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X-Ray Microtomography for Food Quality Analysis

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1. Introduction

In an effort to understand the physical and rheological behavior as well as the mechanical and sensory attributes of foods, processing focus and emphasis have shifted to the microstructure level. Microstructure elements such as air bubbles or cells, starch granules, protein assemblies and food biopolymer matrices contribute greatly to the identity and quality of foods (Aguilera, 2005). The microstructure of food has an influence over the key attributes of a product as evaluated by consumers. Many of these properties are synergetic, therefore having multiple interactions, and are poorly understood as a result. Advances in the last decade in microscopy techniques, along with an improvement in computing capabilities, has made it possible to understand a food's structure; its relation to physical properties (so called structure-property relationships) and how to engineer and control these properties (Aguilera, 2005). Structure-property relationships can strongly affect the physiochemical, functional, technological and even nutritional properties of foods. For example, with regards to solid food foams like bread, extruded cereals, biscuits and cakes, the consumer appreciation of these products is strongly linked to the texture. For texture, sensory properties of solid food foams are related to both mechanical properties and cellular structure. In this context, determining the relationships between a given mechanical property and the cellular structure is thus of prime importance. It has also been found that the structural organization of the components of cheese, especially the protein network, affect the texture of cheese: in particular the stress at fracture, the modulus and work at fracture could be predicted very well from the size of the protein aggregates (Wium et al., 2003). Cheeses having a regular and close protein matrix with small and uniform (in size and shape) fat globules show a more elastic behavior than cheeses with open structure and numerous and irregular cavities (Buffa et al., 2001). The mechanical properties of cocoa butter are strongly dependent from its morphology at microscopic level and, in particular, from the polymorphic transformation of the fat crystals and the coexistence of different polymorphic forms (Brunello et al., 2003). Thorvaldsson et al. (1999) studied the influence of heating rate on rheology and structure of heat-treated pasta dough. They found that the fast-heated samples had pores smaller than the slowly heated one and that the pore dimension affects the energy required to cause a fracture. In particular, the energy required to determine a fracture in the samples having the smallest pores was more than for the

samples having the highest pores. A study carried out on the effects of grind size on peanut butter texture demonstrated that an increase of that variable decreases sensory smoothness, spreadability and adhesiveness (Crippen et al., 1989). Langton et al. (1996) studied correlations existing between microstructure and texture of a particulate protein gel (spherical particles joined together to form strands). They found that the texture, as measured with destructive methods, was sensitive to pore size and particle size, whereas it was sensitive to the strand characteristics if measured with non-destructive methods. Martens and Thybo (2000) investigated the relationships among microstructure and quality attributes of potatoes. They found that volume fraction of raw starch, volume fraction of gelatinized starch and dry matter were positively correlated to reflection, graininess, mealiness, adhesiveness and chewiness and negatively correlated to moistness. From the evidence that microstructure affects food sensorial properties, an important consideration derives: foods having a similar microstructure also have a similar behavior (Kalab et al., 1995). All foods can be analyzed in terms of their chemical composition. This gives limited information about the structure, physical state or sensorial properties. The natural building blocks of foods can be considered as water, air, carbohydrates, proteins and fats. The way in which these are structured during processing ultimately determines the functionality of the food. For example, fat content in meat products is a very important compound influencing the palatability characteristics such as taste, juiciness and texture. In addition, the visual appearance of the fat could affect the consumers overall acceptability of product and therefore the choice when selecting meat product before buying (Helgesen et al., 1998). The design of a food product must account for all these relationships whilst maintaining the high standards expected by consumers. Since microstructure is determined both by nature and processing, food processing can be considered as the way to obtain the desired microstructure (and consequently the desired properties) from the available food components (Aguilera, 2000). As a consequence, knowledge of microstructure must precede the regulation of texture (Ding and Gunasekaran, 1998) and other food attributes. It is also possible to obtain microstructural information by studying mechanical and viscoelastic properties of foods. A food sample submitted to mechanical tests gives rise to a force-time curve from which several parameters related to microstructure can be extrapolated: hardness, cohesiveness, springiness, chewiness, gumminess, stickiness (Martinez et al., 2004). When submitted to a stress (under compression, tension or shear conditions), food samples suffer a strain. The elastic modulus or Young modulus of the analyzed sample can be obtained from the stress-strain curve (Del Nobile et al., 2003; Liu et al., 2003). The viscoelastic properties of a food can be expressed in terms of G' , G'' and $\tan \delta$ parameters. G' takes into account the elastic (solid-like) behavior of a material, G'' is a measure of the viscous (fluid-like) behavior of a material and $\tan \delta$ represents the ratio between G'' and G' . These parameters can be evaluated by performing dynamic-mechanical and rheological tests (Kokelaar et al., 1996; Brunello et al., 2003; Wildmoser et al., 2004; Ross et al., 2004). Therefore, relationships between microstructural and mechanical properties can therefore be analyzed by means of directionally dependent morphological parameters.

2. Imaging techniques for microstructure studies

X-ray microtomography (μ CT) is a miniaturized version of medical CT or CAT (computed axial tomography) scanning and given the enormous success of x-ray computed tomography (μ CT) in medical applications and material science, it is not surprising that in

recent years much attention has been focused on extending this imaging technique to food science as a useful technique to aid in the study of food microstructure. The microstructure of food products determines to a large extent the physical, textural and sensory properties of these products. Developing a proper understanding of the microstructure, particularly the spatial distribution and interaction of food components, is a key tool in developing products with desired mechanical and organoleptic properties. Information about the 3-D microstructure of food products and ingredients can be obtained using various imaging techniques. To-date, commonly used techniques are bright-field, polarising and fluorescence light microscopy (LM), confocal scanning laser microscopy (CSLM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Other techniques such as atomic force microscopy (AFM), ultrasound and magnetic resonance imaging (MRI) are used for specific food applications. LM requires the staining of the different chemical components of a food (proteins, fat droplets, proteins etc.), therefore it is a more suitable technique for the investigation of multicomponent or multiphase foods such as cereal-based foods (Autio and Salmenkallio-Marttila, 2001). LM, SEM and TEM can be used to highlight various aspects of particulate structures, e. g. in a study on micro-porous, particulate gels (Langton et al., 1996), LM was used to visualize pores, TEM was applied to evaluate particle size and SEM was used to detect how the particles were linked together, i.e. the three-dimensional structure. As these techniques require some sample preparation (freezing, dehydration, staining etc.) that may lead to artifacts (Kalab, 1984). On the other hand, CSLM is a more suitable alternative method of analysis of food microstructure, as it requires minimum sample preparation. CSLM has been used for examining the three-dimensional structure of the protein network of pasta samples (Fardet et al., 1998), doughs (Thorvaldsson et al., 1999) and for high-fat foods (Wendin et al., 2000) that cannot be prepared for conventional microscopy without the loss of fat (Autio and Laurikainen, 1997). Atomic force microscopy (AFM) and magnetic resonance imaging (MRI) have been recently introduced into food science as non-destructive techniques. The former is particularly suitable for studying surface roughness, especially in fresh foods (Kaláb et al., 1995) and the latter can be successfully applied for studying processing such as frying, foam drainage, fat crystallization and other operations in which a dynamic study of food structure needs (Kaláb et al., 1995). Takano et al. (2002) and Grenier et al. (2003) used the MRI technique to study qualitatively and quantitatively the local porosity in dough during proving, stage in which invasive analytical methods may cause the dough to collapse. The ultrasound imaging technique is used primarily to investigate the structural properties of meat. It has been used to distinguish crystalline fats from liquid fats (McClements and Povey, 1988) or to determine a food's composition (Chanamai and McClements, 1999). Although, these wide varieties of imaging techniques exist, they are mostly invasive, as they require sample preparation hence, formation of artifacts or are restricted to certain types of food products.

3. X-Ray microtomography – An overview

X-ray microtomography (μ CT), on the other hand, is a non-invasive technique that has several advantages over other methods, including the ability to image low moisture materials. It uses the differences in X-ray attenuation arising, principally, from differences in density within the specimen. A series of X-ray projections are recorded at a number of angles around the specimen (usually over a range of either 180 or 360 degrees). In μ CT, unlike medical CT, the specimen is usually rotated, rather than the X-ray source and

detector. If the projections are taken through a single plane in the specimen, it is possible to reconstruct a cross sectional image of that plane. In most μ CT scanners today, 2D images are recorded, making it possible to reconstruct a complete 3D map of X-ray attenuation. In such cases, because of the divergence of the X-ray beam, it is necessary to use a conebeam reconstruction algorithm. This generally gives only an approximate reconstruction, with errors increasing with distance from the central plane (normal to the rotation axis). By using a spiral locus (translating the specimen along the rotation axis as it rotates) an exact (barring artefacts) reconstruction is possible, but this requires complex reconstruction algorithms, which are currently impracticable for large data sets. In the ideal case, each voxel of data represents the X-ray linear attenuation coefficient (LAC) of the corresponding volume in the specimen only. This is related to the composition and density of the material within that volume. Thus μ CT studies can be used both for pure geometric studies, where the LAC is used only to determine the presence or absence of a phase, and quantitative studies where the LAC is used to determine density or concentration. The latter generally requires a higher signal to noise ratio, requiring high dynamic range detection and long X-ray exposures.

A complete μ CT analysis is normally made by acquiring a number of radiographs (typically about 1000) of the same sample under different viewing angles (one orientation for each radiograph). A series of 2D X-ray images are obtained as a sample is rotated. A final computed reconstruction step is required to produce a three-dimensional map of the linear attenuation coefficients in the material. This three-dimensional map indirectly gives a picture of the structure density. In μ CT, the X-ray source and the detector are placed at the opposite sides of the sample. The spatial resolution of the attenuation map depends on the characteristics of both the detector and the number of X-ray projections. Differences in the linear attenuation coefficients within a material are responsible for the X-ray image contrast. The main contrast formation in μ CT is due to absorption contrast. Manipulation and analyses of μ CT data using special software also allows reconstruction of cross-sections at depth increments as low as 15 micrometer, and along any desired orientation of the plane of cut. A series of non-invasive μ CT slices of the same sample in any direction can provide much more information than just one Scanning Electron Microscopy or optical imaging picture for example. The true 3-D shape of the cells can also be visualized from its 2-D slices (Trater et al, 2005). This technique has been successfully used to observe the stability of gas bubbles in dough during the bread making process (Whitworth and Alava, 2002), the microstructure of foams (Lim and Barigou, 2004.) and ice crystals within frozen foods (Mousavi et. al, 2005).

X-ray microtomography has also proven to be a very useful technique for the non-invasive visualization and measurement of the internal microstructure of cellular food products, such as porous rice kernels and whipped cream (van Dalen et al., 2003), aerated chocolate and muffins (Lim et al., 2004), bread (Falcone et al., 2004; 2005; Lassoued et al., 2007), cornflakes (Chaunier et al., 2007), dough (Mousavi et al., 2005), extruded starches (Babin et al., 2007), French fries (Miri et al., 2006) and biopolymer foams (Trater et al., 2005). X-ray microtomography is non-destructive and provides in-depth information on the microstructure of the food product being tested; therefore a better understanding of the physical structure of the product and from an engineering perspective, knowledge about the microstructure of foods can be used to identify the important processing parameters that affect the quality of a product. Processes are no longer designed from a macroscopic level; knowing the properties of foods on the micro scale determines the process specification.

Hence, X-ray microtomography is fast becoming a very useful tool to aid in the study of food microstructure and is an important development in imaging technology that has eliminated some of the drawbacks of traditional imaging and enabled noninvasive characterization of microstructure food in three dimensions (Flannery et al. 1987; Sasov and Van Dyck, 1998).

As stated above, the fat content in meat products is a very important compound and nowadays, lots of meat products with different fat contents and different physical and chemical features (protein network, moisture content, ingredients, additives and so on) are being manufactured. Consumers of today, require some of these information e.g. total fat content, types fat, ingredients, additives etc. to be stated. Therefore total fat content of meat products (e.g. salami, steak etc) is an important quantity used in numerous studies. Thus, reliable methods for the quantitative analysis of fat from this type of food products are of critical importance. There are several methods to analyze fat content quantitatively (Monin 1998), although the method (AOAC, 1995) that is commonly used is based on chemical analysis, it is quite expensive and time consuming. Furthermore this technique is destructive to the sample, as a result, the same sample cannot be measured more than once and sometimes uses harmful, flammable solvents with health and environmental hazards.

With regards to bread, characteristics such as cell wall thickness, cell size, and uniformity of cell size affect the texture of bread crumb (Kamman, 1970) and also the appearance, taste perception and stability of the final product (Autio and Laurikainen, 1997). Crumb elasticity can be predicted from its specific volume and is strongly affected by the amylose-rich regions joining partially gelatinized starch granules in the crumb cell walls (Scanlon and Liu, 2003). Although, microstructure parameters like size and number density of air cells and their contribution to mechanical properties of solid food foams have been studied before (Barrett and Peleg 1992; Barrett et al. 1994a; Van Hecke et al. 1995; Gao and Tan 1996), the underlying mechanism relating cellular structure to the mechanics of these products is still not well understood. The limitations of traditional imaging techniques like scanning electron microscopy (SEM) and optical microscopy, which are two dimensional (2-D) and destructive in nature and also provide poor contrast, make it difficult to characterize cellular structure accurately. For the last few years, X-ray tomography has been proven to be particularly well suited for the 3D investigation of cellular materials. These studies have shown that tomography images allow describing accurately in three dimensions the complexity of the morphology of cellular food products. In addition, this technique enables dynamic studies. From a mechanical point of view, structure-property relationships of heterogeneous materials are often addressed through theories incorporating more or less realistic microstructural information. In order to understand the relation between microstructure and mechanical structure, mathematical models can be developed. For these models a number of free parameters are needed e.g. initial and final moisture content, structural parameters and type of diffusion. Microstructural parameters can be derived from the 3-D structure visualized by μ CT and mechanical structure parameters can be derived from dynamical mechanical analysis.

A μ CT image is typically called a slice and corresponds to a certain thickness of the object being scanned. Therefore, whereas a typical digital image is composed of pixels (picture elements), a μ CT slice image is composed of voxels (volume elements). An X-ray shadow image corresponds to a two-dimensional projection from the three-dimensional object. In the simplest case, it can be described as a parallel X-ray illumination. In this approximation,

each point on the shadow image contains the integration of absorption information inside the three-dimensional object in the corresponding partial X-ray beam. The X-rays that are transmitted through the object are scattered and/or absorbed. The gray levels in a slice correspond to the X-ray attenuation, which reflects the proportion of X-rays absorbed or scattered as they pass through each voxel. X-ray attenuation is a function of X-ray energy and the density and atomic number of the material being imaged. Directing X-rays through the slice plane from multiple orientations and measuring their resultant decrease in intensity creates a μ CT image. A specialized algorithm is used to reconstruct the distribution of X-ray attenuation in the slice plane. By acquiring a stacked, contiguous series of μ CT images

4. Case studies introduction and objectives

The aim of the following case studies is to demonstrate the capability of X-ray microtomography as a useful technique to study fat distribution and the percentage of fat in meat products such as salami. It also aims to help understand the correlation between the physical aspect and microstructural properties of these food products. It also aims demonstrates how the application of X-ray microtomography technique can aid in the study of cellular food microstructure such as coffee, breadsticks and biscuits with different porosity, by imaging and visualising the internal structure.

4.1 Case study 1: Meat products such as salami

4.1.1 Materials and methods

Five different types of Italian salami, chosen to exhibit variability in terms of visible structure of fat, were used for this experiment: Milano, Ungherese, Modena, Norcinetto and Napoli. They were purchased locally and were tested on the same day of purchase. Three samples were prepared for each type of salami, each 28mm in diameter and a thickness of 18mm. Each sample used for x-ray microtomography (μ CT) analysis was wrapped with parafilm to avoid dispersion of moisture; the parafilm does not interfere with the x-rays. The same samples used for μ CT analysis were also used for chemical analysis of fat composition.

4.1.2 Results and discussion

In order to verify the results of the μ CT analysis, the percentage fat content obtained from the chemical analysis for each sample was compared to the μ CT analysis results. Furthermore, the additional information gained from the μ CT analysis i.e. the geometric parameters mentioned provided the required information to characterize and to see if there is any correlation between the microstructures of the different types of salami.

μ CT technique validation

The percentage dark areas for each x-ray microtomographical image was calculated as a representation of the percentage total fat content within the sample. This value was directly derived from the geometric parameter, Percent object volume (POV). The percentage fat content was also calculated by chemical analysis on the same samples to verify the results obtained by μ CT analysis. There were no statistical differences among the results obtained by chemical analysis and the POV values therefore suggesting that the POV calculated by μ CT analysis for each sample is therefore a true representation of the percentage total fat content present within the sample. Therefore x-ray microtomography has proven to be a useful technique to quantitatively analyse fat content in meat products.

Application of μ CT technique

Table 1 shows the average values obtained for the following six parameters using the CTAn software (Skyscan): Percent object volume (POV), Object surface/volume ratio (OSVR), Fragmentation index (FI), Structure thickness (ST), Structure separation (SS) and Degree of anisotropy (DA) and the results of the statistical analysis carried out as reported below.

	POV	OSVR	FI	ST	SS	DA
milano	22.70a \pm 1.45	1,42c \pm 0.08	-0,041a \pm 0.04	4,23ab \pm 0.19	8,48b \pm 0.69	0,15a \pm 0.05
modena	27.65b \pm 4.64	0,92a \pm 0.10	-0,004a \pm 0.30	5,10c \pm 0.41	8,23bc \pm 0.35	0,21a \pm 0.04
napoli	33.10a \pm 1.32	1,28b \pm 0.07	-0,195a \pm 0.21	4,15a \pm 0.25	10,88c \pm 1.64	0,21a \pm 0.03
norcinetto	32.32c \pm 1.80	0,84a \pm 0.02	-0,287a \pm 0.18	4,94c \pm 0.18	10,43c \pm 1.25	0,21a \pm 0.03
ungherese	21.34c \pm 0.98	1,23b \pm 0.04	-0,309a \pm 0.05	4,65bc \pm 0.19	6,53a \pm 0.31	0,15a \pm 0.06

* All parameters obtained were submitted to one-way analysis of variance (ANOVA) and Duncan’s test (p<0.05) through the statistic package Statistica for Windows (Statsoft, Tulsa, USA).

Table 1. Values for the geometric parameters for the salami samples.

Where FI is the index of connectivity and is a measure of relative convexity or concavity of the total solid surface, based on the principle that concavity indicates connectivity, and convexity indicates isolated disconnected structures (Lim et al., 2004). A lower FI signifies better-connected solid lattices and has a negative index while on the other hand a higher FI indicates a more disconnected solid structure and has a positive index. As the fat in salami is considered generally to be of a concave structure, it can be noted from the table that the FI is negative for all samples and there are no statistically significant differences among the samples for this geometric parameter. The degree of anisotropy (DA) is a measure of the 3D structural symmetry, i.e. in this case it indicates the presence or absence of preferential alignment of the fat along a particular direction (Lim et al., 2004). A value of 0 would correspond to total isotropy, whereas a value of 1 would indicate total anisotropy. According to the results obtained for DA (see table 1), the fat present in all samples have a fairly good degree of isotropy and there are no statistical differences among the samples. POV is the percentage of the total fat content present in the sample as proven above. It can be seen that the POV for the Ungherese salami and the Norcinetto salami are statistically equal and are also the highest. There are also no statistical differences in the POV values between the Milano salami and the Napoli salami. OSVR indicates the fat globule size distribution within the sample, the higher the value, the more finely distributed is the fat present in the sample. It can be seen from the table that the Milano salami has the highest value hence its fat content is more finely distributed. Whilst, the Modena salami and the Norcinetto salami are statistically equal i.e. having approximately the same type of fat globule distribution, their low values indicate that the fat globules are more largely distributed with respect to the Milano salami. The results also show that the Napoli salami and the Ungherese salami are statistically equal, hence they have a similar structure with respect to the fat globule size distribution. ST is the average thickness of fat present and SS is the average distant between the fat globules in the samples. The results for ST from table 1 show that Modena, Norcinetto and Ungherese salami are statistically equal, whilst for SS,

Napoli and Norcinetto are statistically equal and also Milano and Modena. As reported above, in order to group the samples for each geometric parameter, a cluster analysis was run but only the results for ST are reported in this paper, as it could be considered the main explicative parameter for salami microstructure. As can be inferred from the results (not reported), the statistical analysis classified the 5 samples in 2 different groups. A group included Milano and Napoli salami and the other one all other samples. This second group was not homogeneous and could be divided in two different sub-groups: one for Ungherese and another one for Modena and Norcinetto salami. Also from table 1 a correlation of the parameters is not evident therefore cluster analysis was performed on all the geometric parameters in order to group the salami in terms of similarity (results not reported) The results of the cluster analysis showed that the Milano and Napoli salami are similar, this similarity occurs only between the microstructural parameters ST and POV (statistical figures not reported), therefore suggesting that the geometric parameters, structure thickness and percentage object volume are important parameters to consider when defining the microstructure of salami. Due to the lack of literature for this work, further investigation will have to be carried out to help achieve a better understanding of the relationships between the microstructure, mechanical structure and sensory properties of salami.

4.2 Case study 2: Porous foods such as biscuits and breadsticks

4.2.1 Materials and methods

Two different porous food products were used for this experiment, Italian sweet biscuits and breadsticks. Three types of Italian commercial sweet biscuits: 'Campagnole', 'Abbracci' and 'Frollini' were used for the first part of this experiment. Two types of brands were used for each types of biscuits: 'Campagnole' produced by Mulino Bianco and Coop, 'Abbracci' produced by Mulino Bianco and Coop and 'Frollini' produced by Ottimini Divella and Doemi. 'Campagnole' biscuits are made from rice flour and milk, 'Abbracci' are made from cream and chocolate and 'Frollini' are traditional made biscuits. A total of 30 samples were used for this experiment, 10 samples for each type of biscuit, i.e. 5 from each brand. Their dimensions were measured out of twenty to thirty random samples and were as following: 'Marie' thickness: 0.60 ± 0.07 (cm) and diameter 6.7 ± 0.03 (cm), 'Petit Beurre' thickness 0.61 ± 0.01 (cm), great and small axis: 6.23 ± 0.07 (cm), 5.45 ± 0.06 (cm), respectively. Biscuit diameter was measured as the average value of two orthogonal diameters. Thickness was measured with a digital micrometer. For each measurement the average of two to three readings was recorded. For the second part of the experiment, three different brands of breadsticks (Grissini) with the same typology were used: 'Mulino Bianco', 'Coop' and 'Bon'.

4.2.2 Results and discussion

Biscuits

The three-dimensional geometrical parameters were calculated using the CTAn software (Skyscan). Table 2 shows the average values obtained for the following six parameters using the CTAn software (Skyscan): Percent object volume (POV), Object surface/volume ratio (OSVR), Fragmentation index (FI), Structure thickness (ST), Structure separation (SS) and Degree of anisotropy (DA) and the results of the statistical analysis carried out as reported below.

	POV	OSVR	FI	ST	SS	DA
Abbarcci MB	37.91±4.36ab	0.88±0.17a	-0.08±0.03°	5.41±0.48ab	6.09±0.37a	0.36±0.02a
Abbarcci C	39.76±5.18ab	0.85±0.15a	-0.13±0.15a	5.89±1.52ab	6.03±1.17a	0.38±0.02a
Campagnole C	38.02±3.33ab	0.92±0.10ab	-0.12±0.10a	5.13±0.40ab	5.86±0.66a	0.37±0.06a
Campagnole MB	31.23±5.04a	1.18±0.16b	-0.02±0.06a	4.52±0.82a	5.74±0.36a	0.39±0.06a
Traditional D	42.91±5.64b	0.74±0.19b	-0.04±0.11a	6.72±1.34b	5.80±0.39a	0.35±0.09a
Traditional OD	41.03±2.54ab	0.77±0.07a	0.03±0.03a	6.23±0.89ab	5.63±0.16a	0.33±0.01a

* All parameters obtained were submitted to one-way analysis of variance (ANOVA) and Duncan’s test (p<0.05) through the statistic package Statistica for Windows (Statsoft, Tulsa, USA).

Table 2.Values of the geometric parameters for all the biscuit the samples.

Where FI is the index of connectivity and is a measure of relative convexity or concavity of the total solid surface, based on the principle that concavity indicates connectivity, and convexity indicates isolated disconnected structures (Lim et al., 2004). A lower FI signifies better-connected solid lattices and has a negative index while on the other hand a higher FI indicates a more disconnected solid structure and has a positive index. From the results obtained for FI it can be said that the air cells observed in the samples are of a concave structure, as the FI is negative for all samples. It can also be noted that there are no statistically significant differences among the samples for this geometric parameter. The degree of anisotropy (DA) is a measure of the 3D structural symmetry, i.e. in this case it indicates the presence or absence of preferential alignment of the air cells along a particular direction (Lim et al, 2004.). A value of 0 would correspond to total isotropy, whereas a value of 1 would indicate total anisotropy. According to the results obtained for DA (see table 2), the air cells present in all samples have a fairly good degree of isotropy and there are no statistical differences among the samples. POV is the percentage of the total air cells present in the sample. It can be seen that the POV for both the Frollini samples although statistically there are little or no differences among the samples. OSVR indicates the cell size distribution within the sample, the higher the value, the more finely distributed are the air cell present in the sample. It can be seen from the table that the Campagnole MB has the highest value hence its air cells are more finely distributed, this can also be observed from the images obtained. Whilst, both the Frollini samples have the lowest OSVR samples hence their air cells are more largely distributed. ST is the average size of the cells in the samples and SS is the average distant between the air cells in the samples. The results for ST from the table show that there are larger air cells present in both the Frollini sample. From table 2 a correlation of the parameters is not evident therefore cluster analysis was performed on all the geometric parameters in order to group the salami in terms of similarity (results not shown); the results showed that there are three groups for terms of similarity. The Campagnole MB in terms of the geometric parameters cannot be grouped with any of the other samples, whilst, Abbracci C and Campagnole C are similar in terms of their geometric parameters and Frollini D, Frollini OD and Abbracci MB are also similar. Further investigation can be carried out to help achieve a better understanding of the relationships between the geometrical parameters, mechanical structure parameters and sensory properties.

Breadsticks

The three-dimensional geometrical parameters were calculated using the CTAn software (Skyscan). Table 3 shows the average values obtained for the following six parameters using

the CTAn software (Skyscan): Percent object volume (POV), Object surface/volume ratio (OSVR), Fragmentation index (FI), Structure thickness (ST), Structure separation (SS) and Degree of anisotropy (DA) and the results of the statistical analysis carried out as reported below.

	POV	OSVR	FI	ST	SS	DA
Grissini MB	52.39±4.29a	0.58±0.05a	-0.38±0.12a	10.17±0.46ab	4.38±0.15a	0.34±0.02a
Grissini C	57.66±3.25b	0.67±0.67a	-0.30±0.06a	9.65±1.02a	4.48±0.16a	0.42±0.02a
Grissini B	50.26±4.81a	0.89±0.89b	-0.58±0.18b	13.13±1.59b	4.37±0.13a	0.55±0.02a

* All parameters obtained were submitted to one-way analysis of variance (ANOVA) and Duncan’s test (p<0.05) through the statistic package Statistica for Windows (Statsoft, Tulsa, USA).

Table 3. Values of the geometric parameters for all the breadstick samples.

Where FI is the index of connectivity and is a measure of relative convexity or concavity of the total solid surface, based on the principle that concavity indicates connectivity, and convexity indicates isolated disconnected structures (Lim et al., 2004). A lower FI signifies better-connected solid lattices and has a negative index while on the other hand a higher FI indicates a more disconnected solid structure and has a positive index. From the results obtained for FI it can be said that the air cells observed in the samples are of a concave structure, as the FI is negative for all samples. It can also be noted that the FI for the Grissini B sample is statistically higher. The degree of anisotropy (DA) is a measure of the 3D structural symmetry, i.e. in this case it indicates the presence or absence of preferential alignment of the air cells along a particular direction (Lim et al, 2004.). A value of 0 would correspond to total isotropy, whereas a value of 1 would indicate total anisotropy. According to the results obtained for DA (see table 3), the air cells present in all samples are isotropic in nature and there are no statistical differences among the samples. POV is the percentage of the total air cells present in the sample. The Grissini C sample has the highest POV, hence a greater amount of air cells are present in this sample, whilst for Grissini MB and Grissini B the Frollini, statistically there are little or no differences among these samples. OSVR indicates the cell size distribution within the sample, the higher the value, the more finely distributed are the air cell present in the sample. It can be seen from the table that the Grissini B has the highest value hence its air cells are more finely distributed, this can also be observed from the images obtained. ST is the average size of the cells in the samples and SS is the average distant between the air cells in the samples. There are no statistical differences among the samples for ST and SS. From table 3 a correlation of the parameters is not evident therefore cluster analysis was performed on all the geometric parameters in order to group the salami in terms of similarity (results not shown); results obtained show that there are two groups in terms of similarity. The Grissini C in terms of the geometric parameters cannot be grouped with any of the other samples, whilst, Grissini MB and Grissini B are similar in terms of their geometric parameters. As for the case of the biscuits, further investigation can be carried out to help achieve a better understanding of the relationships between the geometrical parameters, mechanical structure parameters and sensory properties.

4.3 Case study 3: Emulsions
4.3.1 Materials and methods

Four different types of commercially produced mayonnaises, chosen for their variability of fat contents, were used for this experiment: ‘kraft’ ‘calvé’, ‘kraft legeresse’ and ‘calvé-mayò’.

They were purchased locally and all tests, microstructural and rheological, were carried out on the same day. Three 10ml samples were prepared for each type of mayonnaise; with regards to μ CT analysis, each sample was placed in a cylindrical tube.

4.3.2 Results and discussion

Application of μ CT technique

Table 4 shows the average values obtained for the following seven parameters using the CTAn software (Skyscan): Percent object volume (POV), Object surface/volume ratio (OSVR), Fragmentation index (FI), Structure thickness (ST), Structure separation (SS), structure modelling index (SMI) and Degree of anisotropy (DA) and the results of the statistical analysis carried out as reported below.

	*POV	*OSVR	Av FI	Av SMI	*Av ST	*Av SS	Av DA
Calvé	65.28 ^a ±1.45	0.48 ^a ±0.04	0.05±0.08	2.06±0.69	5.54 ^a ±0.19	5.18 ^a ±0.05	0.12±0.03
Calvé-mayò	24.12 ^b ±1.32	0.51 ^a ±0.30	0.17±0.10	2.49±1.64	4.76 ^b ±0.07	8.18 ^b ±0.25	0.22±0.08
kraft	69.08 ^a ±1.80	0.60 ^b ±0.21	0.21±0.07	2.57±1.25	2.43 ^c ±0.18	4.69 ^a ±0.02	0.17±0.98
kraft legeresse	21.69 ^b ±0.04	0.61 ^b ±0.18	0.15±0.04	2.64±0.31	2.45 ^c ±0.05	7.80 ^b ±0.28	0.24±0.31

*All parameters obtained were submitted to one-way analysis of variance (ANOVA) and Duncan’s test (p<0.05) through the statistic package Statistica for Windows (Statsoft, Tulsa, USA).

Table 4. Values for the geometric parameters for the mayonnaise samples.

Where FI is the index of connectivity and is a measure of relative convexity or concavity of the total solid surface, based on the principle that concavity indicates connectivity, and convexity indicates isolated disconnected structures (Lim et al., 2004). A lower FI signifies better-connected solid lattices and has a negative index, on the other hand a higher FI indicates a more disconnected solid structure and has a positive index. As it can be noted from the results the FI is positive for all samples and there are no statistically significant differences among the sample, therefore the fat structures in these samples have more or less disconnected solid lattices and therefore convex in structure. The degree of anisotropy (DA) is a measure of the 3D structural symmetry, i.e. in this case it indicates the presence or absence of preferential alignment of the fat present along a particular direction (Lim et al., 2004). A value of 0 would correspond to total isotropy, whereas a value of 1 would indicate total anisotropy. According to the results obtained for DA (see table 4), the fat present in all samples are isotropic and there are no statistical differences among the samples, this in accordance with Langton et al., (1998) whom found out that the fat droplets in mayonnaise samples had no orientation and could be regarded as isotropic. POV is the percentage of the total fat content present in the sample as proven above. It can be seen that the ‘calvé’ and ‘kraft’ mayonnaise samples have the highest POV values that are also statistically equal; therefore these two samples are similar in terms of fat content. On the other hand, the ‘calvé-mayò’ and ‘kraft legeresse’ samples have the lowest values and are also statistically equal. OSVR indicates the oil droplet/fat distribution within the sample. The higher the value, the more finely distributed is the oil droplet present in the sample. It can be observed from the table that the ‘kraft legeresse’ and the ‘kraft’ samples have the highest OSVR values that are also statistically equal; therefore they have a much finer dispersion of oil droplets compared

to the other two samples. On the other hand, the samples, 'calvé' and 'calvé-mayò', have the lowest OSVR values and are also statistically equal i.e. having approximately the same type of fat distribution. Their low values indicate that the oil droplets present in these samples are more largely distributed with respect to the other two samples. ST is the average thickness of the fat structure present; this parameter calculates a volume-based thickness of the structure in three dimensions independent of an assumed structure type. It can be noted from table 4 that the samples, 'calvé' and 'calvé-mayò' have the highest ST values, therefore they have a similar fat thickness. While on the other hand the samples 'kraft legeresse' and the 'kraft' samples have low ST values that are statistically equal. SS is the average distant between the fat structures in the samples, it can be noted from this table that 'calvé' and 'kraft' have low SS values that are statistically equal. On the other hand the samples, 'kraft legeresse' and 'calvé-mayò' light have high SS values this could be due to the fact that the fat content in these samples are very low hence the average distant between each fat globule is greater with respect to the samples; 'calvé' and 'kraft'. No significant differences were found for SMI index in the different mayonnaise samples. SMI parameter, that is a topological index, gives an estimate of the characteristic shape of a structure in terms of plates and cylinders composing the 3D structure (Hildebrand & Rüeggsegger, 1997) and is calculated using a differential analysis of triangulated surface of the structure under examination. The SMI assumes integer values of 0, 3 and 4 for ideal plates, cylinder and spheres, respectively. The SMI values calculated for all the samples ranged from 2.06 to 2.64, these values are fairly close to 3 therefore suggesting that the characteristic shape of the oil droplets present in these commercially produced mayonnaise are cylindrical in nature.

Rheological analysis

It was noted that for the rheological data, G' and G'' values vs. the oscillatory frequency all samples exhibited a viscoelastic behaviour with storage modulus (G') greater than the loss modulus (G'') (Subramanian & Gunasekaran, 1997; Rao & Steffe, 1992). As reported in literature (Ma & Barbosa-Canovas, 1995) emulsions with a greater fat content show higher values of G' . This was found for all samples except for the samples 'calvé-mayò' and 'kraft legeresse' that exhibited a higher storage modulus than sample 'calvé' in spite of their lower fat content. This could be due to the fact that the G' reduction was counterbalanced by the higher carbohydrate content of the 'calvé-mayò' and 'kraft legeresse' samples that structure the emulsions (Munoz & Sherman, 1990; Peressini et al., 1998).

It can also be stated from the results obtained that all the mayonnaise samples exhibited solid-like gel behaviour with rheological spectra resembling that of weak gel (Ross-Murphy, 1988; Richardson et al., 1989). Typical weak gel characteristics were observed: G' was greater than G'' throughout the frequency range, and the moduli showed a slight dependence on frequency.

The experimental data on the frequency sweep tests were correlated using the following power law according to Bohlin's theory of flow as a cooperative phenomenon (Bohlin, 1980):

$$G' = A \cdot \omega \cdot \exp(1/z) \quad (1)$$

On the basis of this theory, emulsions are modeled as a network of rheological units that interact for establishing system structure. The coordination number z gives the level of these interactions and the coefficient A their magnitude. The z and A values were obtained for all

samples. These parameters give an idea of the emulsion stability. From the results obtained (not shown) the ‘calvè’, ‘kraft’ and ‘kraft legeresse’ samples showed statistically similar values of the *z* parameter and higher than that the ‘calvè-mayò’ sample. Therefore, the calvè-mayo light with higher *z* values has a more complex structure and contains more microstructural interactions with respect to the other samples. Regarding the *A* parameter all samples differ significantly. Moreover, the highest and the lowest *A* values were recorded for the ‘kraft’ and ‘calvè’ samples, respectively, therefore the ‘kraft’ sample was more stable (Peressini et al., 1998) with respect to the calvè sample.

Correlation

Table 5 shows the results of the correlation among the microstructural parameters and rheological properties of the mayonnaise samples.

	Av POV	Av OSVR	Av FI	Av SMI	Av ST	Av SS	Av DA	A	z
Av POV	1.00								
Av OSVR	-0.38	1.00							
Av FI	-0.21	<u>0.83</u>	1.00						
Av SMI	-0.51	<u>0.98</u>	<u>0.87</u>	1.00					
Av ST	-0.58	-0.22	0.09	0.00	1.00				
Av SS	-0.68	<u>0.94</u>	0.74	<u>0.97</u>	0.06	1.00			
Av DA	<u>0.86</u>	-0.71	-0.68	<u>-0.84</u>	-0.50	<u>-0.90</u>	1.00		
A	-0.10	0.68	<u>0.97</u>	0.75	0.19	0.59	-0.59	1.00	
z	0.54	0.07	-0.30	-0.15	<u>-0.97</u>	-0.17	0.58	-0.41	1.00

* The highlighted parameters indicate a strong correlation among the parameters for this the EXCEL statistic package for Windows was used.

Table 5. Results of the correlation among the microstructural parameters and rheological properties of the mayonnaise samples.

It can be noted from this table that a correlation exists among some of the following microstructural properties of the mayonnaise samples, OSVR, ST, SMI and FI. With regards to the microstructural and rheological relationship, there is a strong correlation among the coefficient *A* and FI and the coordination number *z* and ST, suggesting that the average thickness, i.e. the volume based thickness, of the fat structure present affects the level of microstructural interactions and the connectivity of the fat surfaces affects the magnitude of these interactions. The correlation thus confirms that as the fat structures in these mayonnaise samples have more or less disconnected solid lattices and being convex in structure there is an amplified level of microstructural interactions and the level of these interactions is correlated to the thickness of the fat structure, hence the results obtained demonstrate that there is a link between some microstructural features and rheological properties. As stated above, structure-property relationships can strongly affect the physiochemical, functional, technological and even nutritional properties of foods. For example, with regards to food emulsions like ice cream, yoghurt, spreads and dressings such as mayonnaise the consumer appreciation of these products is strongly linked to the texture. Therefore, this preliminary investigation allows us to carry out further study on the main process variables that affect the characteristics of mayonnaise that are involved in consumer satisfaction to promote full product acceptability.

4.4 Case study 4: Coffee

4.4.1 Materials and methods

Coffea arabica var. S-795 from India, crop 2008-2009, has been used for this experiment. The green coffee beans were roasted with a Probat model BR74 (220°C, 100 g starting sample) and sampled during roasting process at 2, 3, 4 and 5 minutes. The roasting degree has been determined gravimetrically and expressed in terms of total weight loss (6.8, 10.7, 13.9, and 21.1 % w/w, respectively) it corresponded to very light, light, medium and dark, respectively. Five coffee beans at each roasting time were chosen for the experiment. Each coffee bean was weighed on a digital precision balance ($\pm 0.1\text{g}$) (Gibetini Eurpoe, Italy) to calculate the density of the sample. For x-ray microtomographical analysis, each sample was imaged under the same conditions, using the Skyscan 1172 high-resolution desktop X-ray microtomography system (Skyscan, Belgium).

4.4.2 Results and discussion

Figures 1a, 1b and 1c show as an example the set of flat cross sections that were obtained for these samples after binarisation of the images using the reconstruction software CTAn software (Skyscan), from these images the structure/air matrix is clearly visible. The three-dimensional geometrical parameters were calculated using the CTAn software (Skyscan). From the images of above, it can be noted that the structure and the number of holes in the samples changed and increased with increasing roasting time.

Following data analysis, figure 2 shows the graph for the average distribution of the total volume of the holes present for the coffee bean samples at the five different roasting times.

The values of the total volume represented in the graph does not take into account the large central hole of the coffee bean, as this structure is only air it is therefore excluded in this micro-structural study. It can be noted from this graph that as the roasting time increases, the total volume of holes increases, with a significant peak at roasting time 3 due to the rupture of bonds in the internal structure of the coffee beans, this is in concurrence with Schenker et.al (2000). Statistical analysis, performed on the results of this graph confirmed that there are statistical differences for the total volume between roasting times t_0 and t_3 and roasting times t_3 and t_5 , although there are no statistical differences between the total volumes at roasting time t_4 and t_5 . On the other hand, the percentage hole volume, i.e. the geometric parameter POV, was calculated for each sample as a representation of the percentage total hole content within the sample. From the values obtained for POV (results not shown), it can be noted also in this case that the POV values increase with increasing roasting time. Statistical analysis, performed on the results of confirmed that there are statistical differences among all the POV values at the different roasting times. This result is in accordance with the results for the total volume, as an increase in total volume of holes results in an increase of POV.

Figure 3 shows the graph for the total number of pores present in the samples of coffee examined at different roasting times excluding the large central hole as before.

The trend of the graph shows that at roasting time 2 there is a significant peak due to the increase in temperature and therefore a change of the internal structure, this can also be noted from the images obtained. After roasting time 2 the increase of the total number of holes is gradual and statistical analysis performed on the results confirmed that although there is a statistical difference between the total number of holes at roasting times t_2 and t_5 , there are no statistical differences among the values for roasting time t_3 and t_4 . The average bean densities for the different roasting times are shown in figure 3. There is significant

decrease in bean density due to its increase in pore volume and simultaneous weight loss. This is in accordance with Dutra et. al (2001) whom found also a significant decrease in bean density with increase in roasting time.

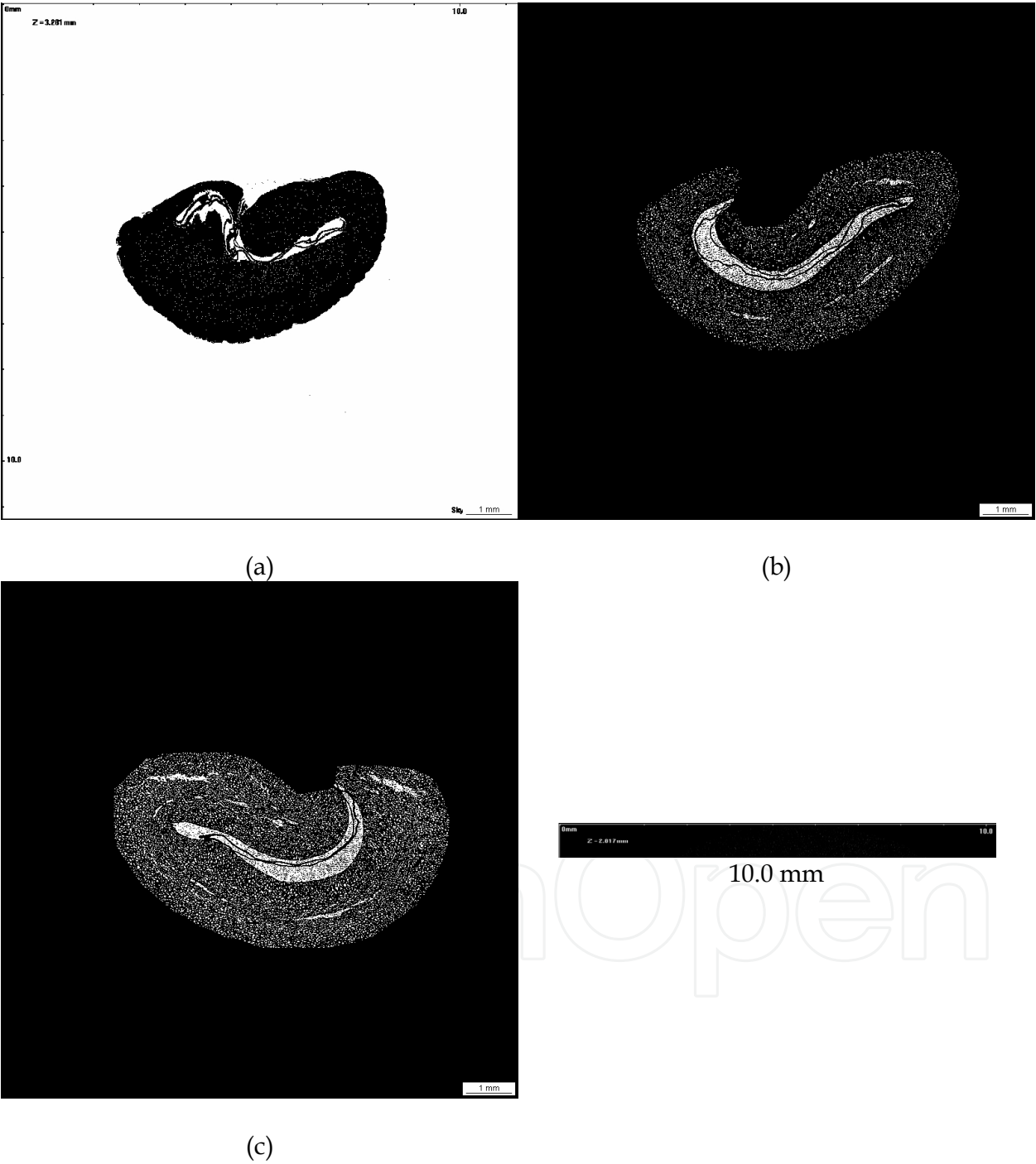


Fig. 1. Examples of the binary tomograph illustrating the separation of the air and structure phases: a) green coffee bean; b) roasted coffee bean at 2 minutes; c) roasted coffee bean at 5 minutes.

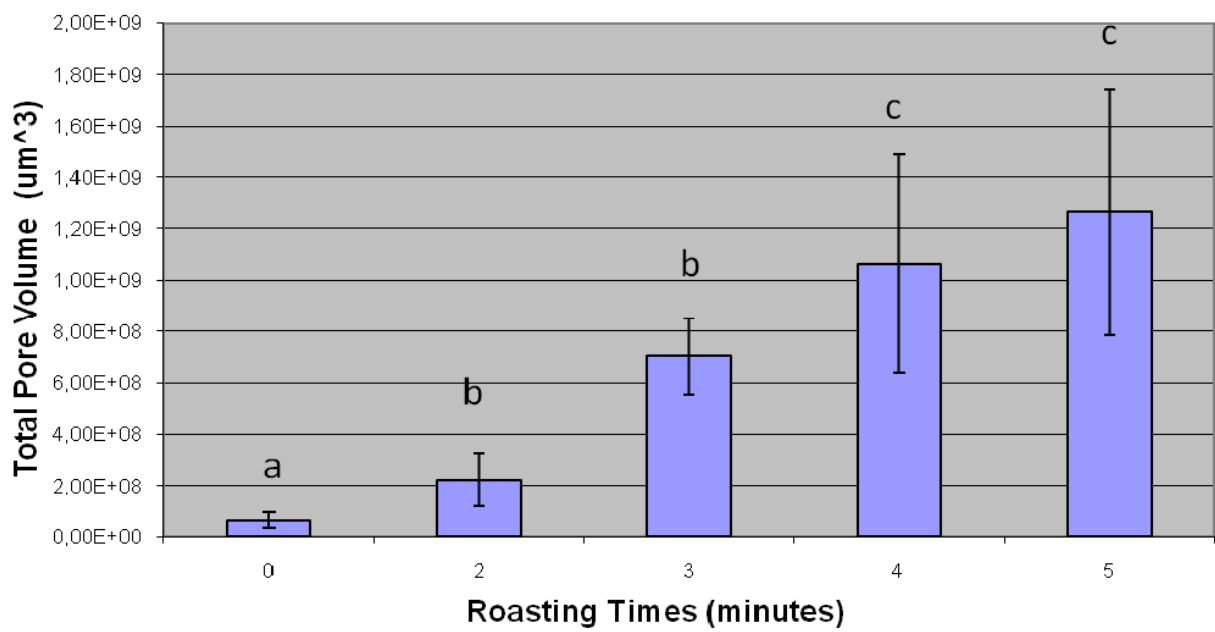


Fig. 2. The average distribution for the total volume of the holes.

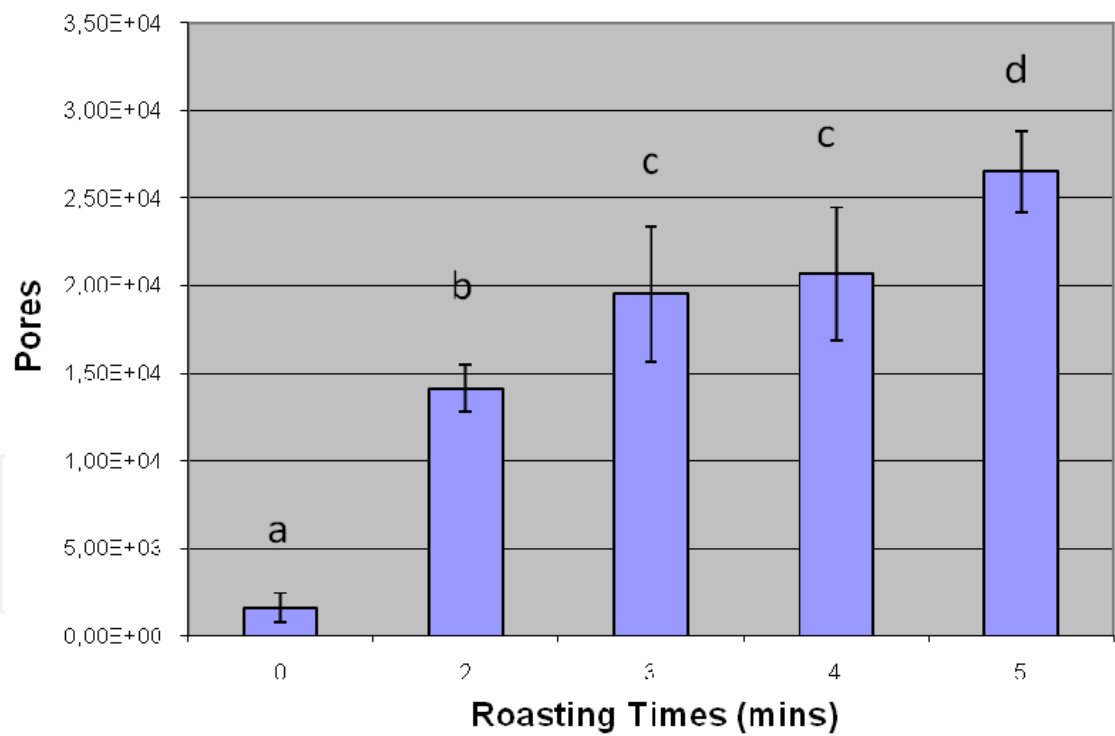


Fig. 3. The total number of pores.

5. Conclusion

X-ray microtomography has proven to be a very useful technique to image the 3-D microstructure of food products. X-ray microtomography can be complementary to other microscopic techniques used for food research. With X-ray microtomography, a full 3-D

image of large samples can be obtained with a voxel resolution of about 0.5 μm and image analysis of the full 3-D microstructure, measuring the size, shape, networking/connectivity and distribution of various phases is possible. These measurements represent the full 3-D microstructure, which is not always possible by 2-D image analysis using statistical techniques.

As stated above, the fat content in meat products is a very important compound and nowadays, lots of meat products with different fat contents and different physical and chemical features (protein network, moisture content, ingredients, additives and so on) are being manufactured. Consumers of today, require some of these information e.g. total fat content, types fat, ingredients, additives etc. to be stated. Therefore total fat content of meat products (e.g. salami, steak etc) is an important quantity used in numerous studies. X-ray microtomography has proven to be a very useful and sophisticated tool for the quantification analysis of fat in meat and meat products. Combined visualisation of the microstructure using X-ray microtomography and other microscopic techniques, extraction of quantitative data obtained by image analysis, and modelling of the microstructure based on characteristics of the structuring elements should point to the optimal food product. The consumer appreciation of solid food foams like bread, extruded cereals, biscuits and cakes is strongly linked to the texture. The control of the sensory properties of such products, which is still a challenge, requires a better understanding of relationships between composition, cellular structure formation mechanisms and the final texture. For texture, sensory properties of solid food foams are related to both mechanical properties and cellular structure. In this context, determining the relationships between a given mechanical property and the cellular structure is thus of prime importance. Since cellular cereal products can be considered, from morphological and topological points of view, like metallic or polymeric foams, it is tempting to address this problem by referring to Gibson & Ashby's model. Such scaling laws are shown to be efficient to assess the effect of the relative density on mechanical properties like Young's modulus or strength of extruded starchy materials or bread. However, for the same density, mechanical properties and sensory properties are also sensitive to microstructural dispersions.

With regards to cellular cereal products, X-ray microtomography to date has proven to be a very useful technique for the non-invasive 3-D visualization and quantitative analysis of the microstructure of cellular cereal products. Further work can be carried out to study the inner cellular structure of the cereal matrix or to assess the integrity of moisture barriers applied on cereal product. The obtained quantitative information can be used as input for simulation models for moisture diffusion. The technique has significant benefits for the design, analysis, and processing science of certain food products. Such an advance in cellular food measurement will undoubtedly open up new horizons for the development of mathematical and computational models that link product microstructure to product mechanical properties and rheology.

With regards to emulsions such as mayonnaise, the fat distribution could be observed and quantified in the microtomographic images, as well as fat droplets size. With μCT , a full 3-D image of large samples can be obtained with high resolution and image analysis of the full 3-D microstructure, measuring the size, shape, networking/connectivity and distribution of various phases is possible. These measurements represent the full 3-D microstructure, which is not always possible by 2-D image analysis using statistical techniques. The correlation of

the μ CT analysis and rheological analysis identified the microstructural-rheological structure relationships. It showed that the average volume base thickness of the fat structure present is correlated to the level of microstructural interactions and due to fact that the fat structures in mayonnaise having more or less disconnected solid lattices and being convex in structure this allows for there to be an amplified level of these microstructural interactions. The identification of the microstructural-rheological structure relationships is of importance as these relationships strongly affect the physiochemical, functional, technological and even nutritional properties of foods.

With regards to coffee beans, the case study demonstrated that with x-ray microtomography a full 3-D image of the bean samples could be obtained with high resolution. Image analysis of the full 3-D microstructure does allow the measuring of size, shape, total volume distribution, porosity and density. These measurements calculated from the 3-D microstructure are not always achievable from 2-D image analysis even by using statistical techniques. Therefore, as proven also in this work X-ray microtomography is a useful and sophisticated tool to provide detail information on the microstructure of porous foods such as coffee beans at different roasting degree: from green to very dark. In fact x-ray microtomography was able to quantify the structural alteration of the microstructure caused by the high internal pressure generated by the large amount of gases released as a consequence of the thermal treatment.

6. References

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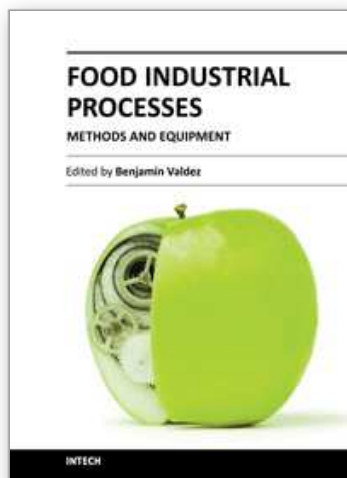
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The global food industry has the largest number of demanding and knowledgeable consumers: the world population of seven billion inhabitants, since every person eats! This population requires food products that fulfill the high quality standards established by the food industry organizations. Food shortages threaten human health and are aggravated by the disastrous, extreme climatic events such as floods, droughts, fires, storms connected to climate change, global warming and greenhouse gas emissions that modify the environment and, consequently, the production of foods in the agriculture and husbandry sectors. This collection of articles is a timely contribution to issues relating to the food industry. They were selected for use as a primer, an investigation guide and documentation based on modern, scientific and technical references. This volume is therefore appropriate for use by university researchers and practicing food developers and producers. The control of food processing and production is not only discussed in scientific terms; engineering, economic and financial aspects are also considered for the advantage of food industry managers.

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