mg/100 g), which functions as provitamin A and is very important for correct vision.

Consumed as part of vegetables, beta-carotene protects humans from various forms of cancer

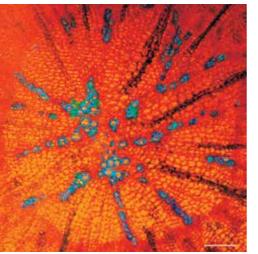


Fig 8: The same carrot sample as in Fig. 7 but the image is formed by autofluorescence at 405 nm excitation and differential interference contrast (DIC). Bar: 200  $\mu$ m.

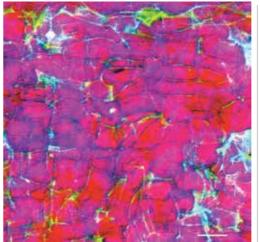


Fig 9: CLSM overlay of a fresh radish section. Autofluorescence (shown in blue/green and red) was observed under 405 nm and 489 nm. Bar: 200  $\mu$ m.

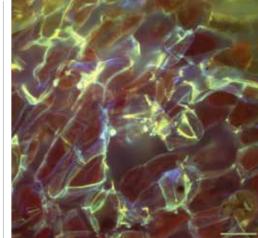
supposedly because it is accompanied by alphacarotene (Brandt et al., 2004). Pure beta-carotene supplements lack the protective property [Internet 10].

In addition, carrots contain approximately 16,000 IU of vitamin A/100 g fresh tissue. Flavonoids present in carrots also help protect from skin, lung and oral cavity cancers. A polyacetylene compound called falcarinol (a phytochemical that protects carrots and other root vegetables from various fungal diseases) was shown to deter major illnesses in animals and in humans (Zidorn et al., 2005). Brandt recommends consumption of one small carrot every day together with other vegetables and fruits to benefit from their health-promoting properties.

#### **Radishes** (Raphanus sativus)

Easy to grow - a seed may sprout in only 3 days - radishes are a very popular member of the Brassicaceae family. The family contains over 330 genera and about 3,700 species. Some vegetable species are well known, e.g., *Brassica oleracea* (e.g. broccoli, cabbage, cauliflower,), *Brassica rapa* (e.g. turnip, Chinese cabbage,), *Brassica napus* (e.g. rapeseed), *Raphanus sativus* (common radish), and *Armoracia rusticana* (horseradish).

Radishes grown as vegetables are round for direct



**Fig 10:** Fresh radish section by fluorescence microscopy. Autofluorescence was observed in ultraviolet light using a combined DAPI/FITC/Rhodamine filter set. Bar: 20 μm.

consumption or elongated for cooking. The colour of their skin varies from white to red but the flesh is mostly white or slightly pink. Special varieties are grown for seeds and their oil.

Radishes are rich in folic acid, vitamins C,  $B_6$  and riboflavin, magnesium, potassium, calcium, copper, and anthocyanins. Similar to all Brassicaceae, radishes contain glucosinolates. Hydrolysis turns them into sulphoraphane and indole-3-carbinol. In conjunction with selenium present in all members of this family, these substances may prevent oral, colon, kidney, stomach, and intestinal cancer (Keck & Finley, 2004). In addition, radishes function as diuretics.

CLSM shows that substances present in fresh radishes autofluoresce at several wavelengths producing colourful micrographs (Figures 9 and 10) which reflect a rich chemical composition. Staining with Calcofluor makes it possible to view the cell walls as thin bluish lines (Figure 11). In contrast to the apparent variety of substances, the structure of radishes is rather simple.

A deteriorating (yellowing) part of a stained radish shows altered fluorescence (Figure 12) compared to fresh tissue, probably as the result of degradation products. This finding suggests that fluorescence could be used to check the freshness of produce.

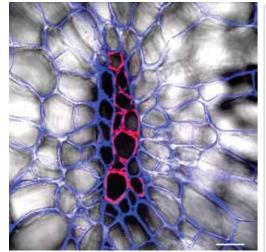
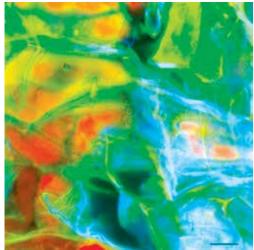


Fig 11: CLSM overlay of a fresh radish section stained with Calcofluor (blue) and Rhodamine (red). Bar: 25 µm.



**Fig 12.** CLSM overlay of a deteriorating radish section stained with Acridine Orange (green). Autofluorescence (shown in blue and red) was observed under 405 nm and 489 nm. DIC shown in yellow. Bar: 200 μm.

# Vegetables growing above the ground

#### Lettuce (Lactuca sativa)

Lettuce is part of a large group of vegetables along with spinach, kale, Swiss chard, collard greens, celery, and parsley to name only a few of nearly one thousand species of plants with edible leaves [Internet 11]. Some (lettuce, spinach) are consumed raw but many others are first boiled or stir-fried. Lettuce contains many phyto-nutrients including vitamins and, like many other leafy vegetables [Internet 12], two carotenoid xanthophylls, lutein (up

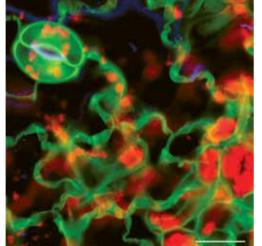
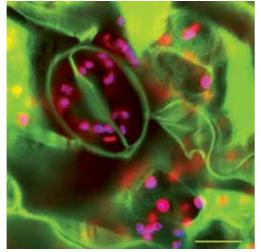


Fig 13: Lettuce leaf constituents by CLSM.A stoma in left upper corner. Cell walls in green and chloroplast in orange. Bar: 20 µm.



**Fig 14:** Lettuce leaf constituents by CLSM.A stoma in left upper corner. Cell walls in green and chloroplast in pink. Bar: 10 µm.

to 1.7 mg/100 g) and zeaxanthin. Both are important for human vision [Internet 13] being concentrated in the retina of the eye. They participate in the prevention of macular degeneration, particularly in elderly people. In the leaves, they are at much lower concentrations than the major pigment, chlorophyll, which is common to all green plants. Chloroplasts are shown in orange or pink by CLSM whereas cell walls in green (Figures 13 and 14) in leaf samples stained with Calcofluor White for cell walls and Acridine Orange for DNA. Stomata figure prominently as openings on the underside of the leaves (Figure 15). They enable gas exchange and



Fig 15: Lettuce leaf stoma by bright-field microscopy. Bar: 5 µm.



Fig 16: Bacteria from contaminated irrigation water may penetrate lettuce leaves through their stomata and propagate inside them. SEM image. Bar: 5  $\mu$ m.

help conserve water in dry weather when they are closed by the guard cells.

Since lettuce and many other leaf vegetables such as spinach are mostly consumed raw, growers must prevent their contamination with microorganisms in the fields. The cause of contamination may be impure irrigation water or wild animals invading the fields. SEM has shown that bacteria contaminating the leaves (Golberg et al., 2011, Kaláb, 2011, Seo & Frank, 1999) congregate around the stomata and

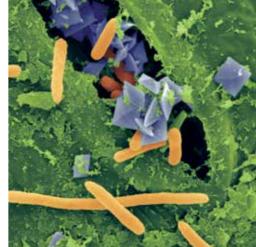


Fig 17: Spinach leaf contaminated with Escherichia coli bacteria. SEM image. Bar: 2  $\mu$ m.

grow inside (Figure 16), where they find water and nutrients.

#### **Spinach** (Spinacia oleracea)

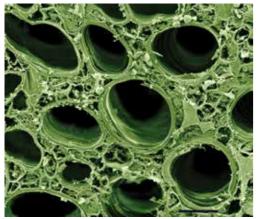
Spinach is consumed either raw (in salads) or cooked. It is nutritionally more valuable when cooked, because quick cooking, steaming or microwaving releases the nutrients by breaking down the cell walls. The same applies to other vegetables (Turkmen et al., 2005) [Internet 14]. Spinach contains all the beneficial nutrients present in leaf vegetables (lutein up to 16.5 mg/100 g) and also glycoglycerolipids, which may confer (Maeda et al., 2010) anticancer properties.

Spinach also contains an elevated concentration of oxalic acid which is known to form insoluble crystals (Bauer et al., 2011) with divalent cations such as calcium, magnesium, and copper; such complexes reduce the mineral bioavailability. For example, the human body can absorb about half of the calcium present in broccoli, yet only around 5% of the calcium present in spinach. The beneficial properties of spinach, however, far outweigh the presence of oxalic acid. Combining spinach with foods rich in calcium such as milk products, particularly cheese, mitigate the effect of oxalic acid.

Similar to lettuce, spinach was the subject of recalls [Internet 15] in 2006 because of bacterial contamination. At that time, spinach leaves were



Fig 18: Spinach leaf by CLSM and DIC showing chloroplasts in orange. Bar: 20 µm.



**Fig 19:** Part of a celery stalk cross section by SEM. Bar: 20 µm. examined by SEM. Figure 17 was obtained with a contaminated leaf specimen briefly dipped in a calcium gluconate solution, rinsed with water, fixed and prepared for conventional SEM. Calcium oxalate crystals developed by a reaction of the leaf juice with the reagent (purple crystals in Figure. 17). Chloroplasts in spinach leaves are shown by CLSM (Figure 18) in orange.

**Celery stalk** (Apium graveolens var. dulce) The crispy stalk of celery is used as a vegetable around the world. All four parts of the plant – the root, stem, leaves, and seeds - are edible and are used as foods [Internet 16]. The stalk, called the petiole, is part of the leaf structure. SEM of a cross section of the stalk shows vascular bundles (Figure 19), which are parts of the water and nutrient transport

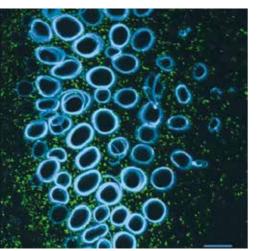


Fig 20: Celery stalk cross section by CLSM. Autofluorescence at 405 and 488 nm excitation. General view. Bar:  $50 \ \mu m$ .

system. They contribute to the crunchiness of the stalks. Autofluorescence of the vascular bundles at 405 nm excitation is shown using CLSM in a general view (shown in blue in Figure 20) and in greater detail in Figure 21.At 488 nm excitation, the chloroplasts are visible and green color has been assigned to them.

**Garden nasturtium** (*Tropaeolum majus*) There are over 60 kinds of edible flowers [Internet 17] which are used as vegetables in meals or in drinks (Mlcek & Rop, 2011). One of these is *Tropaeolum majus*, (Figure 22) known to most people as "garden nasturtium". Its flowers look



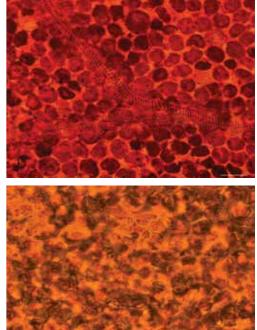
Fig 21: Detail view of a celery stalk by CLSM. Bar: 20 µm.



Fig 22: Tropaeolum majus (Garden nasturtium) yellow-and-red flower

different from the flowers of the Nasturtium genus, best known for the edible watercress (*Nasturtium microphyllum*) which belongs to the family Brassicaceae. Unlike most other plants, all above-ground parts of *T. majus* may be used as vegetables.

The *T. majus* flowers are an example of plant tissues rich in water-soluble pigments called anthocyanins (Horbowicz et al., 2008; Garzón & Wrolstad, 2009). They provide colours to flowers, fruits, and vegetables ranging from orange to red, purple, blue to black. The colours depend on the pH value (acidity) of the medium, being pink in



**Fig 23a and b:** Tropaeolum majus flower petal by bright-field microscopy. a: red flowering plant, b: orange flowering plant. Bar: 25  $\mu$ m. acidic solutions (pH<7), purple in neutral (pH~7), and greenish-yellow in alkaline solutions (pH>7). Anthocyanins are strong antioxidants *in vitro* but this property is lost by digestion.

Under bright field illumination, red and orange nasturtium petals look similar (Figure 23), one being more pigmented than the other. Although red and orange nasturtium petals look similar under brightfield illumination (Figure 23), more pigment can be observed in the red petal. In contrast, when viewed using identical excitation and emission settings on the confocal laser scanning microscope, the autofluorescence characteristics of the two petals are distinctly different.

*T. majus* flowers also contain up to 45 mg of lutein/100 g, the highest concentration of this carotenoid in any edible plant [Niizu & Rodriguez-Amaya, 2005, and Internet 11]. The petals are also very rich in vitamin C, and the unripe seed pods may be used as substitutes for capers.

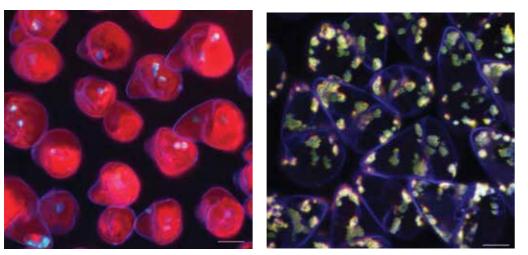


Fig 24a and b: Tropaeolum majus flower petal by CLSM. a: red flowering plant, b: orange flowering plant. Bar: 10 µm.

# **Broccoli** (Brassica oleracea) and cauliflower (Brassica oleracea)

Flower buds such as cauliflower (Fig. 25), broccoli (Fig. 26), globe artichokes, and capers have been established as vegetables for a long time. Unopened rapeseed (*Brassica napus*) flowers are popular as a vegetable called "nanohana" in Japan. Broccoli and cauliflower are two different cultivars of the same species, *Brassica oleracea*. The head of cauliflower is composed of a white inflorescence meristem (i.e., a

tissue consisting of undifferentiated cells). Broccoli heads consist of flower buds; they are harvested before they bloom bright yellow.

Broccoli and cauliflower are members of the cabbage family, such as Brussels sprouts, cabbage, kohlrabi, kale, and savoy. This family is known for cancer fighting phyto-nutrients, particularly sulphoraphane, glucosinolates, and indole-3-carbinol. These substances remain mostly unaffected by steaming,

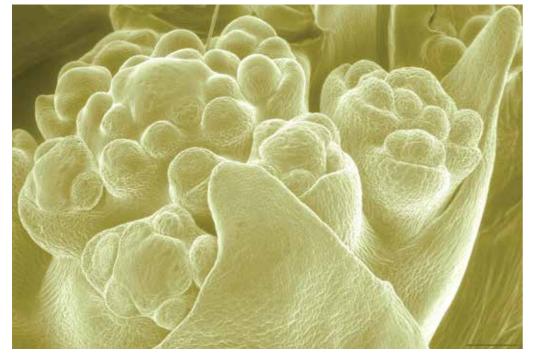


Fig 25: Cauliflower florets by ESEM. Bar: 200 µm.



Fig 26: Broccoli florets by ESEM. Bar: 500 µm.

microwaving, and stir-frying the vegetables but are destroyed by boiling in water (about a 50% loss in 10 min.). Similar to some other green vegetables, broccoli is also rich in lutein.

Glucosinolates in the Brassicaceae family vegetables are being studied for their health benefits. During food preparation and digestion they are converted into a variety of sulphur-containing substances which have several important health maintaining properties in experimental animals. They contribute to general health in humans but their anticancer properties remain controversial.

While the beneficial properties of glucosinolates are acknowledged, their contribution to the development of goiter, i.e., disturbances in the metabolism of iodine in the thyroid gland in developing countries [Internet 18] should also be mentioned.

**Tomatoes** (Solanum lycopersicum) The tomato is a well-known vegetable, the botanical name of which is derived from the carotenoid lycopene, which it contains and which contributes to its red colour. Lycopene is known for its protective

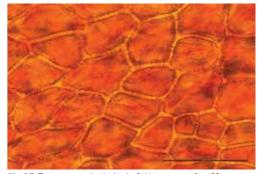


Fig 27: Tomato - raw skin by bright-field microscopy. Bar: 50 µm.

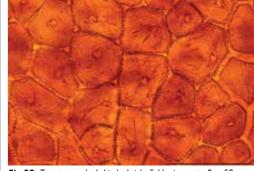
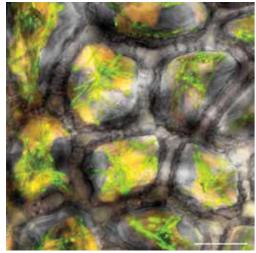


Fig 28: Tomato - cooked skin by bright-field microscopy. Bar: 50  $\mu m.$ 

action against some forms of cancer, particularly prostate cancer [Hadley *et al.*, 2002]. Consumption of cooked tomatoes has been



**Fig 29:** Raw tomato skin by CLSM with green crystals presumably to be lycopene. Bar: 20 μm.

shown to provide the maximum benefit, suggesting that cooking releases lycopene from their tissues including the skin. Lycopene is soluble in organic solvents and in oils but not in water. Thus, tomatoes and olive oil complement each other very well in the so-called Mediterranean diet. Bright-field microscopy shows tomato skin from vine-ripened "banana tomatoes" raw (Figure 27) and cooked (Figure 28). There are some apparent differences such as particulate material (crystals) present in the raw skin but they are more clearly noticeable in micrographs obtained by CLSM at 488 nm excitation. Crystal-like structures (in green) were present in raw tomato skin (Figure 29) but not as many in the cooked skin (Figure 30). Pyke & Howells, (2002) described lycopene crystals emanating from the main chromoplast body in tomatoes as thin straight rods. It may thus be suggested that the green structures in the raw tomato skin in Figure 29 show solid lycopene and cell walls that had been modified by cooking.

In spite of being sold and used as a vegetable, the tomato is botanically classified as a fruit similar to avocado, cucumber, eggplant, okra, pepper, pumpkin, and squash. A fruit develops from a flower and contains seeds. Other plant parts are considered to be vegetables. There are, however, two reasons for viewing the tomato as a vegetable. It is used as such

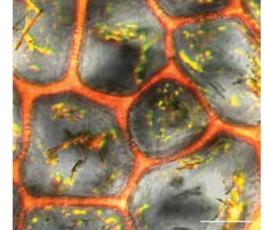


Fig 30: Cooked tomato skin by CLSM. Bar: 20 µm.

by its consumers and the laws [Internet 19] of many countries consider them as vegetables for taxation purposes. So, dear tomato, you are a fruit but we have included your micrographs in this paper on vegetables and we hope that many readers agree.

#### Conclusions

This study has been a rewarding experience for all authors. Collecting information about individual vegetables and viewing them at high magnification has improved our relationship with them - and with other vegetables. We have presented images of starch granules in raw potatoes and images of lycopene in the skin of raw tomatoes. Micrographs of stomata in lettuce and in spinach leaves with bacteria inside tell better than any words how important it is to grow and process these vegetables in strict hygienic conditions. CLSM demonstrates the diversity of substances in the round, seemingly uniform radishes, and the radial microstructure of carrots is equally interesting to view. In addition to flower buds such as broccoli and cauliflower, many colourful "garden" flowers are nutritionally valuable vegetables. What appears as a familiar piece of a typical vegetable on the kitchen counter is, in fact, a unique world of cells, organelles and a variety of beneficial substances.

The different images may raise questions concerning

differences in the composition and food quality of various vegetables and prompt us to search for additional answers. What specific nutrients and in what form do vegetables provide to the human diet which other foods cannot provide? Such questions may initiate studies in greater detail or a desire to look to other plants for an answer to a question as to whether there are indeed almost no weeds but plants which have not yet been recognized as beneficial for our health.

## Acknowledgements

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Internet 5: http://en.wikipedia.org/wiki/Cultivar

Internet 6: http://news.bbc.co.uk/2/hi/health/4246107.stm

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Internet 9: http://en.wikipedia.org/wiki/Resistant starch

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Internet II: http://en.wikipedia.org/wiki/List\_of\_leaf\_vegetables

Internet 12: http://nutritiondata.self.com/foods-011138000000000000-3w.html

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Internet 14: http://bsclarified.wordpress.com/2011/10/03/are-raw-vegetables-healthier-than-cooked-ones/

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Denise Chabot has 25 years of experience as an analytical chemistry research technician at Agriculture and Agri-Food Canada. In addition, she is proficient in both optical and electron microscopy, with a broad scope of expertise from microorganisms to plant organelles to food structure. Her passion for

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Keith has worked as a molecular biology technician at Agriculture and Agri-Food Canada (AAFC) in Ottawa since 1997.

One of the major projects he was involved in was research towards maize crop improvement through genetic modification.

In 2012, he joined the microscopy team at AAFC and has been providing

assistance and expertise in scanning and transmission electron microscopy and also in confocal laser scanning microscopy in a variety of agricultural projects. He also teaches and assists other technicians in this field which has captivated him by its extent and diversity.

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Miloš arrived in Canada from the former Czechoslovakia as a postdoctoral fellow at the National Research Council of Canada in Ottawa in 1966. In 1968, he was hired by then Agriculture Canada to develop new

milk products. To better understand the gelation of milk, he learned electron microscopy in the laboratory of Dr. G.H. Haggis.

Between 1979 and 1993, Miloš helped Dr. Om Johari to organise international meetings of food microscopists and to establish a new journal, "Food Structure" in 1982 (now featured at http://en.wikipedia.org/wiki/Food\_Structure). He served as the Editor-in-Chief until the publisher discontinued the journal in 1993.

In 1982, the American Dairy Science Association conferred the Pfizer Award on Miloš for his microstructural research of cultured milk products. In 1986 he served as a United Nations FAO consultant at the National Dairy Research Institute in Karnal (India) and in 1989 he shared his expertise with food scientists in Tsukuba (Japan) thanks to a grant from the Government of Japan. Miloš has published over 160 scientific and technical papers. After his retirement in 1995, he volunteered as an Honorary Research Associate doing part-time electron microscopy of microorganisms, foods, and blood cells. This is his fourth contribution to infocus.