# Pistachio Deterioration Detected by X-ray Absorption

Alexey PROSHLYAKOV[1], Stavros YANNIOTIS[2] and Jiří BLAHOVEC[1]

[1]Department of Physics, Faculty of Engineering, Czech University of Life Sciences Prague, Prague, Czech Republic; [2]Department of Food Science and Technology, Agricultural University of Athens, Athene, Greece

**Abstract**

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The measurement of the absorption coefficient of X-rays in healthy and fungus infected pistachio kernels was the main objective of this work aimed at detecting the potential injury by insects and/or fungi in the kernel. It was found that the absorption coefficient in the injured parts is about half of that in the healthy parts. The absorption coefficient is also affected by the position in the kernel due to the variations in cell dimensions and the oil and moisture contents in the kernels.

**Keywords**: X-ray image; aflatoxin detection; absorption coefficient; pistachio kernel

Pistachio is cultivated in the Middle East, United States, and Mediterranean countries. Pistachio nut is one of the major agricultural products produced in Iran where more than 50% of the world pistachio production is located (FAO Statistical Yearbook 2005). Pistachio kernels are a good source of fat (50–60%) and contain unsaturated fatty acids (linoleic, linolenic, and oleic acids), essential for human diet (Labavitch *et al.* 1982). They are consumed in confectionery and snack foods. The shell (endocarp) of pistachio nuts splits along their sutures which is a desirable trait because pistachio nuts are usually marketed in-shell for eating out of hand as a snack food.

In the last years, there were many cases of aflatoxin detection in pistachio nuts in amounts above the EU limits taking into account that aflatoxins belong into the hazardous category with the highest number of notifications within EU in nuts (RASFF-Rapid Alert System for Food & Feed Annual Reports). In all such cases, the nuts are not released for human or animal consumption and, therefore, a serious economic loss results due to the direct loss of the product and the indirect cost of the mitigation strategies. Aflatoxin contamination in peanuts and pistachios is considered a major problem in the US (Bhatnagar *et al.* 2004), and also in Asia (Pitt *et al.* 2004; Cheraghali *et al.* 2007) and Africa (Bankole *et al.* 2006) while there are very limited reports available regarding Europe (Battilani 2010). Pistachio aflatoxin contamination is frequently connected with kernel necrosis symptoms that are described in the existing literature (Michailides *et al.* 1995; Chitzanidis *et al.* 2004) as stigmatomycosis disease. Stigmatomycosis of pistachio was reported in Iran (1967), Russia – referring to central Asian countries – (1972), Greece (1979), and USA (1990) (Chitzanidis *et al.* 2004). According to (Michailides *et al.* 1995), kernel necrotic symptoms differ from typical symptoms of stigmatomycosis, which is characterised by the wet, smelly, rancid, slimy appearance of the kernel. Actually, the term stigmatomycosis is the general name for the disease that occurs in nuts which look healthy outside but are deteriorated inside by fungi which have been introduced by insects of the class Hemiptera (Chitzanidis *et al.* 2004).

The aflatoxin danger can be minimised by sorting the individual nuts during processing. Such sorting is currently based on the outside characteristics

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of the nuts such as colour, size, and malformation, but there is also a need for the disease detection inside the kernels. One possible way for such a sorting technique is using X-rays. Continuous line scan X-ray systems are currently available for the food industry for inspection applications involving the removal of foreign materials such as rocks, glass, metals, and bones. X-ray imaging has been studied also for the detection of insect and fungal development in seeds, fruits, and vegetables as well as, for example, fungal infection in wheat, in wheat kernels damaged by the Red Flour Beetle, in wheat kernels infested by *Rhyzopertha dominica*, in Naval Orange worm damage in pistachios (Keagy *et al.* 1993,1996; Karunakaran *et al.* 2004a,b; Pearson & Wicklow 2006; Narvankar *et al.* 2009). X-ray absorption depends on the density and the atomic number of atoms in a material. Fungal infection of foods leads to changes in density. Such density changes can be detected by comparing the features extracted from the X-ray images of healthy and infected foods. In earlier works, we successfully tried to apply X-rays to the detection of necrotic spots in pistachios (Yanniotis *et al.* 2011).

This paper continues the previous research into the application of X-rays for the detection of the injured pistachio kernels. The absorption coefficient is studied of X-rays in different parts of the kernels as well as in the parts injured by fungi.

## MATERIAL AND METHODS

***Experimental material***. Dried pistachio nuts of the Greek cultivar Aegina were obtained from a pistachio processing plant. Samples of about 0.5 kg in weight containing both "healthy" and "injured" nuts were selected for each experiment. The healthy characteristics of the "healthy" nuts are given in Table 1. The data are based on the measurements carried out with 30 typical nuts. The dimensions were measured manually with callipers, the moisture content directly by drying the specimen at 104°C for 4 h, and the density by weighing the kernels in air and water (ISO 10565:1998). The kernels were obtained after manual opening of the shells of the selected nuts.

***Optical and X-ray microscopy***. X-ray images were acquired using a microfocus X-ray source (Thermo Kevex X-ray PXS5-927; Thermo Kevex, Scotts Valley, USA) working at 45 keV. The X-ray beam was directed onto the inspected specimen located in the distance of about 5 cm from the source. The beam, after passing through the specimen, fell on the scintillation plate of a special X-ray detector (CRYCAM-D; CRYTUR, Turnov, Czech Republic). The detection part of the detector consisted of a CCD detector with a resolution of 4008 × 2684 pixels. The thickness of the specimens was 1.28 ± 0.03 mm. In some cases, photographs of the specimens' surface were obtained and/or the specimens were examined by transmission microscopy.

Table 1. The basic properties of individual pistachio nuts ($n$ = 30)

| | Parameter | MV | SD | *CV* (%) |
|---|---|---|---|---|
| Nut | length (mm) | 19.87 | 1 | 5 |
| | width (mm) | 11.5<br>10.5 | 0.57<br>0.7 | 5<br>6.7 |
| | weight (g) | 1 | 0.11 | 11.1 |
| Kernel | length (mm) | 16.98 | 0.82 | 4.82 |
| | width (mm) | 9.27 | 0.56 | 6 |
| | thickness (mm) | 8.22 | 0.65 | 8 |
| | weight (g) | 0.51 | 0.07 | 13.8 |
| | MC w.b. (%) | 9 | 1.8 | 20 |
| | density (kg/m$^3$) | 0.92 | 0.03 | 3.3 |

MV – mean value; SD – standard deviation, *CV* – coefficient of variation; MC – moisture content

The X-rays experiments were based on the application of Lambert's absorption law:

$$I = I_0 e^{-kx} \quad (1)$$

where:
$I_0$ – intensity of the X-ray beam before entering into the specimen
$I$ – intensity of the X-ray beam after leaving the specimen
$x$ – thickness of the specimen
$k$ – absorption coefficient, is the measure of the X-ray absorption by the specimen substance

The absorption coefficient sensitively depends on the substance composition and arrangement, expressed mainly by the specimen density. In the case of specimen inhomogeneity, the absorption coefficient is not constant along the path of the beam trajectory in the specimen and Eq. (1) could give false results. In the experiments with X-rays, instead of beam intensity the grey degree GD can be used and Eq. (1) can be rewritten as:

$$\frac{GDL}{GDO} = e^{-kx} \quad (2)$$

where:
*GDL* – grey degree in the local point of the irradiated specimen
*GDO* – corresponds to the grey degree for the beam that did not pass through the specimen

The CT image values (grey-levels) provide information on the material X-ray absorption coefficient in each point in the image. There is a considerable current interest in the correction of a number of effects, including "beam hardening", which would allow the CT grey levels to be converted to values directly proportional to the local material density (Phillips & Annutti 1997; Burch 2001).

The following experiments were done: (a) determination of the absorption coefficient in the part of the kernel injured by fungi, (b) determination of the absorption coefficient in different directions and different parts of the kernel, (c) determination of the absorption coefficient for tissues with different moisture contents. The experimental arrangement of case (b) above is given in Figure 1.

For optical microscopy, the standard histological method with specimens of 10–15 μm thickness was used (Troyer *et al.* 2002). The specimens were coloured using transparent hematoxylin-eosin. A Nikon Eclipse E600 (Nikon Corporation Instruments Company, Japan) microscope was used.

The cell dimensions were determined from the obtained images using the programme NIS-Elements Version 3.0 (Nikon Corporation Instruments Company, Japan).

***Statistical analysis***. The experimental results were analysed using the standard laboratory software OriginPro Version 8.5 (OriginLab, Northampton, USA).

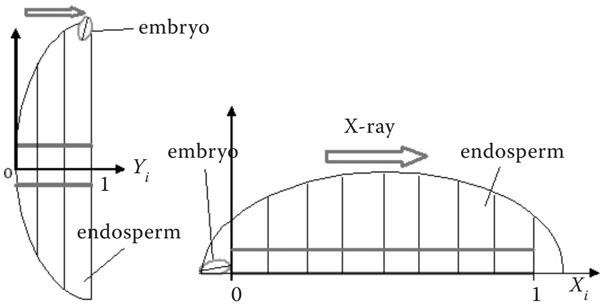


Figure 1. Scheme of kernel cotylydon inspection. On the left: cross inspection by X-rays perpendicular to the individual layers parallel to the basic side of cotylydon, on the right: length inspection by X-rays to the basic side of cotylydon, thickness of the tested individual layers was appr. 1.28 ± 0.03 mm. The relative coordinates $X$ and $Y$ were determined in so manner that its maximum value in the tested individual cotylydon was 1

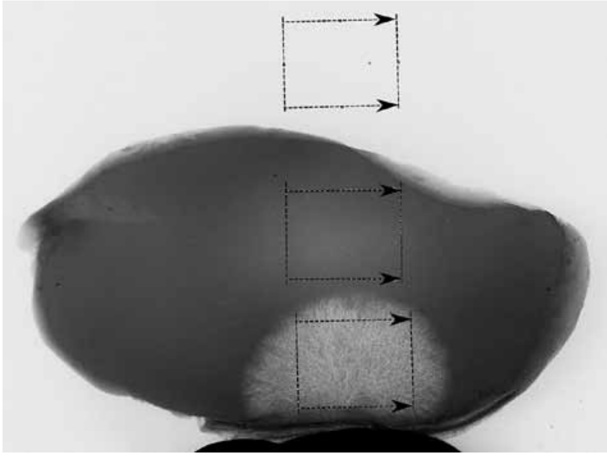

Figure 2. Image of the injury part of pistachio kernel. The specimen was performed by the method described in the left part of Figure 1, thickness of specimen was 1.22 mm. The parts used to determination of coefficient of absorption are denoted by the rectangular lines. The obtained coefficients of absorption were 0.24 mm$^{-1}$ and 0.11 mm$^{-1}$ for basic and injury parts, respectively

## RESULTS AND DISCUSSION

Figure 2 contains a typical part of the pistachio tissue that was injured by fungi. The lower degree of grey in comparison with the other parts of the specimen is clearly visible. Moreover, the structure of the growing fungus is visible. Table 2 contains the data for the absorption coefficients in injured and healthy pistachio tissues. The difference between $k_i$ and $k_b$ was statistically significant and the ratio between X-ray intensities in the injured and healthy parts can be expressed by the intensity ratio, that is the intensity in the injured tissue ($I_i$) to the intensity in the healthy tissue ($I_b$) as:

$$\frac{I_i}{I_b} = e^{-(k_b - k_i)x} \qquad (3)$$

where:
$k_b$ – basic central tissue
$k_i$ – injury part of tissue

Table 2. Coefficient of absorption of pistachio different parts (orientation: left side of Figure 1) ($n$ = 15)

| | MV (mm$^{-1}$) | SD (mm$^{-1}$) | *CV* (%) |
|---|---|---|---|
| Basic central tissue $k_b$ | 0.14 | 0.026 | 18.5 |
| Injury part of the tissue $k_i$ | 0.11 | 0.018 | 16.3 |

MV – mean value; SD – standard deviation, *CV* – coefficient of variation

The intensity ratio is lower than 1 and its value decreases with the increasing thickness of the tissue tested. This forms the theoretical basis for the contrast detection of the injured parts of the pistachio kernels. Under the data from Table 2, the Eq. (3) gives: $I_i/I_b = e^{-0.1x}$.

The absorption homogeneity of pistachio cotylydon was tested by measuring the absorption coefficient in different directions and different parts of the kernel. Figure 3a shows that the absorption coefficient decreases with the increasing $X$, i.e. with the increasing distance from the pistachio embryo. This trend can be crudely approximated by a quadratic equation:

$$k = k_e + a_{x_1}X + a_{x_2}X^2 \qquad R = 0.899 \qquad (4a)$$

where

$k_e$ – absorption coefficient of the tissue close to the embryo (0.197 $mm^{-1}$)

$a_{x_1}$ – gradient of the absorption decrease (–0.141 $mm^{-1}$)

$a_{x_2}$ – parameter at the quadratic term (0.11 $mm^{-1}$)

Equation (4a) determines the lowest value of the absorption coefficient (0.152 $mm^{-1}$) close to the central part of the cotylydon ($X_{min} = a_{x_1}/(2a_{x_2})$ = 0.642). For the opposite part of the cotylydon, Eq. (3) gives the value $k_e + a_{x_1} + a_{x_2}$ = 0.165 $mm^{-1}$. A similar trend was also observed for the absorption in the radial direction that is perpendicular to the previous one. Figure 3b shows the trend that can be described by the formula:

$$k = k_0 + a_yY \qquad R = 0.992 \qquad (4b)$$

where:

$k_o$ – absorption coefficient of the kernel outer part (0.191 $mm^{-1}$)

$a_y$ – gradient of the absorption decrease with the increasing distance from the kernel outer surface (0.0517 $mm^{-1}$)

The observed gradients of the coefficient of absorption can be explained by the inhomogeneous cellular structure inside the cotylydon. Figure 4a shows the microscopic image of the pistachio cellular structure close to the internal surface of the kernel cotylydon. The figure shows that the dimensions of the cells close to the surface are much smaller than those in the internal parts of the tissue. Moreover, another source of absorption is represented by the oil content in the cells. The surface cotylydon layer contained relatively small cells (27.7 ± 1.2 µm), whereas in the central cotylydon parts the cells with a dimension of 42.9 ± 9.0 µm were observed. The relative volume of the cell walls in the product is proportional to $1/r$, where $r$ is the dimension of the cell. It means that the relative cell wall volume in the surface part of the cotylydon is 1.55 times higher than that in its central part; this difference could explain the difference between the observed absorption coefficients in that parts that are given by the ratio of $k_0/k_m \approx 1.26$, where $k_m$ is the minimal value in Eq. (4a).

Figure 4b shows the kernel cell structure on the border between the fungus (injured part) and

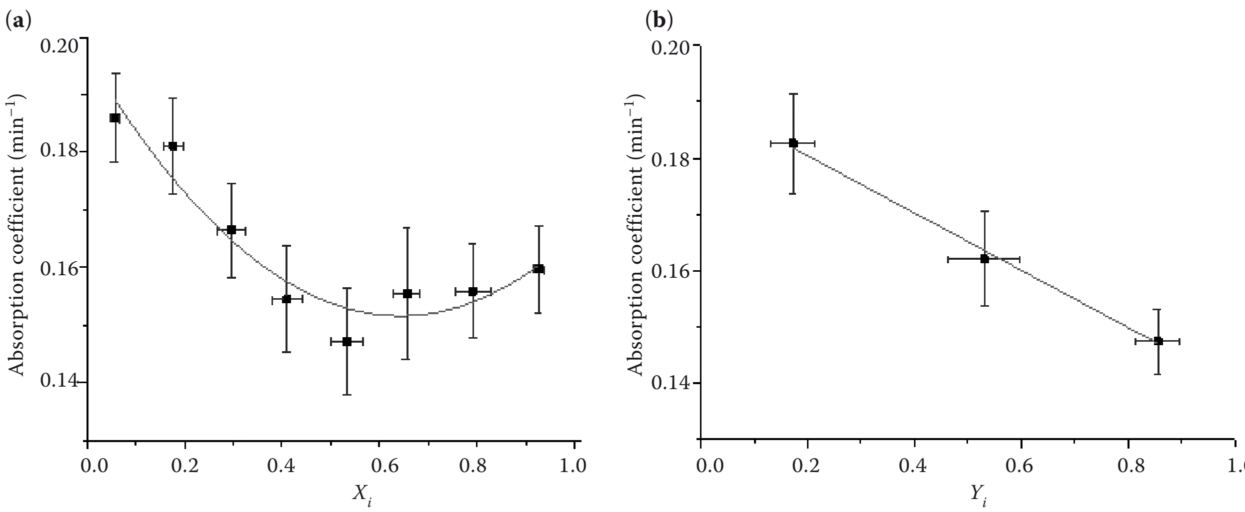


Figure 3. Coefficient of X-ray at 45 keV for different directions and different parts of pistachio kernels: (**a**) versus direction $Y$ (see left side of Figure 1), (**b**) versus direction $X$ (see right side of Figure 1). $Y$ and $X$ are dimensionless coordinates (with values 0–1) calculated as ratios of the individual distances and corresponding kernel dimension. The regression lines (full) and 95% limits (dotted lines) are also given

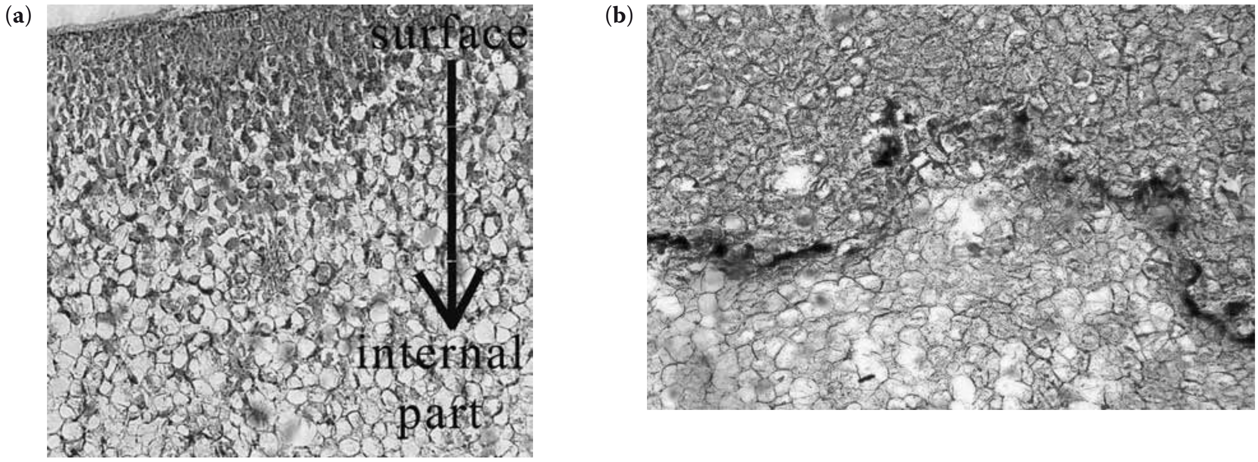


Figure 4. Microscopic images from the pistachio tissue: (**a**) kernel surface layer, (**b**) kernel tissue with fungus located in the lower part of the figure. The oil in the cells is denoted by red colour. Differences in oil concentrations are given: decrease of oil content with increasing distance from the kernel surface (**a**), the emptied kernel cells in the part of fungus framed by the dark border (**b**)

the "healthy" part of the tissue. The most important difference between the two parts is the lack of oil (coefficient of absorption of oil is 0.075 ± 0.010 $mm^{-1}$) in the injured part. The decrease of the absorption coefficient due to the injury caused by fungus represented about 0.14 $mm^{-1}$ which is comparable with the longitudinal gradient of the absorption coefficient in healthy tissue. This may mean that oil is concentrated mainly close to the outer part of the pistachio kernel (see also Figure 4b) and a large portion of the observed gradients is caused by the differences in the location of the pistachio oil.

The effect of the moisture content was demonstrated by inspecting wetted pistachio kernels (wetting for 1 h in distilled water) (Table 3). The observed increase in the moisture content from 9% to 30% led to an increase of the absorption coefficient from 0.14 to 0.22 $mm^{-1}$. This change is comparable to the differences in the absorption coefficient between different parts of the kernel including the injured parts. Therefore, it seems that the moisture content in the kernel must be uniform for a potential sorting device for pistachios using X-rays.

Table 3. Comparison between absorption coefficient of standard pistachio product and the same product after wetting

| State of pistachio | Moisture content w.b. (%) | | Absorption coefficient ($mm^{-1}$) | |
|---|---|---|---|---|
| | MV | SD | MV | SD |
| Standard product | 8.99 | 1.80 | 0.14 | 0.02 |
| Standard product after wetting | 30.02 | 2.29 | 0.22 | 0.02 |

MV – mean value; SD – standard deviation,

## CONCLUSIONS

The absorption of X-rays sensitively depends on the details in the state of the kernels. The variation in the absorption coefficient of up to 50% of the observed maximum values (appr. 0.2 $mm^{-1}$) depends on the product moisture content, distribution of oil, cell dimensions (mainly those close to the kernel surface), and injuries by insects and/or fungi. The injury detection by X-rays is possible due to great differences in the absorption coefficient between healthy and injured tissues. In special cases (moisture content inhomogeneities, dimension, and location of the injured parts), the injury detection could be more complicated.

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*Corresponding author*

Prof. Jiří Blahovec, DrSc., Česká zemědělská univerzita v Praze, Fakulta technická, Katedra fyziky, 165 00 Praha 6-Suchdol, Česká republika; E-mail: blahovec@tf.czu.cz