Journal of Stored Products Research 47 (2011) 372–377

Contents lists available at ScienceDirect

Journal of Stored Products Research

journal homepage: www.elsevier.com/locate/jspr

Synchrotron based infrared imaging study of compositional changes in stored wheat due to infection with *Aspergillus glaucus*

Chandra B. Singh [a], Digvir S. Jayas [a,*], Ferenc Borondics [b], Noel D.G. White [c]

[a] Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB R3T 5V6, Canada
[b] Canadian Light Source Inc., Saskatoon, SK S7N 0X4, Canada
[c] Agriculture and Agri-Food Canada, Cereal Research Centre, Winnipeg, MB R3T 2M9, Canada

ARTICLE INFO

Article history:
Accepted 18 July 2011

Keywords:
Wheat
Fungal damage
Grain quality
Infrared microspectroscopy
Synchrotron

ABSTRACT

Fungi are one of the serious causes of spoilage in stored grain including wheat. *Aspergillus* spp. is one of the most common storage fungi that spoils stored wheat. The damage caused by fungi adversely affects the quality of wheat and reduces its nutritional composition. Present methods of analysing chemical composition of wheat and other cereals using wet chemistry are destructive and use bulk grain and thus rely on bulk analysis. Grains, similar to other biological materials, are highly non-homogenous, hence, bulk analysis which causes damage to intrinsic structure of kernels, cannot be used for characterization of single kernels and studying the compositional distribution within a single kernel. In the present work, synchrotron based high resolution infrared imaging was used to study the compositional changes in stored wheat due to fungal damage. Clear differences between healthy and damaged wheat endosperm spectra were observed at peaks around 1740, 1595, and 1250 cm^{-1}. The difference in the absorption of infrared radiation was likely caused due to reduced lipid (1740 cm^{-1}), lignin (1595 cm^{-1}) and cellulose (1250 cm^{-1}) content in damaged wheat endosperm.

1. Introduction

Wheat is one of the most important cereal grains grown worldwide and consumed in various forms such as bread, pasta, noodles, pastries, cakes, and cookies (Cornell and Hoveling, 1998). A wheat kernel is composed of three major subunits: pericarp, endosperm, and germ. The pericarp is made of outer epidermis layer, hypodermis, several thin-layer cells (cross cells, tube cells), and seed coat or testa (Posner and Hibbs, 2005). Next to the pericarp is the aleurone layer and the inner part is the starchy endosperm. Bran is made up of both pericarp and aleurone layer and accounts for nearly 14.5% of the total kernel mass. The endosperm forms nearly 83% of the kernel and is used to produce wheat flour. In the endosperm, starch granules embedded in protein matrices are filled in cells made of cellulose materials. The endosperm is composed of three types of cells namely peripheral, prismatic, and central. The size and shape of cells and content of starch granules and proteins within the cells vary in the endosperm region. It has high nutritional composition of protein, pantothenic acid, riboflavin, niacin, pyridoxine and thiamine. The germ, which accounts for nearly 2.5% of kernel mass, is removed before grinding operation in wheat flour milling. The quality of the end-product made from wheat flour or semolina is highly dependent on wheat grain used in milling. Healthy wheat kernels will result in high quality flour, hence, better end products. Wheat needs to be stored for a significant period after being harvested at grain storage/grain handling facilities before it is transported to local millers or shipped to international destinations. Shipping might also take up to months and the grain is also stored at milling facilities before it is being milled. Fungal growth during the storage of grain is a serious cause of spoilage in grains. Fungal damage of wheat kernels causes germination loss, discolouration, dry matter loss, increase in free fatty acids, heating, mustiness, and occasional production of mycotoxins (CGC, 2006). Madhyastha et al. (1993) observed compositional changes (lipid and starch) in fungal infected wheat and barley kernels by quantifying the grain composition using wet chemistry. Lipid content was most significantly affected with a dramatic reduction. Starch content was reduced by only a small amount and almost no change was observed in the protein content and its amino acid composition. Though the compositional changes were identified, their spatial distribution within a kernel was not known. Traditional wet chemical analyses (starch estimation by enzymatic analysis, protein by Kjeldahl, and lipid by crude ether extract) of grains are destructive and destroy the original grain structure and its compositional distribution.

* Corresponding author.
E-mail address: Digvir_Jayas@Umanitoba.ca (D.S. Jayas).

0022-474X/$ – see front matter
doi:10.1016/j.jspr.2011.07.001

The kernel tissue is non-uniform; however, wet analysis gives bulk (homogenous) composition of kernels. Near-infrared (NIR) hyperspectral imaging provides spatially resolved spectral information of a kernel and has been used to detect fungal-damaged wheat kernels (Singh et al., 2007). The NIR hyperspectral imaging technique can discriminate healthy and damaged wheat kernels, but despite the strong absorption of NIR radiation by grains, broad and overlapping NIR spectral bands make spectral assignment to a composition very difficult. The mid-IR spectral analysis has many advantages over NIR in terms of sensitivity, spectral assignment, resolution, and quantification (Wilson and Goodfellow, 1994). Mid-infrared spectroscopy has been used to detect *Fusarium* fungi on corn kernels (Kos et al., 2003). Synchrotron based mid-IR imaging has the capability to provide quantitative, compositional, and structural information and distribution of chemical constituents and functional groups in a biological tissue at diffraction-limited spatial resolution over a wide spectral range (Yu, 2007). Therefore, the objective of this research was to study the compositional changes in stored wheat endosperm due to fungal damage using the mid-infrared beamline at the Canadian Light Source, Saskatoon, SK.

2. Materials and methods

2.1. Sample preparation

Canada Western Red Spring (CWRS) wheat sample was artificially infected with storage fungi of *Aspergillus glaucus* group using the method described in Singh et al. (2007). The *A. glaucus* fungus was cultured on old wheat and the infected kernels were plated on filter paper saturated with 7.5 ml aqueous NaCl in petri dishes. After a week, pure fungal lines from infected seeds were placed on potato dextrose agar for 7 days at 30 °C. Then the agar with mould was placed in 200 ml sterilized water in a plastic bottle with one drop of Tween 20 and shaken. One kilogram sample of CWRS wheat was surface sterilized by soaking in 1% sodium hypochlorite for 5 min and then thoroughly rinsed in sterilized water and placed on a paper towel to remove excess water on the surface. The sterilized wheat was filled in plastic bag and mould mixed with water was poured into bag and shaken for thorough mixing. After mixing, the bag was tightly closed with cotton placed on the opening and kept in a control chamber at 70% relative humidity and 30 °C temperature for 2 weeks. After 2 weeks, the infected wheat sample was moved to a freezer and kept at −20 °C for sectioning. The control sample was sterilized and put directly into the freezer to avoid any fungal growth before sectioning.

Wheat samples were cut into 8 μm thin sections using cryomicrotome at the Richardson Center for Functional Foods and Nutraceuticals, University of Manitoba, Winnipeg, MB. The frozen wheat kernels were first cut longitudinally into two halves. Then samples were placed in a supporting plate embedded in a supporting medium Tissue Tech (OCT) and frozen. The freshly frozen samples in supporting plates were mounted on the cryomicrotome and cut into 8 μm thin sections. Three controlled and three damaged wheat kernels were sectioned and 2–3 best sections were collected from each kernel. These samples were collected onto 25 × 1 mm BaF_2 windows (ISP Optics Corp, Loveland, CO) (thaw mounted), placed in a Beta Cell Sample Holder (International Crystal Labs, Garfield, NJ), covered with another 25 × 1 mm BaF_2 window after placing a Teflon spacer and sealed by finger tightening the nylon hex male sample retainer. The upper nylon retainer was removed just before the scanning to provide enough space for microscope focussing. Most of the kernels were highly damaged by the fungi so the kernels with less visible damage were used for sectioning to get better sections as it was very difficult to section highly damaged kernels.

2.2. Mid-infrared (MIR) beamline

The mid-infrared beamline (01B1-1) at the Canadian Light Source (CLS), Saskatoon, SK, was used to measure the wheat samples in transmission mode. The beamline is equipped with a Fourier Transform IR (FTIR) spectrometer and an infrared microscope to supply diffraction-limited spatial resolution (Bruker Optics IFS 66v/S FTIR with Hyperion 2000 confocal microscope). The spectra were collected in the 900–4000 cm^{-1} range at 4 cm^{-1} resolution with 64 scans co-added in each raster point, however, only the 1800–1100 cm^{-1} region was used in the analysis as this region has absorption bands related to lipid, protein, and cellulose. Region below 1100 cm^{-1} was not considered in the analysis due to high noise in the data. Reference spectra were also collected on the clear area of the BaF_2 window at the same resolution with 128 scan co-additions. The size of aperture was 10 μm × 10 μm. Data collection, system control, processing, and analysis were done using the OPUS software (Bruker Optics Inc., Billerica, MA). Multivariate statistical analysis and k-means clustering were accomplished using MATLAB (Mathworks Inc., Natick, MA, USA).

3. Results and discussion

Spectra from both healthy and damaged wheat kernels were extracted. The colour image of healthy wheat section (10 × 10 μm resolution) and absorbance spectra extracted from different regions of healthy wheat kernel are shown in Figs. 1 and 2, respectively. The major absorption bands found in this work were also compared with previous studies (Table 1). The spectra of the outer layer (epidermis and other cells) (grid 1) and seed coat (grid 13) showed similar shape and hence similar composition. These spectra had high absorptions near 1735 cm^{-1} which was contributed by the lipid absorption of stretching vibration of saturated carbonyl ester C═O bonds (Barron and Rouau, 2008). Seed coat contains the

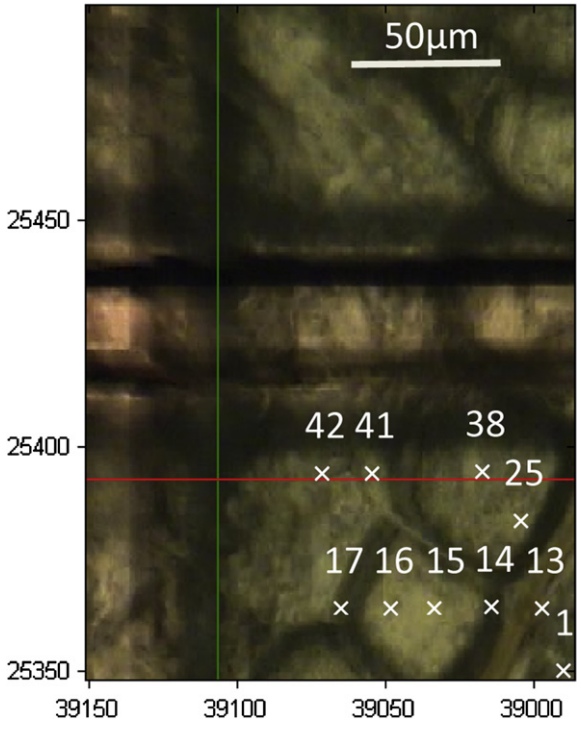

Fig. 1. Longitudinal section (8 μm thick, 10 μm × 10 μm aperture size) of healthy wheat showing pericarp (1), aleurone layer (15) and endosperm (42) and marked grid points.