Chapter 7
Microstructure of Gluten-Free Baked Products

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7.1   Introduction

Celiac disease, which is a chronic inflammatory intestinal disorder, occurs in susceptible people due to the inappropriate immune response to certain grain proteins in wheat, barley, rye, and possibly oat. This immunologic reaction causes damage to the villi, tiny, hairlike projections in the small intestine that absorbs nutrients including iron, folic acid, calcium, and fat-soluble vitamins. Therefore, people with celiac disease should eliminate gluten protein from their diet completely (Demirkesen et al. 2010a). However, gluten is an essential protein to form viscoelastic network in dough. Therefore, eliminating gluten from the products causes some quality problems such as insufficient rise in volume, relatively firmer texture, poor flavor, and rapid staling. In this respect, gluten replacement is still one of the most challenging tasks for many scientists and manufacturers. They seek alternative flour types to wheat flour such as rice, corn, chestnut, chickpea, soybean and sorghum flour, and pseudocereals such as buckwheat and amaranth.

In order to overcome the problems associated with the lack of viscoelasticity, some additives such as starches (Moore et al. 2004; Sanchez et al. 2002), hydrocolloids (Demirkesen et al. 2010a; Ribotta et al. 2004; Schober et al. 2008; Sciarini et al. 2010), emulsifiers (Demirkesen et al. 2014a, b, c; Nunes et al. 2009a; Turabi et al. 2008), enzymes (Moore et al. 2006; Renzetti et al. 2008, 2010; Renzetti

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and Arendt 2009a, b), proteins (Ahlborn et al. 2005; Gallagher et al. 2003; Marco and Rosell 2008; Moore et al. 2004; Nunes et al. 2009b; Schober et al. 2004; Van Riemsdijk et al. 2011), and/or fiber sources (Mariotti et al. 2009; Sabanis et al. 2011) are required to be used. Recently, sourdough breadmaking technique (Moore et al. 2007; Moroni et al. 2011; Schober et al. 2007; Zannini et al. 2012a), infrared–microwave combination baking (Demirkesen et al. 2011, 2013, 2014a, c), and high hydrostatic pressure (HHP) treatment (Hüttner et al. 2009; Stolt et al. 2001; Vallons et al. 2010, 2011; Vallons and Arendt 2009) have been used as potential methods in gluten-free technology. The type of components present, the interactions among them, and the structural organization define the physicochemical (rheology, optics, stability), sensory, nutritional, and transport properties of foods (Aguilera and Germain 2007). These properties are largely affected by the structure of foods varying from the molecular to macroscopic levels.

In line with the general trend in food industry, the quality of gluten-free products is also evaluated manually by trained inspectors, which is tedious, costly, and lacks objectivity, consistency, and efficiency (Du and Sun 2004). There is a great need for more reliable tests and new methods of monitoring product quality (Rafiq et al. 2013). Computer-based image-processing techniques, however, offer the advantage of qualification and quantification of structural features that provide understanding of basic mechanisms of physicochemical changes. These techniques also offer flexibility, accuracy, consistency, and repeatability at relatively low cost in application while eliminating the subjectivity of manual inspections (Du and Sun 2004).

Examination of the macrostructure of gluten-free products gives information about the external attributes such as size, shape, color, and surface texture. Image analysis methods based on a large variety of macroscopic techniques such as charge-coupled device (CCD) and scanning have widely been applied for the quantitative description of macrostructure. The inventions made on food-structure imaging provide deeper information about quality attributes of gluten-free products. Foods are complex multicomponent systems. The majority of structural elements are below the 100-μm range, and therefore it is problematic to detect them in their natural or transformed states (Aguilera 2005; Aguilera and Germain 2007). However, application of special image acquisition techniques such as light microscopy (LM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), confocal laser-scanning microscopy (CLSM), atomic force microscopy (AFM), magnetic resonance imaging (MRI), ultrasonic imaging and X-ray microtomography (X-ray μCT) present potential solutions to the problems for observations of microstructural features of food products. A number of studies have been conducted to evaluate the quality of gluten-free products (Demirkesen et al. 2010a; Nunes et al. 2009a; Turabi et al. 2008). Digital images that were captured using digital scanners have been widely analyzed to characterize the macrostructure of gluten-free products such as bread, cake, biscuit, and pasta (Demirkesen et al. 2013; Mariotti et al. 2011; Turabi et al. 2010). In order to understand the influence of different functional ingredients, microscopic techniques such as LM (Hüttner et al. 2009), SEM (Ahlborn

et al. 2005; Alvarez-Jubete et al. 2010; Hager et al. 2012; Hüttner et al. 2009; Marco and Rosell 2008; Sabanis and Tzia 2011; Schober et al. 2008; Vallons et al. 2010; Vallons et al. 2011), and CLSM (Alvarez-Jubete et al. 2010; Moore et al. 2004, 2006; Renzetti et al. 2008, 2010; Vallons et al. 2010; Vallons and Arendt 2009) have also been used for the observation of the quality attributes of gluten-free dough/ batters and baked products. In recent studies, SEM images have been assessed to obtain not only qualitative but also quantitative information on crumb characteristics of gluten-free breads and cakes (Demirkesen et al. 2013; Turabi et al. 2010). In a recent study, X-ray μCT has also been used for quantitative characterization of bread crumbs by creating 3D representation of the inside structure of bread from 2D image slices (Demirkesen et al. 2014b). In this study, the microscopic changes of gluten-free breads by using X-ray μCT were pointed out, and the crumb microstructure of breads was related to their textural properties.

The aim of the present chapter is to illustrate important imaging techniques and their applications for gluten-free baked products and to summarize the advantages and disadvantages of these techniques. Macro- and microstructural changes of gluten-free baked products by using various imaging techniques and the influence of different functional ingredients and prospective methods on microstructure of gluten-free dough/batters and/or products were also reviewed.

7.2   Structural Analysis of Bakery Products

The quality of a baked product, which depends on appearance, texture, volume, and sensory properties, is significantly affected by its structure from molecular to macroscopic levels (Zghal et al. 1999). Thus, understanding of macro- and microstructure by image analysis techniques is critical. The obtained information from image analysis provides the conversion of the food system complexity to numerical data that will be analyzed to improve the understanding of structure–function relationships of materials (Chanona-Perez et al. 2008).

Application of special image acquisition techniques such as several microscopy, scanning, and spectrometric techniques that allow visualization of changes in structure at both macroscopic and microscopic levels without intrusion have been applied as convenient tools for image acquisition (Demirkesen et al. 2013; Falcone et al. 2006). In recent years, image analysis based on various macroscopic and/or microscopic techniques (e.g., flatbed scanning, SEM, X-ray μCT) has been used as a quantitative tool for description of baked product structure (Datta et al. 2007; Farrera-Rebollo et al. 2012; Kelkar et al. 2011; Ozkoc et al. 2009; Ronda et al. 2009; Rouillé et al. 2005; Sanchez-Pardo et al. 2008; Sapirstein et al. 1994; Zayas 1993; Zghal et al. 2002), which further on was adapted for the characterization of gluten-free products (Demirkesen et al. 2013, 2014b; Mariotti et al. 2011; Schober et al. 2008; Sozer 2009; Turabi et al. 2010).

7.2.1   Macrostructure of Gluten-Free Bakery Products

Macrostructural analysis provides information about the external attributes of food materials such as size, shape, color, and surface texture. Image analysis methods based on a large variety of macroscopic techniques such as CCD and scanning have widely been applied for quantitative evaluation of the macro- and microstructure of food products. Quantitative examination of crumb of baked products, such as measuring gas cell sizes and their distribution, can be done by image analysis to provide structural information.

CCD camera, which converts light into electrical charges, is a cost-effective way for image acquisition. It is commonly included in digital still and video cameras (Du and Sun 2004). It has the ability to produce high-quality and low-noise images with lots of pixels and excellent light sensitivity. It has widely been applied to examine physical characteristics and quality of food. The multiple uses of CCD cameras can be applied when it is difficult to evaluate food quality in ordinary spectral region. CCD cameras can be connected to microscopes and flatbed scanners in order to obtain images in digital form (Du and Sun 2004).

The most widely applied imaging technique in macrostructural food research is flatbed scanning. The use of a flatbed scanner for image acquisition and the assessment of appearance and/or color offer all the advantages of previously investigated camera-based systems. In addition, the acquisition of 2D images by flatbed scanning offers some advantages over camera-based systems such as being fast, easy to use, economical, robust, independent of the external light conditions, and providing good accuracy. However, one of the disadvantages of this technique is the lack of a standardized technique for food evaluation. The differences in methodologies such as scanning resolution and the range of air cells also result in different data for similar products (Farrera-Rebollo et al. 2012). Hence, comparing information among published reports is challenging. The most common use of flatbed scanners in the area of cereal research is for characterization of bread crumb structure (Demirkesen et al. 2014b). Pore size, pore distribution, wall thickness, and number of pores were measured for quantitative characterization of gluten-free bread crumb (Demirkesen et al. 2013, 2014b; Schober et al. 2008; Van Riemsdijk 2011; Zayas 1993). In addition, scanners were used for image acquisition of other types of baked products such as cake and biscuit (Ronda et al. 2009; Turabi et al. 2010). The sample images of scanned gluten-free cake and bread are shown in Figs. 7.2 and 7.4.

7.2.2   Image Analysis Process

Image analysis process includes the following five steps: image acquisition, preprocessing, segmentation, object measurement, and classification (Du and Sun 2004).

7.2.2.1 Image Acquisition

Images, which are the spatial representations of objects, stored as matrixes of columns *(x)* by rows *(y)* containing thousands of cells known as pixels. Each pixel consists of a numerical value that is called digital number (Falcone et al. 2006). Image acquisition, which is the first stage of the image analysis process, is the capture of an image in digital form. In order to obtain high quality from a captured image, lighting conditions are an important prerequisite of image acquisition and have a critical role on the improvement of the accuracy and the success of image analysis (Du and Sun 2004). The use of several different image acquisition techniques allows the observation of the structure of food from macroscopic to microscopic level and the translation of images into digital form. CCD camera, flatbed scanners, X-ray μCT, ultrasonic imaging and MRI are widely used image acquisition techniques.

7.2.2.2 Preprocessing

Preprocessing of the raw images is used to enhance the quality of the original images and hence to obtain correct information for image processing. For this purpose, the correction of geometric distortions, removal of distortions and noise, gray-level correction, correction of blurring, etc., are applied analysis steps in preprocessing. Based on the size of the pixel neighborhood, which is used for the calculation of a new pixel, there are two different types of image preprocessing approaches used in food quality evaluation: pixel preprocessing and local preprocessing.

Pixel preprocessing, which is a simple but important image-processing technique, converts an input image into an output image, where each output pixel is fitted directly to the input pixel having the same coordinates, but the values are changed according to the specified transformation function (Aguilera and Germain 2007; Du and Sun 2004). The most applied pixel preprocessing method in food quality evaluation is color space transformation. Although most of the color images are commonly obtained as 24-bit RGB (24 bits: 8 red, 8 green, 8 blue) images, most programs are capable of operating on gray scale (8 bit, monochrome; Falcone et al. 2006), in which images use only 1 byte of 8-bit data per pixel instead of 3 bytes. In addition, the byte still consists of values of 0 (black) to 255 (white), to represent 256 shades of gray. Therefore, the transformation of the RGB digital color image either in a gray-scale image or in three monochrome images (monochrome red, monochrome green, and monochrome blue) is the first stage after image acquisition. However, RGB cannot present all the colors that people can see, and people do not perceive color as a combination of red, green, and blue. Therefore, it is not the most adequate space for color representation, and it is preferable to transform the RGB color space into one of the more representative color spaces for the analysis of foods such as the Commission Internationale de l'Eclairage (CIE) Lab color space (Aguilera and Germain 2007). CIE contains a larger scale than the RGB space. It is device independent and presents a more consistent color measurement regardless

of the device used to acquire the image. The CIE chromaticity diagram is a 2D plot and utilizes three coordinates to locate a color in a color space, which are CIE XYZ, CIE L*a*b*, and CIE L*C*h*. For color analysis, the RGB image is transformed into XYZ tristimulus values, which are assigned to the red, green, and blue curves, respectively. The CIE XYZ data are then converted to the CIE L*a*b* coordinates, where L* reflects the luminance or lightness component, a* indicates the content of red or green, and b* defines the content of yellow and blue (Aguilera and Germain 2007). Hue, saturation, intensity (HSI) color space has also been used for the discrimination of different parts of food material. Intensity is indicated by brightness and moves from 0 to 1 as colors become brighter. Like HIS, hue, saturation, lightness (HSL) and hue, saturation, value (HSV) color spaces are known as human-oriented color spaces. They are two similar sets of coordinates that distinct the gray-scale brightness (L for luminance or V for value) from the hue which moves from 0 to 1, representing color variation from red, yellow, green, cyan, blue, magenta and back to red, and the saturation which moves from 0 to 1 with the increasing amount of black in the color.

Local preprocessing uses a small neighborhood of a pixel in an input image to eliminate nonuniform illumination and obtain a new brightness value in the output image. It can be also regarded as a filtering step, and different types of filters are used as a function of the noise magnitude (Falcone et al. 2006). Edge enhancement and noise reduction in the input images are needed before binarization since they may cause errors in the processing of images. Binarization is the transformation of the color or gray-level image into a black-and-white image. Binarization allows relating a value of black or white to each pixel. After binarization, the image will be ready for the extraction of artifacts and noise and to apply other functions. Then, the binary image can be used for quantitative analysis (Falcone et al. 2006).

7.2.2.3   Image Segmentation

Image segmentation is the process of partitioning the digital image into objects and background. Since the subsequent measurements depend on the accuracy of this operation, it is usually considered the most important step in the image analysis process (Aguilera and Germain 2007). It may be done either manually or automatically (Falcone et al. 2006). Based on different approaches, the techniques used in image segmentation can be classified into three categories: thresholding-based, region-based, and edge-based segmentation. Among these methods, the thresholding-based and region-based are the most widely applied methods (Du and Sun 2004).

Thresholding-Based Segmentation
Thresholding-based segmentation is the simplest way to perform segmentation, and it is the most commonly employed segmentation technique in image-processing applications. This method is based on a threshold value to turn a gray-scale image into a binary image, in which all the pixels that form part of the objects present (foreground) are set to black, while the background pixels are set to white (Aguilera

and Germain 2007). In this way, the object can be distinguished from the remaining part of an image. Thresholding works efficiently when different regions in an image have a different frequency distribution, and the objects of interest have uniform interior gray level. If the image consists of several objects with different surface characteristics, multiple thresholding can be applied for segmentation (Aguilera and Germain 2007).

Region-Based Segmentation
Region-based segmentation is a method used to determine the region directly, and thus algorithms are used to group the neighboring pixels within one region having similar values. It can be categorized as region growing-and-merging and region splitting-and-merging. In region growing-and-merging method, pixels or sub-regions are grouped into larger regions based on a set of homogeneity criteria, while in region splitting-and-merging, the image is successively divided into smaller and smaller regions until certain criteria are satisfied (Du and Sun 2004). Although it is computationally more expensive, it has the capability to use several image properties directly and simultaneously to determine the final boundary location that makes it a powerful approach in the segmentation of food products.

Edge-Based Segmentation
In edge-based segmentation, an edge filter is applied to the image to determine whether the pixels are on edge or not, depending on the filter output. A numerical test for image gradient or curvature, or other properties to classify pixels, is used in this technique (Aguilera and Germain 2007).

7.2.2.4   Object Measurement

Object measurement is the stage used for quantifying the individual features of each object. These features can be classified into four classes such as size (area, perimeter, length, and width), shape (roundness, elongation, compactness, etc.), color, and texture (smoothness, coarseness, graininess, etc.; Du and Sun 2004).

7.2.2.5   Classification

The aim of this step is the discrimination of multiple objects from each other within an image. It comprises a broad range of decision-theoretic approaches to the identification of objects by classifying them into one of the individual categories, comparing the measured features of the new object with those of a known object or other known criteria. A wide variety of approaches, such as the statistical, fuzzy, and neural network methods, are used to classify image objects (Du and Sun 2004). In statistical classification, probability models are used to classify objects. The fuzzy classification method categorizes objects into classes without defined boundaries, and thus the degree of similarity of the considered object with respect to the others can provide more information about the confidence of the class assignment.

Artificial neural network methods use statistical methods with the machine-learning objective of emulating human intelligence (Du and Sun 2004).

7.2.3 Microstructure of Bakery Products

Physical properties of bakery products are strongly related to structural organization of foods at molecular, microscopic, and macroscopic levels. In particular, microstructure and interactions of food components critically contribute to the transport, physical, and sensory properties of foods and hence determine their texture (Falcone et al. 2006). Image analysis techniques (e.g., electron microscopy (EM), CLSM, MRI, AFM, X-ray), which are a convenient and useful key to qualify and quantify the characteristics of crumb structure, can be used to observe the microstructure of gluten-free baked products (Demirkesen et al. 2013). The selection of the most suitable image acquisition technique is influenced by several factors such as the physical properties of the sample, magnification and resolution level requirement, and equipment availability (Aguilera and Germain 2007). One of the main limitations of these microscopic techniques is optical illusion (Du and Sun 2004). Furthermore, sample preparation artifacts are another challenge for the application of microscopic techniques. However, the application of a variety of different image analysis techniques to the same sample and comparing results that were obtained by applying these techniques may be a solution for this problem (Falcone et al. 2006).

LM provides specific staining of different chemical components of a food (proteins, fat droplets, etc.), which makes it a suitable imaging technique for investigating multicomponent or multiphase foods, such as cereal-based foods. LM has low magnifications and resolutions as compared to EM. Thus, the internal structures of micro-components cannot be observed by LM. In order to enhance the contrast of specific structures on samples, bright-field illumination, phase contrast, differential interference contrast, and polarizing light are commonly used methods (Aguilera and Germain 2007). In this method, image acquisition is created by connecting a CCD camera to the microscope objective lens. In the study of Hüttner et al. (2009), both SEM and LM imaging techniques were used to determine the impact of HHP on the microstructure of oat batters and to explain the changes in the major flour components, starch and protein. SEM micrograph revealed that the starch granules of batters treated at 500 MPa were swollen and slightly disintegrated. Treatment at increasing pressures also affected oat batter proteins, as revealed by bright-field microscopy (Fig. 7.1).

EM gives information about tomography, morphology, and composition. EM uses a beam of electrons as the illumination source instead of light. Thus, the images are obtained by projecting the electrons through a thin specimen. The differences in the affinity of heavy metals such as ruthenium, lead, uranium, and osmium allow the differentiation of structure components. Colorful micrographs cannot be obtained, but the colors can be added to the micrographs (Falcone et al. 2006). Since it works under vacuum conditions, sample preparation procedures such as
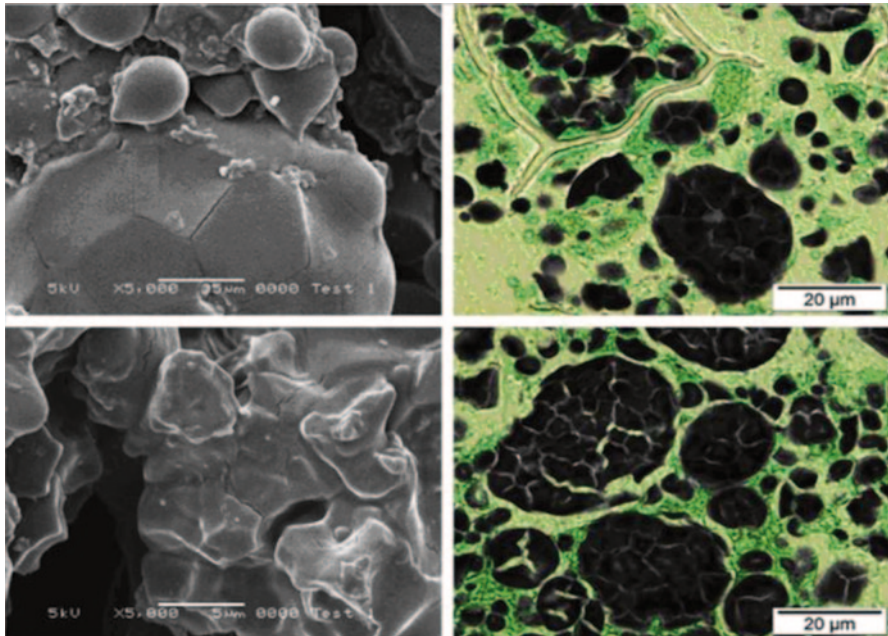
Fig. 7.1 SEM *(left)* and bright-field microscopy *(right*; proteins are stained in *green* and starch in *brown)* analysis of untreated oat batters *(first row)* and oat batters treated at 500 MPa *(second row)*. (For the interpretation of colors in the figure legend, the reader is referred to the web version of the chapter. Reprinted from Hüttner et al. 2009, copyright 2009, with permission Elsevier)

dehydration, freezing, or freeze-drying are required, and they may cause artifacts. EM is categorized into TEM and SEM. Compared with LM, SEM and TEM allow a higher resolution. TEM increase magnifications up to over 10,000 allowing the observation of smaller structures. However, it has many drawbacks. For the application of TEM, samples have to be cut extremely thin for observation (50–100 nm), and that may cause artifacts. Moreover, samples have to be extremely dry and strong enough to resist damage from the electron beam that requires high vacuum conditions during observation. The maximum magnification levels of an LM are about $1000\times$, while TEM can reach magnifications levels of up to over $10,000\times$. The magnification levels of SEM, on the other hand, are between that of LM and TEM, and it can magnify from 20 to $10,000\times$ (Aguilera and Germain 2007). SEM is one of the most important image analysis techniques since it provides the combination of high magnification, large depth of focus, great resolution, and fast and easy sample observation (Demirkesen et al. 2013). SEM also offers the detailed observation of either whole samples or surface/internal structure of samples. Images can be recorded at various magnifications with one objective lens. However, the cost of this method is relatively higher. Furthermore, the sample's surface should be coated in order to avoid charging, and that requires a special training. Different characteristics of particulate structures can be determined by the combination of different

imaging techniques such as LM, TEM, SEM, and CLSM (Falcone et al. 2006). In the study of Langton et al. (1996), LM was used to observe pores, and TEM was used to evaluate particle size, while SEM revealed how the particles were linked together. Up to date, some SEM studies have been assessed to determine the changes that occur during baking qualitatively (Ozkoc et al. 2009; Polaki et al. 2010; Rosell and Santos 2010; Sanchez-Pardo et al. 2008). In recent studies by Demirkesen et al. (2013) and Turabi et al. (2010), SEM has been used to obtain quantitative information on macro- and microstructure of gluten-free breads and cakes. These will be discussed further in Sects. 7.4.1, 7.4.2, and 7.6.2.

Concerning sample preparation, CLSM represents a suitable alternative imaging technique since it requires a minimum sample preparation compared to EM. One of the other advantages of this method is providing the optical slicing of the sample. Thus, as opposed to LM, TEM, and SEM, it is a noninvasive technique. The topographic information can be obtained from a set of confocal images taken over a range of focal planes. A 3D topographical map of the object is obtained from a series of optical slices of the images at different focus positions. It provides volumetric data, which can be very easily quantified. It works best when it is used to provide extraordinary thin, in-focus, high-resolution optical sections through a thick specimen (Aguilera and Stanley 1999). It also provides specific staining of food components. However, CLSM has a high cost. Up to date, CLSM has been used to evaluate the 3D structure and protein network of gluten-free doughs and breads (Moore et al. 2004, 2007; Renzetti et al. 2008; Schober et al. 2007).

MRI, which is also known as nuclear magnetic resonance (NMR) imaging (NMRI), or magnetic resonance tomography (MRT), has found widespread applications in food quality evaluation as a nondestructive and noninvasive imaging technique. The working principle of MRI is based on the absorption and emission of energy in the radio frequency range of the electromagnetic spectrum, and thus it provides image contrast on the basis of the molecular ability (Falcone et al. 2006). Images are acquired based on the absorption or emission of energy by the atomic nuclei in an external magnetic field, and they are created from the Fourier transform of the signal. It also uses physicochemical properties of water to obtain images (Kelkar et al. 2011). MRI allows the collection of images of the system undergoing changes as a function of time from a unique specimen (Prior-Cabanillas et al. 2007). The signal of each voxel varies based on the physical properties of the sample such as proton density, relaxation times, temperature, diffusion, flow, and local differences in magnetic susceptibility (Mariette et al. 2012). MRI can be used to acquire 2D and even 3D images of food material without any preliminary sample preparation. It gives information about the 3D distribution of water, fat, and salt content of food material. It is usually applied in water-containing materials in order to monitor the variation of water content with time (Oztop 2010). It can also be applied to study the transport of solvents into solid systems in real time. Heat and mass transfer within food materials can be studied by MRI (Mariette et al. 2012). Another application of MRI is to understand and follow up the physicochemical changes during food processing. In addition, NMR parameters such as relaxation time and diffusion coefficient can be used for the definition of the interactions among water

and other molecules, which provides information about the physical and chemical properties of food material (Clarke and Eberhardt 2002). However, the main disadvantage of this expensive technique is that it can be used only for such food materials which have sufficient water content (13 % ≤). There are limited studies on MRI of gluten-free baked products (Lodi and Vodovotz 2008). In the study of Lodi and Vodovotz (2008), water and other components of the gluten-free bread matrix were characterized in fresh and stored soy and soy-almond bread samples using a variety of thermal and magnetic-resonance-based techniques. In this study, it has been suggested that changes in water and lipid mobility reflected the microscopic basis of the different loaf quality attributes of soy and soy-almond breads.

AFM is a powerful and revolutionary method that enables information in nanoscale. AFM produces an image by scanning with a sharp stylus, which is attached to a flexible cantilever, across the sample surface. The tip is brought into contact with or close to the sample, and the surface is scanned beneath the tip. When the sample is raster scanned (zigzag movements), the change in surface topography or in probe-sample forces causes the cantilever to deflect (Leite and Herrmann 2005; Yang et al. 2007). The deflection of the cantilever is determined by using laser light reflected off the back surface of the cantilever onto a position-sensitive photodiode detector. Due to that deflection, a line-by-line image of the sample is produced (Leite and Herrmann 2005). Thus, images are created as a result of the measurement of the magnitude of a multitude of forces interacting between the probe and surface of the sample (Yang et al. 2007). AFM can be operated with or without feedback control modes. In feedback control mode, positioning piezo responds to any changes in force and changes the tip-sample separation to restore the force to a predetermined value. As a result of this topographical or height mode, an image can be created. The operation of AFM is done in constant-height or deflection mode in without-feedback mode. Contact, noncontact, and tapping modes are the three basic operation modes in AFM. In contact mode, the tip is attached to the end of the cantilever, and it maintains contact with the sample surface at all times. In noncontact mode, an alternating current (AC) signal is obtained from the cantilever as a result of the cantilever oscillation at a frequency that is slightly above the cantilever's resonance frequency. In tapping-mode AFM, the tip attached to the end of the oscillating cantilever intermittently contacts the surface of the sample at the lowest point of the oscillation (Yang et al. 2007). High resolution, ease of use, and simple sample preparation are the advantages of AFM. In addition, the specimen can be imaged in air or liquid. Furthermore, AFM imaging method provides information about the individual molecules of a material, and it can be used for imaging biological nonconducting materials (Falcone et al. 2006).

Ultrasound is a sound wave having a frequency higher than the hearing limit of the human ear (~20 kHz; Awad et al. 2012). Ultrasound can be used to inspect internal features. The images are created as a result of high-frequency sound waves that are translated into information. The ultrasonic velocity, attenuation coefficient and the acoustic impedance are essential physical factors that depend on the composition and microstructure of a material. The relationship between measured ultrasonic parameters and microstructural properties of a material can be created by a

calibration curve or, theoretically, by using equations that relate the propagation of ultrasound through the material (Falcone et al. 2006). Therefore, 2D images and 3D images are generated. As opposed to light-scattering studies, which require the dilution of food material, ultrasound can measure concentrated food properties, and this allows the analysis of nonhomogeneous foods such as solidifying fats, dynamically changing dairy food systems, dough, and emulsions (Falcone et al. 2006). Ultrasound technology is nondestructive, rapid, and easily modified for on-line measurement. On the other hand, one of the disadvantages of ultrasound technology is that it can be used for imaging concentrated and optically opaque materials. In addition, the presence of small gas bubbles in a sample can weaken the ultrasound, and the signal from the bubbles may interfere with the signals obtained from other components. Moreover, it has limited use for complex biochemical and physiological events. Furthermore, design and development of efficient power ultrasonic systems, which support large-scale operations, are needed (Awad et al. 2012). Based on frequency range, the applications of ultrasound in food industry can be categorized as low- and high-energy ultrasound. Low-energy ultrasound is also known as low-power, low-intensity ultrasound, and it has frequencies higher than 100 kHz at intensities below 1 W/cm^2. High-energy ultrasound, on the other hand, has intensities higher than 1 W/cm^2 at frequencies between 20 and 500 kHz. In bakery industry, it has been applied for online dough quality control (Ross et al. 2004), characterization of the fermentation phase during breadmaking (Elmehdi et al. 2003; Skaf et al. 2009), defining the changes in wheat flour dough consistency induced by proteins and gelatinization of the starch (García-Álvarez et al. 2011), for estimation of the textural characteristics of bakery products such as bread, cakes, crackers, and wafers (Petrauskas 2007; Povey and Harden 1981), and for evaluating rheological characteristics of dough (Fox et al. 2004; Gómez et al. 2008; Salazar et al. 2004). Although there is no literature focusing on the use of ultrasound imaging for gluten-free baked products, we believe that this imaging technology could be applied also for this type of products.

X-ray μCT, which is usually used in medical applications, uses ionizing radiation in the form of X-rays. The major advantage of this nondestructive and powerful imaging technique is the ability to generate cross-sections of the 3D image of an object from a large series of 2D images taken around a single axis of rotation (Kelkar et al. 2011). Another advantage of this technique is its ability to produce the contrast-enhanced imaging without any sample preparation that helps to overcome typical artifacts in the visualization of structure. In addition, the final image results can be recorded by 3D rendering, by 2D slices, or projections following arbitrary directions, and these images can be stored digitally (Besbes et al. 2012; Falcone et al. 2004, 2005; Primo-Martín et al. 2010; Wang et al. 2011). This method also provides greater spatial resolution of X-ray microscopy over other types of microscopes. X-rays have the ability to penetrate through food materials and, in most cases, to capture 3D details of the inner microstructure. However, most other existing microscopes have the ability to focus only at the object surface or a transmission image through a thin section; thus, the internal 3D structure can only be

obtained invasively (Lim and Barigou 2004). X-ray μCT imaging technique requires an X-ray source, a rotation stage, and a radioscopic detector, and analysis is made by scanning the sample under different viewing angles. This imaging method has been used for quantitative characterization of bakery products such as bread, cake, cookie, and muffin (Besbes et al. 2012; Falcone et al. 2004, 2005; Lape et al. 2008; Lim and Barigou 2004; Pareyt et al. 2009; Primo-Martín et al. 2010; Wang et al. 2011), to study the bubble structure of aerated food products, and to obtain quantitative information on the number and size of bubbles present (Babin et al. 2006; Bellido et al. 2006). Demirkesen et al. (2014b) used X-ray μCT to study the effects of different gums and gum blends additions on the crumb structure of gluten-free breads. In this study, microscopic changes of gluten-free breads were related to textural properties of breads.

7.3   Celiac Disease and the Role of Gluten in Baked Products

Celiac disease (gluten-sensitive entropathy), is a multi-symptom, autoimmune disorder, which is triggered by the response of the body's immune system to prolamins found in wheat (gliadin), rye (secalin), barley (hordein), and their crossbreeds (Bower 2006). When people with celiac disease consume gluten, their immune system generates antibodies against this protein causing damage to the tiny hairlike projections in the small intestine; in severe cases, the lesion extends to the ileum colon (Demirkesen et al. 2014a). Hence, a permanent withdrawal of gluten from the diet of celiac patients is required throughout their life span. Recent studies showed that celiac disease is more common than previously reported, and the incidence is $1:100 - 30:100$ in the general population of Europe and the USA (Catassi et al. 2002).

Bread is one of the most important basic items of the human diet. Wheat, which is the major cereal in breadmaking, consists of starch (70–75 %), water (14 %), proteins (10–12 %), and non-starch polysaccharides (2–3 %), particularly arabinoxylans and lipids (2 %; Goesaert et al. 2005). Wheat flour consists of two groups of proteins: non-gluten proteins, which have either no or just a minor role in breadmaking, and the gluten proteins, which have a major role. Non-gluten proteins, which represent 15–20 % of the total protein content of wheat, are mainly present in the outer layers of the wheat kernel. These proteins are mostly structural proteins and genetically related to the major storage proteins in legumes and in the cereals of oats and rice. Gluten is the major storage of protein in wheat and contributes 80–85 % of the total wheat protein. They are found in the endosperm cells of the mature wheat grain, where they form a continuous matrix around the starch granules (Van Der Borght et al. 2005). It is essential to form a strong protein network for the desired viscoelasticity to obtain high-quality breads. Therefore, the quality and quantity of gluten have critical role in the quality of breads. Glutenin and prolamin

are the major fractions of gluten. Glutenin molecule is linked by intermolecular di-sulfide bonds, giving a network structure. In contrast, monomeric gliadin molecule is linked by intramolecular disulfide bonds, creating a globular confirmation for the the proteins (Tronsmo et al. 2002). Therefore, while prolamin provides viscous properties and extensibility in a dough system, polymeric glutenin is responsible for the elastic and cohesive properties of the dough (Gujral and Rosell 2004). Together, the two are important for crumb structure of cereal-based products, and the relative proportions of gliadin and glutenin affect the overall quality. When flour is mixed with water, gluten proteins provide cohesive viscoelastic properties to the dough that is responsible for retaining gas produced during fermentation and oven rise, so a high volume and soft texture can be obtained.

Consumer expectations for gluten-free breads are greatly influenced by tradi-tional bread attributes. Therefore, gluten-free product developers should determine their aims based on wheat bread. Gluten-free dough formulations tend to contain higher water level, so it resembles a batter rather than dough, and they cannot be shaped like wheat dough. Thus, mixing is done mechanically, often in a kitchen mixer or in a mixer using a batter attachment (Lazaridou and Billiaderis 2009). While traditional wheat bread production consists of mixing, bulk fermenting, di-viding, proofing, and baking, gluten-free bread production steps are only mixing, proofing, and baking. Furthermore, gluten-free dough formulations need shorter proofing and baking times than their wheat counterparts (Arendt et al. 2008). Stud-ies showed that the standard farinograph curve for a gluten-free dough mixture requires longer time to reach the consistency of 500 Brabender unit (BU) and a continuous increase in consistency during mixing time (Lazaridou and Billiaderis 2009). This result suggested that gluten-free dough has poor dough characteris-tics for baking with relaxing stretchable properties and with limited machinability, which creates handling problems during mixing. Due to the handling problems of gluten-free dough, researchers have used quality measurements such as volume, texture, and macro- or microstructural analysis of crumb to evaluate the effect of water absorption and mixing time on structure instead of using farinograph mea-surements. Wheat dough is a colloidal system with a continuous phase containing hydrated biopolymers and a dispersed phase consisting of CO_2. On the other hand, gluten-free dough is a colloidal system with starch particulates that make up the continuous phase and water constituting the dispersed phase (Dobraszczyk et al. 2001). Although, starch gelatinization provides some structure to gluten-free dough, it is not enough to provide resistance to shear and entrapment of CO_2. Thus, the rate of CO_2 release in gluten-free dough is higher (22 μmol/min) than that in wheat dough (5 μmol/min) at 23 °C (He and Hoseney 1991). As a result, manufacturers and scientists have tried to decrease CO_2 diffusion of gluten-free dough to obtain high volume, good crumb structure and flavor, and longer shelf life from the gluten-free breads. For this purpose, they are especially focused on the use of additives such as hydrocolloids, emulsifiers, enzymes, protein sources, and fibers.

7.4 Cereal Sources for Gluten-Free Products

Since gluten creates a continuous protein network, which helps to retain gas produced from yeast fermentation and oven rise, gluten-free baked products on the market are of poor quality with low volume and poor texture and flavor. Moreover, they lack the adequate amount of vitamins, minerals, and fiber that deteriorates the nutritionally unbalanced diet of celiac sufferers (Bardella et al. 2000; Demirkesen et al. 2010b). For these reasons, producing high-quality gluten-free products is one of the most challenging issues for cereal food technologists and scientists (Demirkesen et al. 2010b; Moore et al. 2007). Traditionally, most gluten-free products have been produced by replacing wheat flour by alternative flours (rice, corn, chestnut, chickpea, soy, soybean and sorghum flour, pseudocereals such as buckwheat and amaranth, etc.) as well as by using some additives (e.g., starches, hydrocolloids, emulsifiers, enzymes, protein, and/or fiber sources) to mimic the viscoelastic properties of gluten (Demirkesen et al. 2010b).

7.4.1 Rice Flour

Rice flour is the most suitable cereal flour for preparing gluten-free products due to its several noteworthy characteristics: It is natural, hypoallergenic, colorless, and has a bland taste. It contains a very low amount of protein, sodium, and fat, and a high amount of easily digested carbohydrates. Rice has a very low amount of prolamins (2.5–3.5 %) (Demirkesen et al. 2010a). It can also be used in combination with other types of gluten-free flours. Despite its numerous advantages, rice proteins have poor functional properties. Moreover, they are insoluble because of their hydrophobic nature, and this prevents the formation of viscoelastic structure in dough (Rosell and Collar 2007). As a consequence, rice products have low volume, firm texture, short shelf lives, and they stale rapidly. Several studies in the literature have demonstrated the potential use of rice flour for the development of gluten-free products (Ahlborn et al. 2005; Demirkesen et al. 2010a, b, 2011, 2014a, b, c; Moore et al. 2006; Rosell and Collar 2007; Sivaramakrishnan et al. 2004; Turabi et al. 2008). There are various studies in the scientific literature that show the application of different imaging tools for the evaluation of macroscopic and microscopic characteristics of gluten-free baked products containing rice flour (Demirkesen et al. 2013, 2014b; McCarthy et al. 2005; Turabi et al. 2010). In these studies, researchers used different gums, enzymes, and dietary fibers to develop gluten-free formulations. Turabi et al. (2010) studied the effects of different gums (xanthan, guar, LBG, k-carrageenan and xanthan–guar gum blend) on the macro- and microstructure of gluten-free rice cakes. The cakes were baked by using two different baking methods: conventional and infrared–microwave combination baking. In order to characterize the cake crumbs quantitatively, flatbed scanner and SEM were
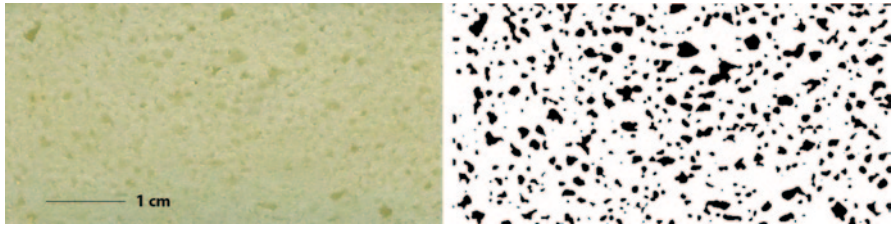
Fig. 7.2 a Scanned image of rice cake containing xanthan gum. **b** Binarized image of this cake. (Reprinted from Turabi et al. 2010, copyright 2010, with permission Elsevier)

used as imaging tools. Scanned images were used to observe the macrostructure of gluten-free rice cakes, while the microstructure of cakes was examined by using SEM images. During the preprocessing step, the scanned images of the cake crumb (Fig. 7.2a) were converted into binarized images (Fig. 7.2b).

Image analysis software ImageJ was used for quantitative analysis of pores. The pore area fraction values of the macrostructure of cake crumbs were significantly affected from both gum type and baking mode. Cakes baked in infrared–microwave combination oven had higher porosity than those baked in conventional oven. The addition of xanthan and xanthan–guar gum blend gave higher pore area fractions than the other gums when they were baked in infrared–microwave combination oven. The higher porosity values of cake crumbs were related to the entrapment of more air bubbles in batters containing these gums due to their higher viscosity values. To obtain microstructural information about rice cakes, SEM images of cake crumbs at $30 \times$ magnification were used (Fig. 7.3).

Pore area fraction values of these images were analyzed by image analysis. It was found that cakes containing xanthan gum and xanthan–guar gum blend and baked in infrared–microwave combination oven had higher pore area fractions as compared to other formulations. This result was in agreement with the data obtained for the macrostructure of cake crumbs. The authors hypothesized that the variation in dielectric properties of gums might affect the porosity of cake crumbs. High dielectric properties of the batters might cause more interaction between the cake batters and microwaves and hence higher gelatinization. Thus, it was postulated that the higher porosity of cakes containing xanthan was due to higher dielectric properties of xanthan than the other gums (Turabi et al. 2010). Different hydrocolloids were added in gluten-free bread formulations based on rice flour and cornstarch in order to investigate their effects on dough rheology and bread quality (Sabanis and Tzia 2011). In this study, concentrations of ingredients (cornstarch, rice flour and hydroxypropylmethylcellulose (HPMC)) were optimized for a fiber-enriched gluten-free bread formulation. SEM images of dough samples were used to show the structural integrity of hydrocolloid-containing gluten-free bread crumbs. The SEM micrographs of xanthan-gum-containing dough samples showed a large number of very small gas cells in an interrupted hydrocolloid–starch matrix, presenting a dense structure. On the other hand, a continuous matrix that was obtained between starch and HPMC exhibited a more aerated structure. The baking and sensory
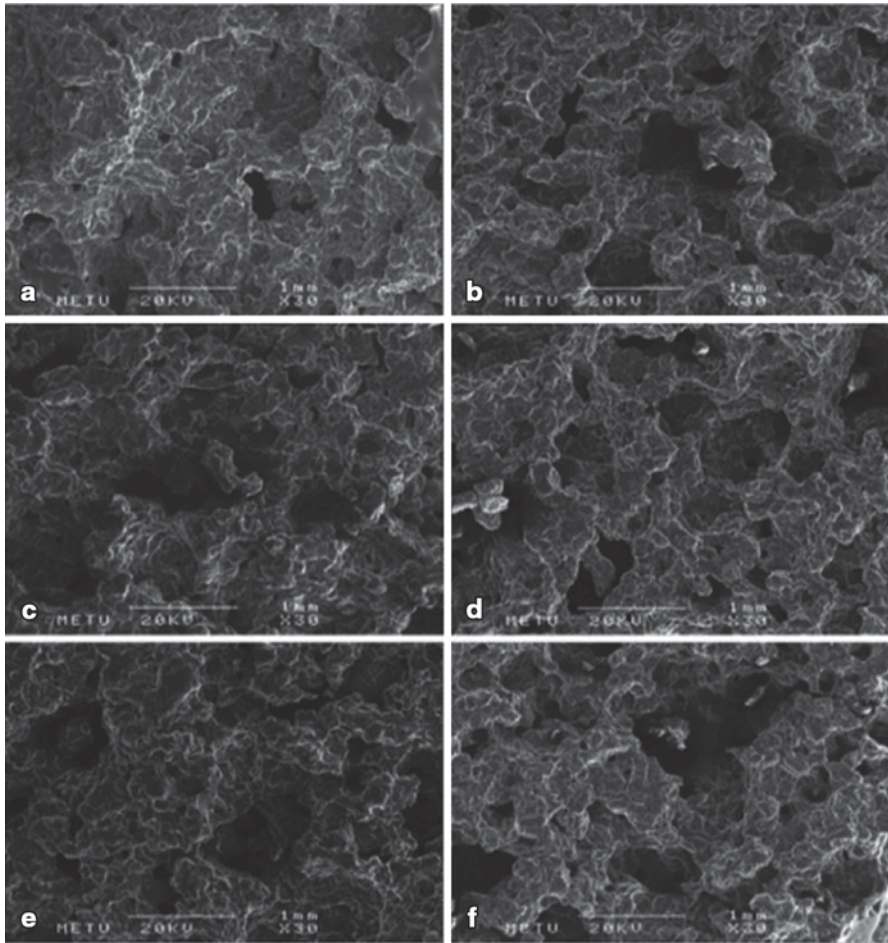
Fig. 7.3 SEM micrographs (30×) for cakes baked in conventional oven (**a** control, **b** xanthan, **c** guar, **d** xanthan–guar, **e** locust bean, **f** kappa-carrageenan). (Reprinted from Turabi et al. 2010, copyright 2010, with permission Elsevier)

tests indicated that the addition of HPMC resulted in higher loaf volume, uniform and finely grained crumb texture, better color, longer shelf life, and higher sensory scores, and thus HPMC was found to be the most effective gum in structuring gluten-free rice cakes.

7.4.2   Chestnut Flour

Chestnut flour has high-quality proteins with essential amino acids (4–7 %), a relatively high amount of sugar (20–32 %), starch (50–60 %), dietary fiber (4–10 %),

and a low amount of fat (2–4%). It also includes some important vitamins, minerals, and phenolics (Blaiotta et al. 2012; Chenlo et al. 2007; Sacchetti et al. 2004). Besides its health and nutritional benefits, the ingredients of chestnut flour may provide some functional properties to the dough. While the fiber content of chestnut flour may assist the emulsifying, stabilizing, texturizing, and thickening properties of dough, the sugar content of chestnut flour may improve the color and flavor properties of gluten-free products when it is used at a certain level (Demirkesen et al. 2013). Demirkesen et al. (2013) studied the effects of the replacement of rice flour with chestnut flour and xanthan–guar gum blend emulsifier–diacetyl tartaric acid ester of mono- and diglycerides (DATEM) mixture addition on the macro- and microstructure of rice breads baked in different types of ovens (conventional and infrared–microwave combination ovens). In this study, flatbed-scanned and SEM pictures were analyzed by image analysis program in order to obtain quantitative information about the macro- and microstructure of gluten-free breads. The scanned images of gluten-free bread samples prepared with different formulations and baked in different ovens are depicted in Fig. 7.4.

Control breads, which were prepared without using chestnut flour and gum-emulsifier blend had heterogeneous crumb structure with their larger pores. This result was related to insufficient air incorporation of dough during the fermentation and baking processes due to its low viscosity and viscoelastic properties. The higher fiber content of chestnut flour improved the viscoelastic properties of dough leading to entrapment of more air bubbles. Therefore, even in the lack of additives, the replacement of rice flour with chestnut flour prevented the formation of very large pores (Fig. 7.4c and g). Hydrocolloids enhanced bread quality by improving water absorption and viscoelastic properties of dough, and emulsifiers lowered the surface tension of dough leading to the subdivision of the entrapped air bubbles into more and smaller bubbles during mixing. Therefore, when xanthan–guar gum blend–DATEM mixture was incorporated into dough, the pores of gluten-free breads were found to be smaller and more uniform in size (Fig. 7.4b, d, f, h). The high internal heat generation in infrared–microwave combination baking produced higher internal pressure, which created a puffing effect, and this puffing effect resulted in the formation of looser and more porous structure. Consequently, among all gluten-free breads, the most homogenous structure was obtained in the presence of chestnut flour, xanthan–guar gum blend–DATEM mixture addition, and infrared–microwave combination baking (Fig. 7.4). The pore area fractions of gluten-free breads prepared with different formulations and baked in different ovens based on scanned images are presented in Fig. 7.5.

The lowest pore area fraction values were obtained from control breads. However, the replacement of rice flour with chestnut flour increased the pore area fractions of breads. Furthermore, the incorporation of xanthan–guar gum–DATEM mixture and the use of infrared–microwave combination oven resulted in higher pore area fraction values. Therefore, the qualitative information based on scanned images was found to be in agreement with the quantitative data obtained by image analysis. In order to obtain quantitative information about the microstructure of breads, SEM images at magnification of 20× were used for the image analysis. Similar to scanned images, the highest pore area fraction values were obtained from breads
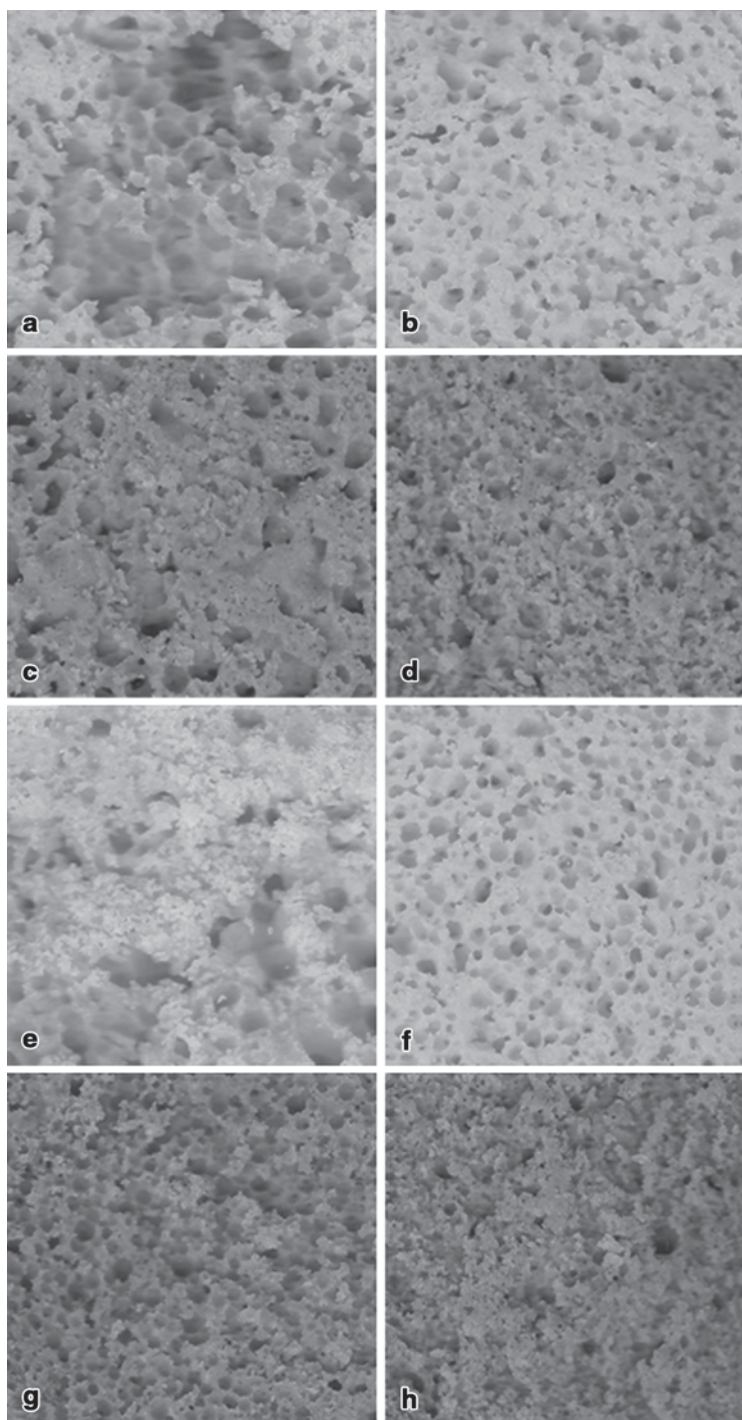
Fig. 7.4 Scanned images of different gluten-free bread formulations baked in different ovens.
a Rice bread baked in a conventional oven. **b** Rice bread containing xanthan–guar gum blend–
DATEM mixture and baked in a conventional oven. **c** Chestnut-rice breads baked in a conventional
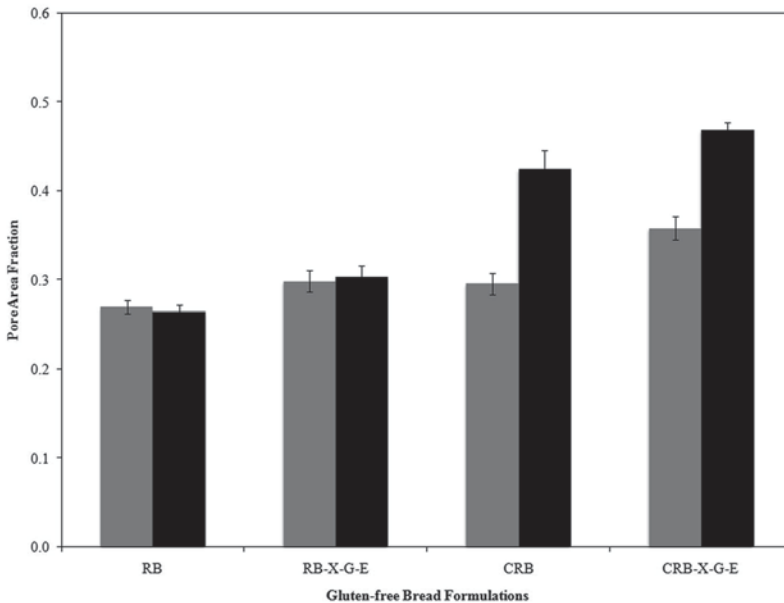
Fig. 7.5 Based on scanned images, pore area fractions of different gluten-free bread formulations baked in conventional *(gray)* and infrared–microwave combination ovens *(black)*. (*RB* rice bread, *RB-X-G-E* rice bread containing xanthan–guar gum blend–DATEM mixture, *CRB* chestnut-rice bread, *CRB-X-G-E* chestnut-rice bread containing xanthan–guar gum blend–DATEM mixture). (Reprinted from Demirkesen et al. 2013, copyright 2013, with permission Springer)

formulated with chestnut flour with xanthan–guar gum blend–DATEM mixture addition and baked in an infrared–microwave combination oven (Fig. 7.6).

In this study, SEM pictures of the bread crumbs at 1000× magnification were also obtained (Fig. 7.7). Control breads, especially conventionally baked ones, had less-developed pores due to the entrapment of insufficient amount of air bubbles into the dough (Table 7.1 and Fig. 7.7a, e). More homogenous structure was obtained in breads prepared with the replacement of rice flour with chestnut flour (Table 7.1 and Fig. 7.7c, d, g, h).

The enhancement of viscosity and decrease in starch-protein binding in the presence of fiber might lead to the formation of more homogenous structure. It was also suggested that the significant relationship between starch granule size and gas retention might also affect final crumb appearance. Flours that have larger starch

oven. **d** Chestnut-rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **e** Rice bread baked in an infrared–microwave combination oven. **f** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in infrared–microwave combination oven. **g** Chestnut-rice breads baked in an infrared–microwave combination oven. **h** Chestnut-rice bread containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven. (Reprinted from Demirkesen et al. 2013, copyright 2013, with permission Springer)
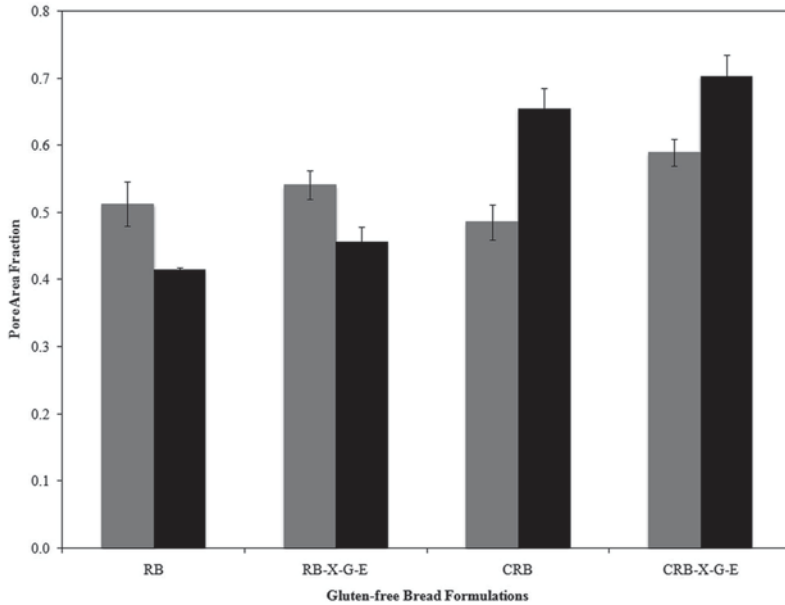
Fig. 7.6 Based on SEM, pore area fractions of different gluten-free bread formulations baked in conventional *(gray)* and infrared–microwave combination ovens *(black)*. (*RB* rice bread, *RB-X-G-E* rice bread containing xanthan–guar gum blend–DATEM mixture, *CRB* chestnut-rice bread, *CRB-X-G-E* chestnut-rice bread containing xanthan–guar gum blend–DATEM mixture). Magnification: 20×. (Reprinted from Demirkesen et al. 2013, copyright 2013, with permission Springer)

granules, like chestnut starch granules, might release more amylose during baking since they contain more amylose as compared to small rice granules. Thus, a film-like structure was formed by the interaction between that amylose and protein which might coalesce less during the baking of chestnut-flour-containing breads. Like scanned images of breads, SEM images also showed that among all breads prepared without any additives, the most homogenous structure was obviously obtained from breads formulated with chestnut flour and baked in an infrared–microwave combination oven (Fig. 7.7g). More homogenous pore distributions were observed in gluten-free breads containing xanthan–guar gum blend–DATEM mixture (Fig. 7.7b, d, f, h).

7.4.3   Sorghum Flour

Sorghum is a member of the grass family of Graminae and tribe Andropoggonae. It is regarded as a primary ingredient in bread in most urban areas due to its easier growing conditions and low prices (Arendt and Moore 2006). Although, sorghum is reported to have very similar chemical composition to maize, it has slightly lower protein (11–12%) and starch digestibility. Furthermore, sorghum flour is also
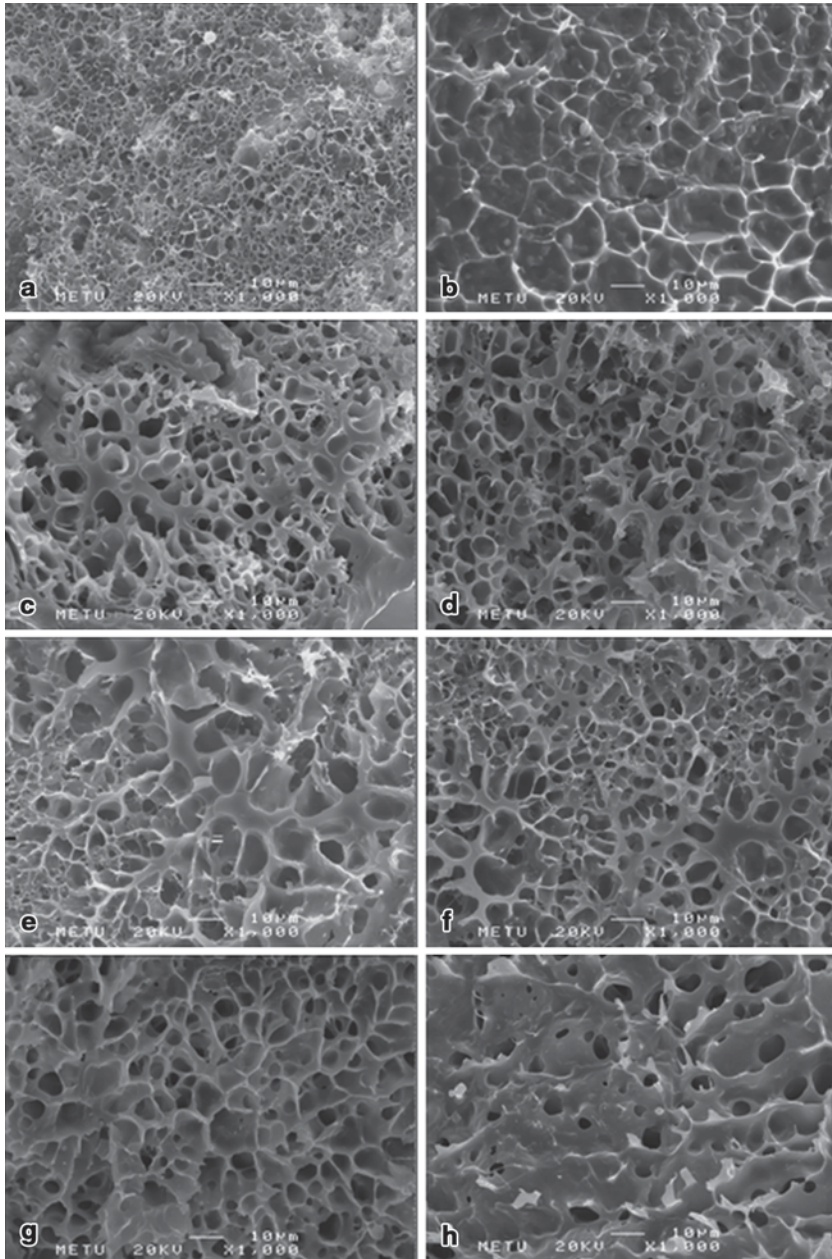
Fig. 7.7 SEM micrographs of the outside of gluten-free bread crumb samples baked in different ovens. **a** Rice bread baked in a conventional oven. **b** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **c** Chestnut-rice breads baked in a conventional oven. **d** Chestnut-rice bread containing xanthan–guar gum blend–DATEM mixture and baked in a conventional oven. **e** Rice bread baked in an infrared–microwave combination oven. **f** Rice bread containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–

Table 7.1 Pore area distribution of gluten-free breads prepared with different formulations and baked in different ovens. (Reprinted from Demirkesen et al. 2013, copyright 2013, with permission Springer)

Number of pores					
Range of pore area (mm^2)	Oven type	RB-X-G-E	RB	CRB-X-G-E	CRB
	Conventional				
0–5		161	98	278	339
5–10		62	23	54	32
10–20		24	18	28	24
>20		3	11	1	5
Total number of pores		250	150	361	400
	Infrared–microwave combination				
0–5		259	271	291	292
5–10		54	25	27	26
10–20		16	23	26	25
>20		0	5	17	19
Total number of pores		329	324	361	362

RB rice bread, *RB-X-G-E* rice bread containing xanthan–guar gum blend–DATEM mixture, *CRB* chestnut-rice breads, *CRB-X-G-E* chestnut-rice bread containing xanthan–guar gum blend–DATEM mixture

neutral in flavor and light in color. As in the case of maize and millet, the protein content of sorghum is not considered to have a large role in creating structure in foods. However, sorghum proteins are reported to form cross-links with themselves or with other constituents during processing as well as cooking that directly impact the functional properties of dough and the quality of sorghum-based products (Hamaker and Bugusu 2003). Gluten-free breads made from sorghum present technical difficulties. Thus, very limited studies have been conducted on gluten-free sorghum dough and bread characteristics. Schober et al. (2004) evaluated crumb characteristics of gluten-free breads made from different sorghum varieties by digital image analysis and texture profile analysis (TPA). Significant differences in crumb structure in terms of number of pores, pore size, and hardness were found and were related to differences in kernel hardness and damaged starch. It has been postulated that a coarse and open crumb structure might provide less resistance to the probe during TPA, thus resulting in softer crumb. In a recent study of Hager et al. (2012), crumb grain characteristics of gluten-free breads prepared with sorghum, oat, buckwheat, rice, maize, quinoa, and teff flours were investigated by image analysis. SEM was also used for the observation of microstructure of dough and bread samples, while a C-cell bread imaging system was used for the quantitative description of the crumb grain characteristics. Digital image analysis revealed that

microwave combination oven. **g** Chestnut-rice breads baked in an infrared–microwave combination oven. **h** Chestnut-rice bread containing xanthan–guar gum blend–DATEM mixture and baked in an infrared–microwave combination oven. Magnification: 1000×. (Reprinted from Demirkesen et al. 2013, copyright 2013, with permission Springer)

several gluten-free samples prepared with quinoa, teff, maize, and buckwheat were characterized by a dense structure with a low number of cells as a percentage of a slice area. On the other hand, breads made from oat and rice had an open aerated structure with a higher number of cells. The SEM images of the breads also reflected a more aerated crumb structure of oat and rice breads, and this result was found to be in accordance with the macrostructure observations obtained by image analysis. By regarding the results of both digital image analysis and quality tests (specific volume, crumb hardness, springiness, loaf height, and sensory), the authors concluded that only breads produced from oat flour had a similar quality to wheat bread.

7.4.4 Pseudocereal Flours

Buckwheat, amaranth, and quinoa are classified as pseudocereals, and their seeds show similarity with true cereals in terms of their function and composition. Pseudocereals contain a high amount of starch (52–69 %), which make them important energy sources. The protein content of pseudocereals ranges approximately from 11 to 18 %. Pseudocereals are also rich in sources of good-quality protein with the acceptable levels of essential amino acids, and the structural characteristics of these proteins influence their techno-functional properties. They also have a high amount of dietary fiber and lipids rich in unsaturated fats. Moreover, they contain adequate levels of important micronutrients, such as minerals and vitamins, and significant amounts of other bioactive components (Alvarez-Jubette et al. 2010). Due to their excellent nutritional value, the latest studies have focused on the use of pseudocereals in the formulation of high-quality, healthy, gluten-free products. In the study of Alvarez-Jubette et al. (2010), technological characteristics (i.e., batter and baking properties) of pseudocereals (amaranth, quinoa, and buckwheat) as ingredients in the production of gluten-free breads were evaluated. In this study, SEM images were used to evaluate properties of flours. CLSM images obtained from the baked breads were used to obtain qualitative information about microstructure (Fig. 7.8).

On the other hand, C-cell bread imaging analysis was conducted on the bread slices to obtain quantitative information (Fig. 7.9).

SEM images of the flours indicated that the size of flour particles had significant differences, and the smallest particle size was observed in potato starch and wheat flour, followed by rice, buckwheat, amaranth, and quinoa flours. The results for the crumb grain analysis of the baked breads showed that the largest number of cells was obtained from buckwheat bread, followed by quinoa, control, and amaranth breads. The smallest cell volume was found in gluten-free control bread, followed by quinoa, amaranth, and buckwheat breads. The cell wall was the thinnest in quinoa bread, followed by control, buckwheat, and amaranth breads in increasing order. Significant variations were obtained between CLSM images of different breads (Fig. 7.8). Starch gelatinization, which was characterized by fusing starch granules together and losing their original structure, occurred at a greater degree in the gluten-free control bread compared with the pseudocereal-containing gluten-free
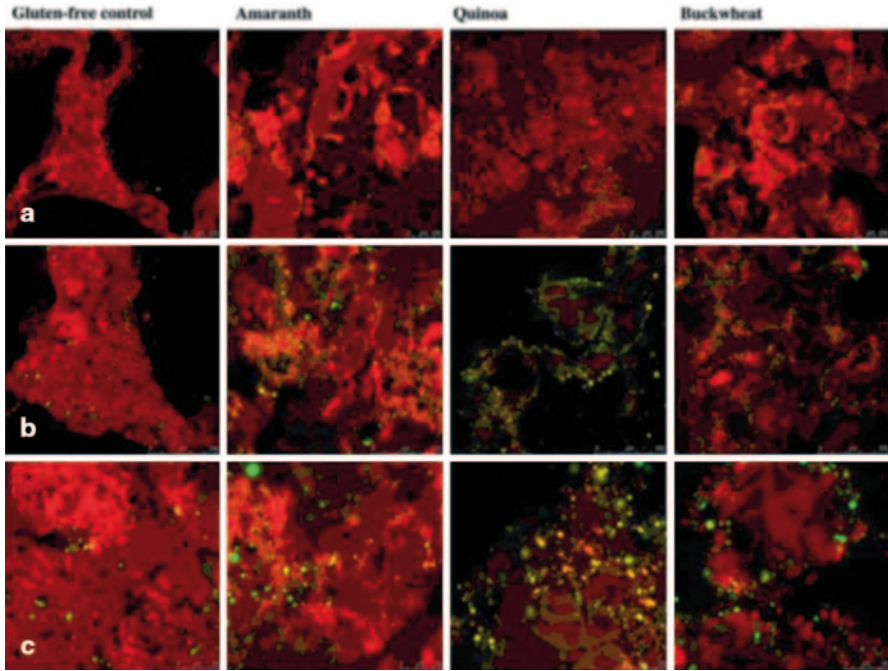
Fig. 7.8 Confocal laser-scanning micrographs of gluten-free control, amaranth, quinoa, and buckwheat breads. Scale bars row **a** 0–250 μm, **b** 0–250 μm, **c** 0–50 μm. (Reprinted from Alvarez-Jubete et al. 2010, copyright 2010, with permission Springer)
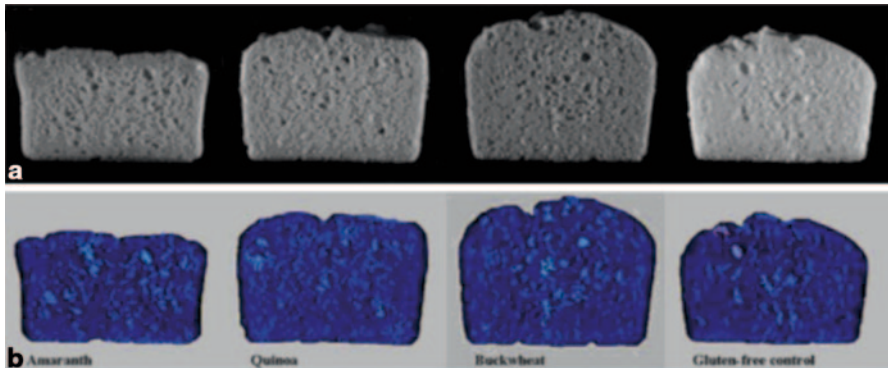
Fig. 7.9 Raw (**a**) and cell (**b**) images of amaranth, quinoa, buckwheat, and gluten-free control breads. (Reprinted from Alvarez-Jubete et al. 2010, copyright 2010, with permission Springer)

breads. Pseudocereal-containing gluten-free breads had a more homogenous structure with less gas voids and a more even distribution of fat, protein, and starch. The pseudocereal-containing breads were characterized by a noticeably softer crumb texture that was attributed to the presence of natural emulsifiers in the pseudocereal flours and confirmed by the CLSM images (Fig. 7.8). Thus, the authors suggested

the application of pseudocereal flours as feasible ingredients in the manufacture of good-quality and healthy gluten-free breads.

## 7.5   Ingredients and Additives Used to Improve the Structure of Gluten-Free Baked Products

Nowadays, the use of additives, which have the ability to mimic the viscoelastic properties of gluten, has commonly been applied in the gluten-free industry. Hydrocolloids have been widely used in the gluten-free bakery industry since they can improve dough viscoelasticity, enhance moisture retention, improve gas holding, texture, and shelf life as well as retard starch retrogradation, modify gelatinization of starch and act as fat replacer in formulations (Arendt et al. 2008). There has been growing interest in the use of hydrocolloids as gluten substitutes in gluten-free bread formulations (Acs et al. 1997; Anton and Artfield 2008; Brites et al. 2010; Demirkesen et al. 2010a, b; Demirkesen et al. 2011, 2013, 2014a, b, c; Gambus et al. 2001; Leray et al. 2010; Peressini et al. 2011; Ribotta et al. 2004). The most commonly used hydrocolloids in the gluten-free industry are xanthan gum, guar gum, locust bean gum (LBG), HPMC, and carboxymethylcellulose (CMC).

McCarthy et al. (2005) optimized a gluten-free bread formulation based on rice flour, potato starch, HPMC, and skim milk powder using a response surface methodology. Crumb structure was evaluated using a digital image analysis system. The crumb grain parameters used in this study were as follows: number of small cells ($0.05 < x < 4.00$ mm^2), number of large cells (> 4.00 mm^2), number of cells/cm^2 (including small and large cells), and mean cell area in mm^2. Both sides of three central slices of each loaf were used for crumb grain measurements (60×60 mm cropped scanned images of the crumb), yielding 24 images per formulation in the optimization experiment and 12 images per replicate in the shelf-life trial. It has been stated that increasing HPMC and water level decreased crumb firmness and increased specific volume. However, high levels of HPMC and water had adverse effects on crumb structure by reducing the number of cells per unit area. When optimized bread formulation was used, the resulting bread was found to be comparable with a wheat bread in terms of mean cell area, but was still lower in terms of number of cells/cm^2. Schober et al. (2008) showed that the addition of HPMC resulted in a regular, fine, gluten-free crumb, a round top, and good aeration. HPMC also stabilized gas bubbles. CLSM revealed finer zein strands in the dough when HPMC was present. Sciarini et al. (2010) conducted a study to assess the influence of the addition of different hydrocolloids (carrageenan, alginate, xanthan gum, CMC, and gelatin) on gluten-free batter properties and bread quality and to obtain information about the relationship between dough consistency and bread quality. Images analysis was done using ImageJ software. Cell average area (mm^2) and number of cells per square millimeter were determined (0.15 and 10.00 mm^2 were the lower and upper area limit values, respectively, for being considered as cells by the software). The ratio of small cells ($0.15 < x < 2.00$ mm^2) to large cells ($2.00 < x < 10.00$ mm^2)
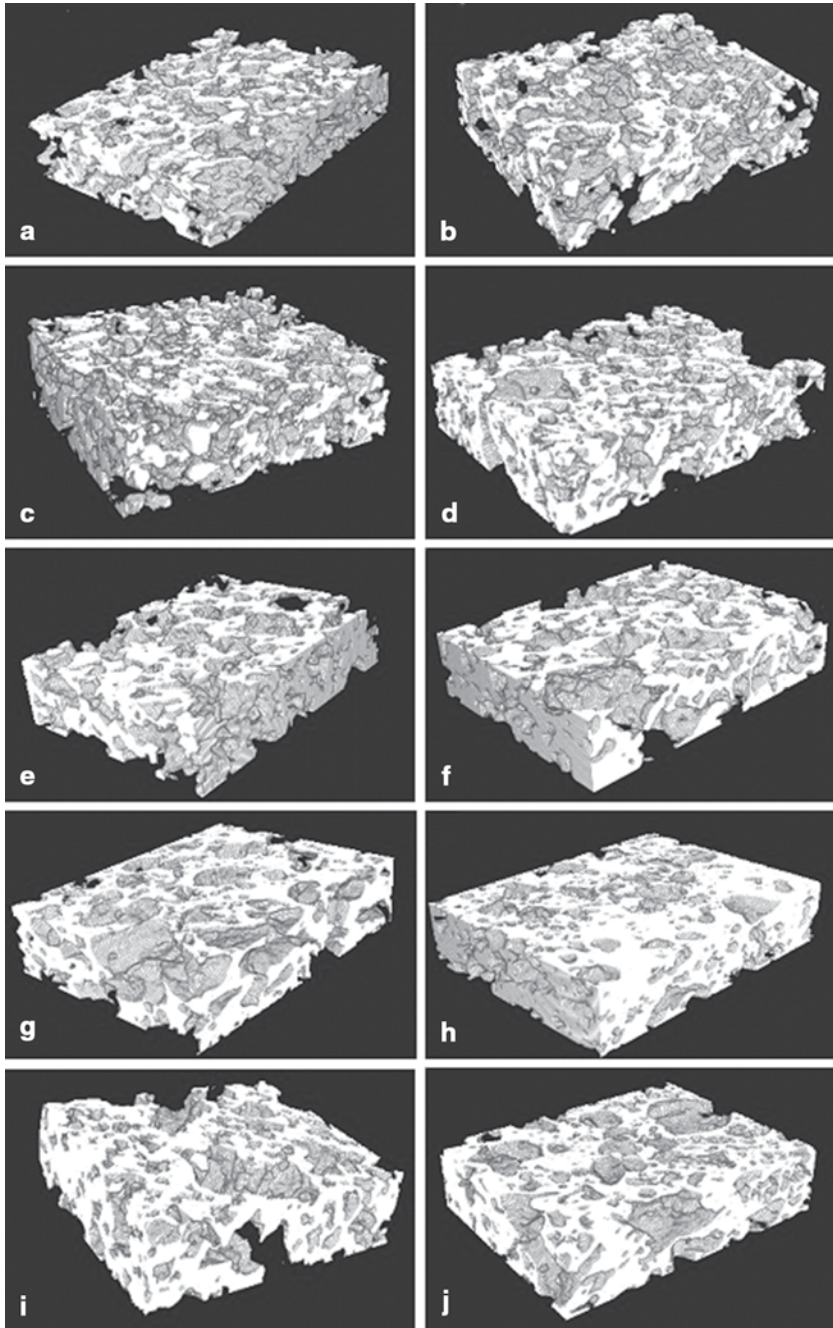
was calculated. The highest consistency and specific volume were observed for xanthan-containing batters and breads. The addition of xanthan gum enhanced crumb, thus leading to the formation of a more aerated structure with increased cell size and reduced shrinkage. Xanthan gum incorporation also decreased crumb firmness and retarded staling.

In addition to hydrocolloids, emulsifiers are also commonly used in bakery products to assist blending and emulsification of ingredients, to enhance the properties of the shortening, and to obtain a softer crumb. Moreover, they enhance dough-handling ability; improve rate of water absorption; provide greater tolerance to resting and fermentation; improve crumb structure and loaf volume; increase uniformity in cell size; advance gas retention resulting in lower yeast requirements, better oven spring, and faster rate of proof; and provide longer shelf-life of bread (Stampfli and Nersten 1995). Although the synergic interaction between hydrocolloids and emulsifiers is well known, there are limited publications on the use of both hydrocolloids and emulsifiers in gluten-free bread formulations (Demirkesen et al. 2010a, b, 2011, 2013, 2014a, b, c; Nunes et al. 2009a; Onyango et al. 2009). Demirkesen et al. (2014b) examined the effects of adding the emulsifier DATEM and different gums (xanthan, guar, LBG, agar, methylcellulose (MC), CMC and HPMC) and gum blends (xanthan–guar and xanthan–LBG) on crumb structure of gluten-free breads by X-ray μCT. In this study, porosity, number of pores, average pore size, aspect ratio, and roundness of pores were used as parameters to describe the internal structure of the crumbs, and the characteristics of the crumb structure were related to the hardness, cohesiveness, and springiness of breads. 2D and 3D X-ray μCT images of gluten-free bread samples prepared with the addition of different gums or different gum blends indicated that in the lack of hydrocolloids and the emulsifier DATEM, control breads had a very open sponge-like structure (Figs. 7.10 and 7.11). The crumb structures of gluten-free breads prepared with MC and agar showed similarity to the control bread crumb structure. It was observed that these breads exhibited heterogeneous structures with lots of void spaces (open pores). The reason for undesirable, heterogeneous crumb appearances of these breads might be related to the nonuniform distribution of open pores as well as the wide distribution of closed cells in breads. In addition, control, MC, and agar were not capable of providing high viscosity and viscoelastic structure to dough during mixing, which might be another reason for their heterogeneous crumb appearance with nonuniformly distributed void spaces (Figs. 7.10a–c and 7.11a–c). On the other hand, except MC and agar, the presence of a relatively higher number of smaller pores of gluten-free breads prepared with additives provided the capture of more gas bubbles and moisture in their closed pores, resulting in finer crumb structure (Figs. 7.10d–j and 7.11d–j). Porosity, number of pores, averages size of pores, aspect ratio, and roundness values of pores are shown in Table 7.2.

Among all gluten-free breads, gluten-free breads prepared with the addition of MC, agar, and no additives had the highest porosity values with the lowest number of pores, which indicated a nonuniform crumb structure with very large pores. The highest average areas of pores observed for these breads were related to the interconnection between all gas cells (Table 7.2, Figs. 7.10a–c and 7.11a–c).
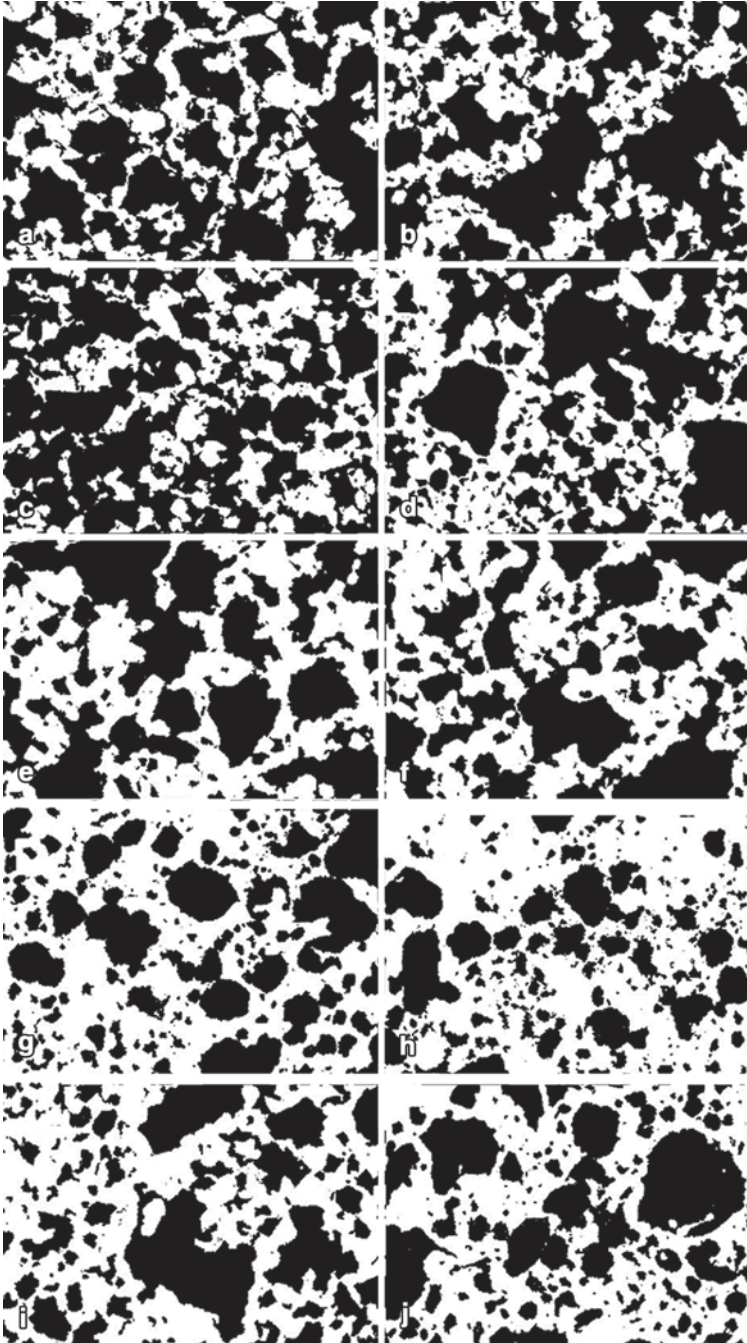
Fig. 7.10 2D X-ray μCT images of gluten-free bread slices prepared with different gums or gum blends. **a** Control breads. **b** Breads prepared with methylcellulose. **c** Breads prepared with agar. **d** Breads prepared with locust bean. **e** Breads prepared with guar. **f** Breads prepared with xanthan. **g** Breads prepared with carboxymethylcellulose. **h** Breads prepared with hydroxypropylmethylcellulose. **i** Breads prepared with xanthan–locust bean gum blend. **j** Breads prepared with xanthan–guar gum blend. (Reprinted from Demirkesen et al. 2014b, copyright 2014, with permission Elsevier)

Fig. 7.11 3D X-ray μCT images of gluten-free bread samples prepared with different gums or gum blends. **a** Control breads. **b** Breads prepared with methylcellulose. **c** Breads prepared with agar. **d** Breads prepared with locust bean. **e** Breads prepared with guar. **f** Breads prepared with xanthan. **g** Breads prepared with carboxymethylcellulose. **h** Breads prepared with hydroxypropylmethylcellulose. **i** Breads prepared with xanthan–locust bean gum blend. **j** Breads prepared with xanthan–guar gum blend. (Reprinted from Demirkesen et al. 2014b, copyright 2014, with permission Elsevier)

Table 7.2 Quantification of the porous structure per slice thickness (0.036 mm) and per identical size (~0.688 cm³) of the gluten-free bread samples prepared with different gums or gum blends. (Reprinted from Demirkesen et al. (2014b), copyright 2014, with permission Elsevier)

Gluten-free bread samples	Porosity values of identical size (~0.688 cm³)		Number of pores per 1.85 cm² area		Average size of the pore (cm²)	
		SE		SE		SE
Control	0.568[a]	1.65	8.56[c]	3.079	0.165[a]	1.20
MC	0.629[a]	2.05	5.10[c]	2.015	0.298[a]	2.07
A	0.602[a]	1.72	3.99[c]	1.091	0.478[a]	4.26
LBG	0.539[b]	1.21	11.0[b]	1.094	0.088[b]	0.19
G	0.510[b]	1.34	11.4[b]	2.096	0.083[b]	0.24
X	0.453[c]	0.98	13.9[a]	2.052	0.055[c]	0.13
CMC	0.471[c]	1.16	15.5[a]	1.092	0.050[c]	0.09
HPMC	0.382[c]	0.87	14.3[a]	2.006	0.040[c]	0.07
X-LBG	0.502[c]	1.76	12.3[a]	2.047	0.071[b]	0.18
X-G	0.423[c]	1.18	14.4[a]	2.073	0.047[c]	0.15

Analysis was done in duplicate. Pores >0.01 cm² considered. Values are means of 100 slices
SE standard error of 100 slices *MC* methylcellulose, *A* agar, *LBG* locust bean gum, *G* Guar gum, *X* Xanthan, *CMC* carboxymethylcellulose, *HPMC* hydroxypropyl methylcellulose
[a, b, c] Formulations having different letters are significantly different ($p \leq 0.05$)

A significant negative correlation between porosity and number of pores ($r = -0.90$) and a positive correlation between porosity and average size of pores ($r = 0.80$) were also found in that study. This result also indicated the noticeable effect of void spaces on porosity of crumb structure. As opposed to breads containing MC and agar and control breads, the lowest porosity, the lowest average pore area, and the highest number of pores were observed for gluten-free breads prepared with the addition of xanthan, CMC, xanthan–guar, xanthan–LBG, and HPMC. A negative correlation observed from the data between number of pores and average size of pores ($r = 0.94$) indicated the finest crumb structure of these breads with the highest number of smaller pores. The aspect ratio of all the bread samples, which expresses the relationship between the width of the pore and its height, was found to be under 2, representing the ellipsoidal nature of the pores. The roundness of pores in gluten-free bread samples prepared with different additives was found to be between 0.559 and 0.637 indicating their noncircular shape.

Recently, enzymes such as amylase, cyclodextrin glycosyltransferases (CGT), transglutaminase (TG), glucose oxidase (GO), laccase, and proteases have been successfully used in the gluten-free baking industry in order to improve dough-handling ability and water-binding capacity as well as texture and shelf life of products. Since the proteins in cereals except wheat gluten do not aggregate to continuous network in dough at room temperature, protein functionality can be modified by cross-linking. Recently, protein-cross-linking enzymes, such as TG, have been used in the gluten-free industry since they are responsible for the formation of a protein network—through the formation of intra- and intermolecular cross-links between the polypeptide chains. TG is responsible for the formation of new covalent cross-links in proteins via lysine and glutamine residues. It can be applied as a protein

modifier, and it is able to modify the functional characteristics of a wide range of proteins, such as casein and albumen from milk, animal protein from eggs and meat, soy protein, and wheat protein (Arendt et al. 2009). Moore et al. (2006) evaluated the impact of TG at different levels (0, 0.1, 1, and 10 U of TG/g of protein) on the quality of gluten-free bread prepared by rice flour, potato starch, corn flour, xanthan gum, and various protein sources (skim milk powder, soy flour, and egg powder). For image analysis, the images of the bread were captured using a flatbed scanner and supporting software. Brightness levels were adjusted, and a square field of view was evaluated for each image, which in turn captured the majority of the crumb area of each individual slice of bread. The crumb grain feature chosen was the number of cells per square centimeter in digital photographs. From the image analysis based on the scanned images, it was observed that TG inclusion increased the number of cells per square centimeter of bread crumbs when milk and egg powder were used. This result suggested an improvement in the crumb structure due to protein cross-linking. Image characteristics of breads were also evaluated by using CLSM. CLSM images indicated that no differences could be detected between the microstructure of batters containing TG and their controls, and no orientated protein network was visible within these batters. The microstructure of the breads indicated that TG application improved continuity in the protein network within skim–milk- and egg-powder-containing gluten-free breads. It was concluded that it is possible to form a protein network in gluten-free bread with the addition of TG, but the efficiency of the enzyme changes depending on both the protein source and the level of TG concentration. To promote protein cross-linking, Renzetti et al. (2008) studied the incorporation of 0.1 and 10 U of TG into six different gluten-free cereals (brown rice, buckwheat, corn, oat, sorghum, and teff). CLSM was used to observe the modifications caused by TG on the microstructure of batters and breads made with brown rice or buckwheat (Figs. 7.12 and 7.13).

The microscopy images of control buckwheat and brown rice batters, which were incubated for 30 min and prepared without addition of any enzyme revealed a homogeneous distribution of proteins in the system (Fig. 7.12). The incorporation of 10 U of TG after 30 min incubation resulted in buckwheat and brown rice proteins appearing to be distributed in aggregates. 180 min of incubation caused larger protein agglomerates formation in the system, and no differences could be detected for the other cereal batters. The incorporation of 10 U of TG enhanced the continuity of the protein phase in breads (Fig. 7.13).

The result of the study indicated that upon the addition of TG, the rheology of batters and the quality of breads from buckwheat, rice, and corn flour improved, but no improvement occurred when oat, teff, and sorghum flours were used (Renzetti et al. 2008). Thus, it was suggested that TG can be successfully applied to gluten-free flours to improve their breadmaking potentials by promoting network formation, but the protein source is a key element in determining the impact of the enzyme. The use of CLSM confirmed the improvements brought by TG on the microstructure of batters and breads. The impact of the different levels of laccase, GO, and protease on the breadmaking performance of gluten-free oat flour was also tested (Renzetti et al. 2010). The addition of laccase and protease enzymes significantly
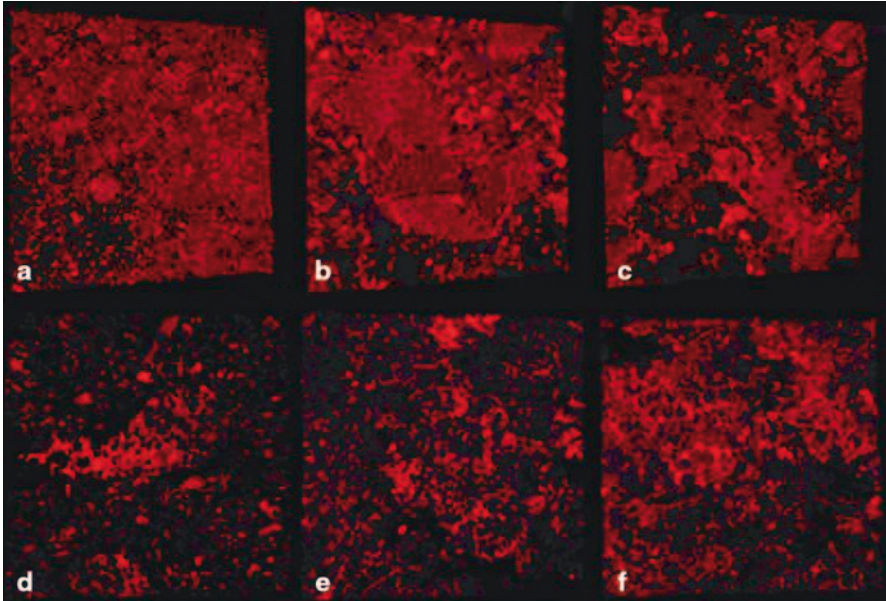
Fig. 7.12 3D elaboration of confocal laser-scanning microscopy (CLSM) images of buckwheat (BW) and brown rice (BR) batters (40× magnification): **a** BW control (0 U of enzyme) after 30 min at 301 C, **b** BW 10 U of transglutaminase (TG) batter after 30 min at 301 C, **c** BW 10-U batter after 180 min at 301 C, **d** BR control (0 U of enzyme) after 30 min at 301 C, **e** BR 10 U of TG batter after 30 min at 301 C, **f** BR 10-U batter after 180 min at 301 C. Proteins appear *red*. With increasing incubation time, the proteins in the batter change from a homogeneous dispersion (**a** and **d**) to protein aggregates (**b** and **c, e** and **f**). (Reprinted from Renzetti et al. 2008, copyright 2008, with permission Elsevier)

enhanced the quality of oat bread as the enzymes increased the specific volume and decreased crumb hardness and chewiness. The improved quality of breads upon the addition of these enzyme preparations was attributed to their noticeable levels of endo-β-glucanase side activity. CLSM of bread crumbs revealed that the addition of GO resulted in larger protein aggregates in the bread crumbs, and these globular aggregates negatively affected the breadmaking performances of oat bread. In contrast, protease addition showed the presence of smaller protein clusters dispersed in the system, resulting in oat breads of the best textural quality. Renzetti and Arendt (2009a) studied the impact of GO and protease on the breadmaking performance of four different gluten-free flours (buckwheat, corn, sorghum, and teff). GO enhanced corn and sorghum bread quality by increasing specific volume and reducing collapsing at the top. The improvements of the bread quality were related to the aggregation of the dispersed protein structures as revealed by CLSM and resulted in enhanced continuity of the protein phase and elastic-like behavior of corn and sorghum batters. In another study of Renzetti and Arendt (2009b), the effect of protease treatment was investigated on the baking properties of brown rice bread.
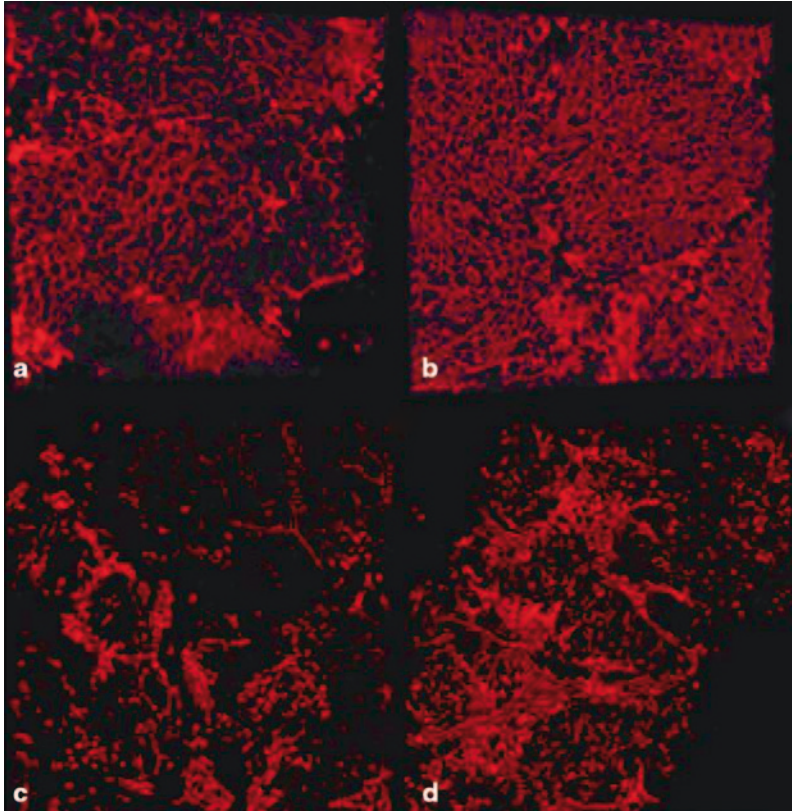
Fig. 7.13 3D elaboration of confocal laser-scanning microscopy (CLSM) images of buckwheat (BW) and brown rice (BR) bread crumb (40 × magnification): **a** BW control bread (0 U of enzyme), **b** BW 10 U of transglutaminase (TG) bread, **c** BR control bread (0 U of enzyme), **d** BR 10 U of TG bread. Proteins are stained *red* together with yeast cells, which appear *round shaped*. For BW, the control bread shows a continuous protein network, characteristic only of BW breads. The addition of the enzyme results in a crumb with a strengthened, finer-meshed protein network. For BR, the control bread shows the absence of any kind of protein network. The addition of the enzyme enhances the continuity of the protein phase. (Reprinted from Renzetti et al. 2008, copyright 2008, with permission Elsevier)

It was shown that protein degradation could be beneficial in improving the bread-making performances of brown rice flour, by increasing the specific volume of the bread and decreasing crumb hardness and chewiness. CLSM images confirmed that the gelatinized starch phase was the main structuring component in protease-treated breads.

In order to improve both the nutritional and functional properties of gluten-free products, the replacement of gluten with protein sources such as dairy, egg, soy, and maize has long been used in gluten-free baking technology (Ahlborn et al. 2005; Gallagher et al. 2003; Marco and Rosell 2008; Nunes et al. 2009b; Schober et al.

2004; Van Riemsdijk et al. 2011). The incorporation of two important milk proteins, caseins and whey proteins, has been established in gluten-free baking industry in order to increase water absorption and, therefore, enhance the handling properties of the batter and bread characteristics. Besides, supplementing the gluten-free formulation with high-protein-content dairy powders also increases the protein content of the breads (Gallagher et al. 2003). Schober et al. (2004) tested the influence of milk proteins and rice starch on crumb structure (pore size, number of pores, and crumb hardness) by using digital image analysis and TPA. Bread slices were scanned by a flatbed scanner and digital image analysis of the crumbs was performed using a digital image analysis system. Mean cell area and total number of cells were selected as crumb structure features. It was found that the addition of milk proteins and rice starch in gluten-free sorghum breads resulted in the formation of more open structure and hence decreases in the number of cells per square centimeter. The more open structure provided less resistance to the probe during TPA, and thus softer textures were obtained from the bread supplement with milk proteins. Moore et al. (2004) produced gluten-free breads using dairy protein sources. Due to its high resolution and ability to scan through thick samples, CLSM proved to be very useful in detecting the desirable protein structure elements of bread crumb. From CLSM images it was observed that the dairy-based gluten-free bread crumb had a network-like structure, which resembled a gluten network in wheat bread crumb. This study showed that the formation of a continuous protein phase and film-like structures was critical for an improved quality of gluten-free bread based on starch retrogradation. Ahlborn et al. (2005) compared the microstructure of wheat bread with low-protein starch bread and with gluten-free bread supplemented with egg proteins and milk proteins by SEM. Both low-protein starch bread and gluten-free bread contained xanthan gum and HPMC. It was observed that both cell diameter and lamellae thickness were larger in the rice and low-protein starch breads as compared to wheat bread. SEM images made it possible to see a web-like structure in gluten dough and gluten bread. On the other hand, in the low-protein starch bread, coalescence of the gas cells as evidenced by larger cell diameters and thicker lamellae was observed. The results of this study demonstrated that the combinations of rice, tapioca, egg, and milk proteins with xanthan gum and HPMC could create a bicontinuous matrix with starch fragments, similar to gluten. Nunes et al. (2009b) indicated the successful use of low-lactose dairy powders in gluten-free recipes. In this study, the impacts of different low-lactose dairy ingredients (sodium caseinate, milk protein isolate, whey protein isolates, and whey protein concentrate) on gluten-free dough rheology and bread quality were determined. Better quality in terms of specific volume and hardness is attained with whey proteins. CLSM images showed that the microstructure of casein-containing breads was dominated by a starch phase, where no protein network could be observed, while the incorporation of whey protein formed a protein network. It has been noted that the differences in thermal stability of casein and whey protein might play a major role in the resulting microstructure of the bread crumbs containing casein or whey protein.

7.6 Methods to Improve the Microstructure of Gluten-Free Products

As stated before, producing high-quality gluten-free products is one of the most challenging issues for cereal technologists and scientists. Up to date, most gluten-free products have been produced by replacing wheat flour by alternative flours as well as using some additives to mimic the viscoelastic properties of gluten. In order to meet the rising demand of celiac patients for high-quality products, sourdough technology, infrared–microwave combination baking, and HHP treatment have also lately been studied as potential methods in gluten-free technology.

7.6.1 Sourdough Technology

Sourdough, which is a mixture of flour and water fermented with lactic acid bacteria (LAB) and/or yeast, is used to improve the quality of wheat breads (Moroni et al. 2009). In order to meet the rising demand of celiac patients for high-quality and healthier products, this old biotechnological process has recently been applied as a new approach in gluten-free breadmaking. It has been stated that the problems associated with gluten-free dough and bread characteristics may be overcome by the application of sourdough. By the application of this technology, the rheological properties of gluten-free dough as well as the quality of gluten-free breads in terms of volume, texture, crumb structure, flavor, and nutritional value may be improved (Moroni et al. 2011; Schober et al. 2007; Zannini et al. 2012a). The positive effects of sourdough on the retardation of the staling process and protecting gluten-free bread from mold and bacterial spoilage have also been demonstrated by researchers (Moore et al. 2007).

Moore et al. (2007) conducted a study on the effects of adding different strains of LAB on gluten-free sourdough, batter, and bread. As controls, chemically acidified (80 % lactic acid and 20 % acetic acid) and nonacidified batters/breads were used. CLSM, which presents advantages to produce optical sections through a 3D specimen without damaging the structure, was used to observe microscopic changes. Digital image analysis was used to characterize the crumb structure. The images of the bread were captured using a flatbed scanner, and the number of cells was chosen as the crumb structure characteristic. CLSM showed remarkable structural changes in sourdough over time due to the action of the LAB. As compared to the initial microstructures of sourdoughs, smaller and more scattered proteins within starch granules were observed in microimages of sourdoughs at 24 h. This result indicated that the protein particles in sourdough-containing breads were degraded over the fermentation process. Since the degradation was found to be less obvious in gluten-free sourdough, it was concluded that gluten-free sourdoughs did not exhibit oriented protein network or degraded network, which is generally seen in wheat-based sourdough systems. The microimages of gluten-free breads indicated

that gluten-free breads prepared with 20% sourdough incorporation had a higher amount of aggregated and larger particles as compared to nonacidified control gluten-free breads. The pore walls in both nonacidified control and gluten-free batters prepared with 20% sourdough incorporation were characterized by the presence of large denatured soy globules and gelatinized starch granules. Moreover, none of the breads had network formation. Therefore, the control and the breads prepared with the addition of 20% sourdough did not indicate any significant difference in terms of loaves volume and height, number of cells, or mean cell area. However, the addition of sourdough delayed the onset of staling, which showed that the addition of sourdough in gluten-free bread formulation may improve the quality of the resulting bread. Schober et al. (2007) used sourdough fermentation in order to improve the quality of gluten-free sorghum breads. Flatbed scanning images were used to examine the microstructure of breads, while CLSM was applied to understand the microstructure that contributes to bread quality. In the case of sourdough breads, the height of breads was significantly improved. This might be due to the stronger starch gel in sourdough breads. For a further understanding of the mechanisms, the authors compared bread crumbs prepared with the addition of HPMC with those of sourdough breads at microscopic level and also included chemically acidified bread crumb to address the effect of acidification alone. CLSM revealed aggregated protein in both gluten-free breads prepared with HPMC and chemically acidified gluten-free breads. In the case of chemically acidified breads, protein aggregation might be attributed to poor solubility of sorghum proteins at acidic pH values. However, in the case of breads prepared with sourdough fermentation, only small isolated patches of protein bodies embedded in matrix protein remained. Thus, the authors suggested that unlike protein bodies, the specific sourdough fermentation of this study could degrade some other proteins, which would otherwise aggregate upon baking as in the case of bread crumb prepared with the addition of HPMC. The authors concluded that as opposed to gluten proteins, aggregated proteins in the case of gluten-free breads prepared with HPMC and chemically acidified gluten-free bread were clearly not associated with improved bread quality. In contrast, proteins in sourdough-containing breads can no longer cross-link, and therefore they do not aggregate upon baking, which may be attributed to the improved quality of sourdough bread.

7.6.2   Infrared–Microwave Combination Baking Technology

Microwave heating offers a number of advantages such as energy efficiency, faster heating, space saving, precise process control, selective heating, and food with high nutritional quality, but microwave-baked products do not meet with consumer acceptance because of their quality problems (Sumnu 2001). Infrared–microwave combination heating provides selectivity that improves moisture distribution inside food by heating the surface of a food faster. Therefore, moisture can be easily removed from the surface of the product, and the food remains crisp (Datta et al.

2005). This technology combines the browning and crisping advantages of near-infrared heating and the time-saving and energy efficiency advantages of microwave heating. The advantages of infrared–microwave combination heating over microwave heating have been realized over the past few years. Previous attempts have been made to overcome the problems associated with microwave baking, and in these studies the combination of microwave heating with infrared heating has been successfully used by several researchers (Datta et al. 2007; Demirekler et al. 2004; Keskin et al. 2004, 2005; Sakiyan et al. 2011; Sumnu and Sahin 2005). This baking technology may also be a good alternative to conventional baking to produce gluten-free products with comparable quality but in shorter times (Demirkesen et al. 2011, 2013, 2014a, c; Sumnu et al. 2010; Turabi et al. 2008). In recent studies, the images obtained by a flatbed scanner and SEM were used to obtain qualitative and quantitative information about the macro- and microstructure of gluten-free bread and cake products (Demirkesen et al. 2013; Turabi et al. 2010). In the study of Demirkesen et al. (2013), both quantitative and qualitative information were obtained related to the macro- and microstructure of gluten-free breads prepared with rice and chestnut flour and xanthan–guar gum blend–DATEM mixture addition, and baked in conventional and infrared–microwave combination ovens. Quantitative information about crumb cells of the scanned images and SEM micrographs at a magnification of 20× were obtained using Image J software. Binarized images were used for quantitative analysis of pores. Crumb structures of gluten-free bread samples were analyzed by calculating pore area fraction, pore size distribution, and mean roundness values by this software. As discussed before, the macrostructure images of gluten-free breads, which were obtained by the help of a scanner, showed that gluten-free breads baked in infrared–microwave combination oven were looser and more porous (Fig. 7.4). The puffing effect that occurred during infrared–microwave combination baking also increased the pore area fraction values and the total number of pores of gluten-free breads (Fig. 7.5). It has been demonstrated that baking method also affected the pore size distribution of breads significantly. Infrared–microwave combination baking increased the number of small pores in rice breads but large pores in chestnut-rice breads (Fig. 7.5). The quantitative data obtained by SEM images showed that infrared–microwave combination baking resulted in higher pore area fraction values (Fig. 7.6). It has been shown that gluten-free breads baked in an infrared–microwave combination oven had more starch granules, which did not lose their identity and did not disintegrate completely (Fig. 7.7). The authors hypothesized that shorter processing time during infrared–microwave combination baking might affect swelling and gelatinization and lead to incomplete disintegration of starch granules. Turabi et al. (2008) also used flatbed-scanned and SEM images to obtain quantitative and qualitative information on the macro- and microstructure of gluten-free rice cakes containing different types of gums and baked in different ovens. Macrostructure of gluten-free cakes indicated that infrared–microwave combination baking provided more porous structure to cakes, which may be caused by higher pressure gradient during the infrared–microwave combination baking mechanism. Infrared–microwave combination baking increased the pore area fraction values and the total number of pores of gluten-free

cakes and resulted in the formation of pores having larger area. From SEM images it was also observed that conventionally baked cakes showed more starch granule deformations (Fig. 7.3), while cakes baked in an infrared–microwave combination oven had granular starch residues and deformed starch structure together. The studies of Demirkesen et al. (2013) and Turabi et al. (2010) demonstrated that there was a close relationship between the quantitative information obtained from the macro- and microstructure of gluten-free products.

7.6.3 High Hydrostatic Pressure Technology

HHP technology, which consists in applying pressures between 100 and 1000 MPa to foods, creates new structures and textures by modifying functional properties of biopolymers such as proteins and starches (Zannini et al. 2012b). Therefore, HHP may offer a good alternative for enhancing the viscoelasticity of gluten-free doughs and the quality of resulted bakery products. Moreover, this alternative nonthermal technology may prevent the necessity of the use of additives in gluten-free products. It has been stated that HHP treatment induces starch swelling and gelatinization without the disruption of granule integrity that offers advantages over products such as high moisture retention, improvement of gas retention capacity of the batter, hence increased volume, and enhanced shelf life of baked products. HHP also alters the structure and functional properties of proteins which may be due to its pronounced effect on the folded–unfolded equilibrium of proteins, on the weakening of electrostatic and hydrophobic interactions, and on the thiol–disulphide exchange reactions resulting in the disruption of non-covalent interactions within proteins, with subsequent reformation of intra- and intermolecular bonds within or between proteins (Vallons et al. 2011). Recent studies showed that HHP could be utilized as a potential tool to improve the functionality of gluten-free products (Hüttner et al. 2009; Stolt et al. 2001; Vallons et al. 2010, 2011; Vallons and Arendt 2009).

In the study of Vallons and Arendt (2009), the effect of HHP treatment on structural properties of sorghum starch was evaluated by using rheological and microscopic techniques. In addition, pressure-induced gelatinization of sorghum starch was compared with heat-induced gelatinization of sorghum starch. CLSM was used to determine the loss of birefringence during gelatinization. CLSM pictures showed loss in birefringence of starch granules at high-pressure and temperature treatment but preservation of granular structure upon pressure and temperature treatment. In order to obtain finer details about the effect of pressure and temperature on the starch granule morphology, SEM analysis was used. Both CLSM and SEM images revealed that granular integrity was retained within the gelatinization interval in the presence of pressure and temperature changes. It was also observed that there were no significant differences between the microstructure of the pressure-treated samples and the temperature-treated samples within the gelatinization intervals. In order to evaluate the potential use of pressure-treated sorghum in the production of sorghum breads, another study was conducted on the application of high-pressure

processing of sorghum batters (Vallons et al. 2010). The impacts of high pressure on the structure of sorghum breads were examined by SEM. The untreated batter and batter treated at low pressures did not show visual differences. However, at higher-pressure treatment, starch granules became swollen and deformed, while their granular structure remained intact. The results of this study suggested that pressure treatment has the potential to delay staling in gluten-free products. In another study, the effect of HHP on the microstructure properties of three gluten-free batters made from buckwheat, white rice, and teff was examined (Vallons et al. 2011). SEM was used to observe the changes in the microstructure of the batters induced by the HHP treatment. Clearer changes were observed in the buckwheat images. Although, most granules retained their granular shape after treatment with 600 MPa, they were swollen, deformed, and collapsed. In the case of the microstructure of the gluten-free batters made from rice and teff, the effects of HHP on the granule structures were found to be less obvious; however, the structure of the batters looked smoother after HHP treatment. These structural changes in batters showed the noticeable effect of HHP treatment on the modifications occurring in starch and proteins. Hüttner et al. (2009) conducted a study on the effects of HHP on the microstructure of oat batters. SEM and bright-field microscopy were used to examine the structural changes of batters through HHP treatment. It was observed that both proteins and starch granules were affected by the HHP treatment, and higher pressures resulted in more noticeable changes in the structures of batters. Both bright-field microscopy and SEM revealed that higher pressures led to swollen and slightly disintegrated starch granules of batters and a more continuous distribution of proteins (Fig. 7.1). In another study, Hüttner et al. (2010) examined the use of HHP as a tool to improve the breadmaking performance of oat four. SEM and bright-field microscopy were used to investigate the effect of HHP treatment on the oat batter constituents. Both protein and starch were affected by HHP treatment, and more evident alterations were observed with higher-pressure treatment. Although the majority of oat starch granules retained their granular structure, modifications in their surface appearance such as swelling and slight disintegration of some granules were found to be visible. As compared to the untreated oat batter, more continuous distribution of proteins with higher-pressure treatment was observed. It was suggested that HHP treatment could be successfully applied for the improvement of oat flour functionality and bread quality by selecting the suitable pressure levels and amounts of flour. Protein modification and changes in the moisture distribution positively influenced the quality by retarding the staling of oat bread.

7.7 Conclusion

Gluten is responsible from the formation of the basic structure necessary for the appearance, texture, flavor, and shelf life of many baked products; therefore, eliminating gluten from the products causes quality problems. Replacing gluten in gluten-free products requires the use of alternative flour types to wheat flour

as well as several functional ingredients such as starches, hydrocolloids, emulsifiers, enzymes, protein, and/or fiber sources. In addition, sourdough breadmaking technique, infrared–microwave combination baking, and HHP treatment have been used as potential methods in gluten-free technology. The physicochemical, sensory, nutritional and, transport properties of foods are largely affected by the structure of foods varying from molecular to macroscopic levels. Although quantitative description of baked products is generally done by image analysis methods based on macroscopic techniques, microstructural analysis provides more detailed information about quality attributes of multicomponent sophisticated food products.

Recently, LM, SEM, CLSM, MRI, and X-ray μCT have been the popular techniques used in image acquisition for the quality of dough/batters and gluten-free baked products. Since CLSM has the ability to produce optical sections of a 3D specimen without damaging the structure and provides specific staining of components, it has been extensively used as a valuable tool in gluten-free baked products in order to provide deeper understanding of the role of ingredients and their interactions in the microstructure of gluten-free batters and breads. SEM imaging technique has recently been used to obtain quantitative information about crumb characteristics of gluten-free breads and cakes. As a powerful imaging technique, X-ray μCT has been recently used for quantitative characterization of gluten bread crumbs by creating 3D representation of bread structure from 2D image slices. In the future, widespread application of novel and powerful imaging techniques for the examination of the microstructure of gluten-free products can provide deeper knowledge about the quality attributes of gluten-free baked products.

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