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Quantification of Microstructures in Freeze-Dried Carrots using µCT

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Abstract. The characterization of the microstructures of freeze-dried food products determines the speed of and the properties after rehydration. The pore size distribution is an important characteristic of such textures. Spatial homogeneity and the orientation of voids and cracks inside the samples are studied.

Keywords: freeze-drying, fruit, vegetables, microtomography, granulometry, pore size

1 Introduction

Dried fruits and vegetables (F&V) are important ingredients in many convenience food products like soups, sauces and complete meals. However, the current products are a compromise between convenience and sensorial/nutritional quality. Most F&V are generally dried convectively with heated air. A disadvantage is the substantial degradation in appearance (shrunken, shirvelled, darkened), nutrients and flavour and the low rate of rehydration. Higher quality products can be obtained using freeze-drying (FD) methods. However, the very porous structure leads to a loss of texture and increase of friability. So far no systematic approach that considers the underlying microstructure during the fresh-dried-rehydrated processing chain has been applied. A major barrier to embark on such an approach has been the lack of adequate measurement technologies that enable decision making based on sound microstructural data. Hence we have deployed X-ray microtomography (µCT) \cite{4,5} to investigate the microstructural impact of FD. 3D image analysis methods were developed to obtain quantitative information about the porous microstructure. This information will further be used to develop mathematical models for simulation of the moisture transport within the product. These models will focus on the control of ice crystal growth, microstructure and the speed of rehydration. Finally, these models will be validated using MRI. In this paper, the usability of 3D image analysis methods will be demonstrated on two individual carrot samples.
2 Material and methods

2.1 Carrots

Cylindrical samples with a diameter and length of 10 mm were cut from winter carrots. These samples include parts of the central stele and peripheral cortex, containing different types of cells having different size, shape and orientation. Samples were frozen at two different temperatures: \(-28^\circ\)C and \(-196^\circ\)C. Freeze drying is based on the dehydration by sublimation of a frozen product. It results in a very open structure with large cavities promoting fast rehydration. These cavities are not the plant cells but are created by the ice crystals. The size of these cavities is therefore mainly influenced by ice crystallisation behaviour or the cooling rate.

2.2 X-ray microtomography (\(\mu\)CT)

The internal porous structures of the samples were visualised using a SkyScan 1172 desktop X-ray micro-tomography system. A stack of approximately 2000 horizontal cross sections (4000 × 4000 pixels) was produced with a pixel size of 4.0 \(\mu\)m. The contrast in \(\mu\)CT images is based on the difference in X-ray attenuation of the constituents of the sample (e.g. solids and air), which is influenced by the density and composition of the constituents.

2.3 Image processing

The \(\mu\)CT images of freeze-dried carrots as shown in figure 1 clearly show differences between both drying methods. The sample on the left shows elongated voids oriented in a diagonal direction. Close inspection reveals two types of tissues, one on the bottom left and one on the top right side of the sample. The sample on the right shows more clearly distinct tissues: fine structures on the right and a very porous tissue on the left, both containing long cracks.

A characterization of these textures can be achieved in several ways. One of the quantitative methods is the computation of the distribution of the pore sizes, also called a granulometry. The minimum pore width is taken as a characteristic for the pore size. The minimum pore size can be related to the speed of the ingress of water during the rehydration.

A second quantification is based upon the homogeneity of the sample. Two types of homogeneity can be distinguished: homogeneity in pore size, indicated by a narrow size distribution, and spatial homogeneity, which means that each subset of a spatially homogeneous sample will generate ‘the same’ granulometry. Spatial inhomogeneity may be an indication that more than one type of tissue is present in the sample.

A third quantification is the orientation of pores, cracks and voids in a sample. As can be observed, the pores are not scattered randomly, but they are more or less parallel, due to the biological composition of the sample.
Granulometry  

The distribution of the sizes of the pores can be obtained using morphological sieving [1,2]. The distribution is obtained by a closing scale-space, using a sphere with increasing diameter. The spherical shape of the structuring element guarantees rotation invariance, so it is independent of the orientations of the sample and of the structures within the sample. The morphological closing is now defined as a dilation of the image with a specific structuring element, subsequently followed by an erosion with the same structuring element. As a result, all voids smaller than the size of the structuring element are filled in.

\[ C(x, d) = (I(x) \oplus S(d)) \ominus S(d) , \]  

where \( \oplus \) and \( \ominus \) denote the dilation and the erosion respectively, \( I(x) \) is the image and \( S(d) \) is the structuring element of diameter \( d \). By choosing a set of increasing spherical structuring elements, a cumulative size distribution \( G(d) \) can be obtained, as

\[ G(d) = \frac{\int C(x, d)dx - \int C(x, 0)dx}{\int C(x, \infty)dx - \int C(x, 0)dx} , \]  

where the closing \( C(x, 0) \) is equal to the original image and \( C(x, \infty) \) is the closing with an infinite structuring element, which is equal to the image with all voids filled in.

Finally, we need a test to decide whether acquired granulometries differ significantly. In statistics, a Kolmogorov-Smirnov test is one of the most useful methods to compare empirical cumulative distribution functions. Suppose \( F_{1,N_1} \) and \( F_{2,N_2} \) are two cumulative distribution functions generated from respectively \( N_1 \) and \( N_2 \) independent and identically distributed observations. To test the equality of those distributions, a null hypothesis is formulated: the two distributions are equal. This hypothesis is rejected as

\[ H = \sqrt{\frac{N_1N_2}{N_1 + N_2}} \sup_x \left| \frac{F_{1,N_1}(x) - F_{2,N_2}(x)}{K} \right| > 1 \]  

where \( 1 < K < 2 \) is a critical value, depending on the significance of the test [3]. However, this test assumes that the observations are independent. The number of observations \( N \) for each granulometry is required in the test, but this cannot be the number of pixels that contribute to a particular scale. Note that each point in the curve represents an area of pixels, within the diameter of the corresponding structuring element. Here, we need a parameter which indicates the number of degrees of freedom. Since these pixels are not independent, they will not yield a reliable test. Instead of the number of pixels, the number of spheres may be a more accurate estimate for \( N \). This value is calculated from the granulometry curve as the total area of the voids divided by the area of the characteristic diameter, i.e. the diameter where the granulometry equals one half.

Spatial homogeneity  

The spatial homogeneity can be defined in several ways. An obvious method is to divide the image into blocks and compute a granulometry for each block. If the sample is spatially homogeneous, these granulometries
will appear the same. Again, a Kolmogorov-Smirnov test is required in order to decide whether two granulometries from different parts of the sample are equal or not. A second method can be the analysis of the contribution from a specific closing diameter. After closing an image with a structuring element with a specific diameter, the spatial distribution of the remaining voids can be computed. This spatial distribution is an indication of spatial homogeneity.

**Orientation** As can be seen in the images, the voids and cracks are not oriented randomly. Most voids are elongated in the radial direction (with respect to the center of the whole carrot). Cracks appear in these samples both along and perpendicular to the radial direction.

As an approach in the determination of the main directions of the voids, one can take the projection of the sample from a specific angle. This leads to a direction dependent mass density. If the voids are aligned with the projection, the mass density shows peaks (summed cell walls) and low values (summed voids). As a result, the variance of this signal is large. If the voids are not aligned, the signal will be more homogeneous. Rotation of the sample over 180°, will lead to a direction dependent variance.

### 3 Results

#### 3.1 Granulometry

The grey-valued µCT images are thresholded in order to get a binary image. After thresholding, the images are despeckled and masked in order to define the outer edge of the carrot sample. Cracks are regarded to be part of the sample. The acquired binary three-dimensional datasets are used as an input for the computation of the pore size distribution.

Computation of the granulometry for all datasets leads to the results shown in figure 2. The curves are in good agreement with visual inspection. The −196°C-curve rises earlier than the other curve, due to the fine structures present in the right half of the sample. For larger diameters (above 60 µm), the curve flattens and finally rises to 1 at a diameter of approximately 1000 µm. This is due to the large hole in the center left part of the sample. Application of the Kolmogorov-Smirnov test leads to $H = 287$. Experimentally, it has been determined that comparable distributions lead to $H$-values considerably smaller than 100. As a result, the hypothesis that both distributions are equal, is clearly rejected.

#### 3.2 Spatial homogeneity

The second image (freeze-dried at −196°C) shows two distinct tissues, one on the left side with large voids and one on the right with very fine structures. The image is now cut into four equally sized blocks (2000×2000×2000 pixels) and the granulometry is computed for each quadrant. Figure 3 shows the results. As can be seen, two types of curves can be distinguished. The curves from the right part
**Fig. 1.** Cross sections of two carrot samples, freeze dried at different temperatures. On the left at −28°C and on the right at −196°C.

**Fig. 2.** Granulometries of carrot samples, freeze-dried at different temperatures.

**Fig. 3.** Granulometry of four quadrants of the carrot sample, freeze-dried at −196°C.

**Fig. 4.** The variance of the mass density as a function of the projection angle.

**Fig. 5.** Two angles from figure 4, plotted in the sample.
do not differ much from each other and show a steep rising around 50 µm, which is the characteristic pore size of the fine structures. The other two curves show that a significant part of the area has a minimum pore size of 50 – 1000 µm, which is in agreement with the expectations. The granulometry of the whole sample, as shown in figure 2, is the area-weighted mean of those four curves. Comparison of these curves leads to large values ($H > 150$) when a left quadrant is compared with a right quadrant and significantly lower values when the two left quadrants or the two right quadrants are compared ($H = 56$ and $H = 27$ respectively).

### 3.3 Orientation

The variance of the mass density of the first sample (−28 °C) is shown in figure 4. As can be seen, between the angles $\alpha_1$ and $\alpha_2$, the signal is relatively large. In figure 5, these two angles have been plotted on top of the sample. The range between those two angles covers the directions of the elongated voids on the bottom left part of the sample.

### 4 Conclusion and further work

The granulometry is an important measure to characterize microstructures. It is also a valuable characteristic to discriminate between two or more types of tissue. Spatial homogeneity and orientation of voids and cell walls have been studied and proven to be useful.

Future work includes a more in-depth study of the orientation and shape of voids and wall thicknesses, and the correlation between granulometry and MRI measurements. For the modeling of the rehydration, the speed of water ingress has to be related to the connectivity of the voids and the tortuosity of the sample.

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**References**