Food Research International 100 (2017) 74–86

Contents lists available at ScienceDirect

Food Research International

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

Review

Non-destructive techniques for the detection of fungal infection in cereal grains

Irene Orina[a,b], Marena Manley[a], & Paul J. Williams[a,*]

[a] Department of Food Science, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch 7602, South Africa
[b] Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000, Nairobi, Kenya

ARTICLE INFO

Keywords:
Cereal grains
Fungal detection
Mycotoxins contamination
Non-invasive techniques

ABSTRACT

Infection of cereal grains by fungi is a serious problem worldwide. Depending on the environmental conditions, cereal grains may be colonised by different species of fungi. These fungi cause reduction in yield, quality and nutritional value of the grain; and of major concern is their production of mycotoxins which are harmful to both humans and animals. Early detection of fungal contamination is an essential control measure for ensuring storage longevity and food safety. Conventional methods for detection of fungal infection, such as culture and colony techniques or immunological methods are either slow, labour intensive or difficult to automate. In recent years, there has been an increasing need to develop simple, rapid, non-destructive methods for early detection of fungal infection and mycotoxins contamination in cereal grains. Methods such as near infrared (NIR) spectroscopy, NIR hyperspectral imaging, and electronic nose were evaluated for these purposes. This paper reviews the different non-destructive techniques that have been considered thus far for detection of fungal infection and mycotoxins in cereal grains, including their principles, application and limitations.

1. Introduction

Cereal grains constitute major sources of dietary energy and protein for humans and livestock. Maize, wheat, rice and barley represent the key staple cereal grains worldwide (Haard, 1999). Other cereal grains such as sorghum, oats, rye and millet are also relatively important. A chronic problem with cereal grains worldwide is the infection by fungi, belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria* (Wagacha & Muthomi, 2008). The growth of fungi in the grains results in discolouration; contributes to heating and losses in dry matter through utilisation of carbohydrates as energy sources; degrades lipids and proteins or alter their digestibility; produces volatile metabolites giving off-odours; causes loss of germinability, hence affect their use as seed; and loss of baking and malting quality (Christensen, 1973). Of major concern, however, is the production of toxic fungal secondary metabolites, mycotoxins, which pose health hazards to humans and animals (Hussein & Brasel, 2001; Naresh, David, & Sanchis, 2004). The major mycotoxins that occur in cereal grains include aflatoxins produced by *Aspergillus*; ochratoxins produced by *Penicillium* and *Aspergillus*; and fumonisins, deoxynivalenol, trichothecenes and zearalenone produced by *Fusarium* (Pascale, 2009; Pereira, Fernandes, & Cunha, 2014). Although these toxins are typically present in levels as low as parts per million (ppm) or parts per billion (ppb), acute or chronic exposure to mycotoxins has been associated with immuno-suppression (Wagacha & Muthomi, 2008), impaired growth in children (Gong et al., 2004), malnutrition (Ramjee, Berjak, Adhikari, & Dutton, 1992), liver cancer (Williams et al., 2004) and death in some incidences (Lewis et al., 2005). Early detection and, if possible, removal of fungal contaminated grains is an important control measure in ensuring storage longevity, seed quality and food safety (Pasikatan & Dowell, 2001).

Traditional methods used to detect fungal infection and/or mycotoxin contamination include: culture and colony techniques (Gourama & Bullerman, 1995); chemical analyses (Lin & Cousin, 1985); enzyme linked immunosorbent assay (ELISA) (Meirelles et al., 2006); adenosine triphosphate (ATP) bioluminescence, the polymerase chain reaction (PCR) method (Boutigny et al., 2012), chromatographic techniques (Pereira et al., 2014) and biosensors (van der Gaag et al., 2003). Although reliable, specific and sensitive; these methods are time-consuming and labour-intensive; they involve tedious sample preparation which lead to destruction of the sample. Hence, efforts have been made to develop simple, rapid, accurate and non-destructive methods for detection of fungal contamination in cereal grains. Non-destructive techniques for fungal detection and mycotoxin contamination have thus become a major area of interest recently.

The emergence of modern imaging acquisition techniques, in conjunction with image processing methods has offered many potential

* Corresponding author.
E-mail address: pauljw@sun.ac.za (P.J. Williams).

http://dx.doi.org/10.1016/j.foodres.2017.07.069
Received 26 May 2017; Received in revised form 31 July 2017; Accepted 31 July 2017
Available online 02 August 2017
0963-9969/

avenues for non-destructive evaluation of agricultural products (Chen & Sun, 1991). Techniques such as colour imaging (Tallada, Wicklow, Pearson, & Armstrong, 2011), Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) (Greene et al., 1992), electronic nose (Paolesse et al., 2006), near infrared (NIR) spectroscopy (Berardo et al., 2005), hyperspectral imaging (Siripatrawan & Makino, 2015), thermal imaging (Chelladurai, Jayas, & White, 2010), neutron tomography (Cleveland et al., 2008) and X-ray micro-computed tomography (Narvankar, Singh, & White, 2009) have been investigated for their application of fungal and/or mycotoxin detection in grains. The present paper provides a review of the non-destructive techniques that have been utilized to determine fungal infection and mycotoxin contamination (where applicable) in cereal grains along with their limitations.

2. Colour imaging

Colour is a vital visual attribute of cereal grains used in grain inspection and grading. Characterisation of different grains and their varieties is based on kernel colour and discolouration due to grain damage (Luo, Jayas, & Symons, 1999). Colour images are described either using the primary colours red, green and blue (RGB system) or by converting the RGB component value of the object image to the main factors of human colour sensation namely hue, saturation and intensity (HSI system) (Gunasekaran, 1996; Majumdar, 1998). A colour imaging system essentially consists of a sample holding platform, digital camera for capturing the image, image capture board (frame grabber or digitiser) for digitising the image, light source for proper illumination, and computer hardware and software to process the images (Fig. 1) (Vithu & Moses, 2016). A digital image is acquired by incident light in the visible spectrum falling on a partially reflective surface of the sample. The scattered photons are gathered up in the camera lens and then converted to electrical signals by either a vacuum tube or charge-coupled device (CCD), and saved on a hard disk for further image display and image analysis (Wu & Sun, 2013).

A system consisting of a high-pixel resolution CCD chip and associated hardware is the commonly used method for generating digital images (Patel, Kar, Jha, & Khan, 2012). The acquired digital images of the object are then pre-processed for the purpose of enhancing the image quality or for removing irrelevant sources of variation (Vithu & Moses, 2016). Image acquisition and image analysis are the two vital steps for the application of colour imaging. A high-quality image can help to reduce the time and complexity of the subsequent image processing steps. The colour features of an object are extracted by examining every pixel within the object boundaries (Du & Sun, 2004). Colour image features including the mean, histograms of the red, green and blue colour, intensity, range of hue, saturation and textural features derived from grey level co-occurrence matrices (GLCM) are extracted using appropriate image processing algorithms (Gunasekaran, 1996). These features are then used as input to statistical discriminant classifiers to differentiate the grains. The advantage of colour imaging is the possibility of analysing each pixel of the entire surface of the grain and quantifying surface characteristics and defects (Brosnan & Sun, 2004; Du & Sun, 2004).

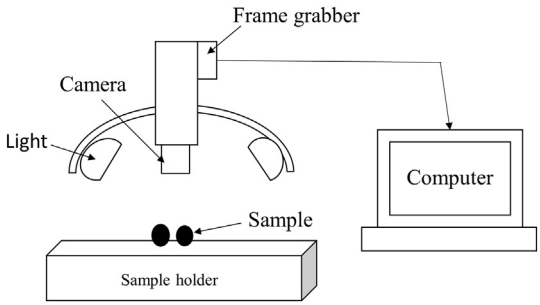

Fig. 1. Schematic diagram of a typical colour imaging system, adapted from Vithu and Moses (2016). Digital images of the sample are acquired using the camera and processed further in the computer to extract useful information.

Fungal damage of cereal grains is usually associated with discolouration and fissures on the seed coats, and the degree of discolouration varies with the type of fungi (Wang, Dowell, Ram, & Schapaugh, 2004). Colour imaging has been used to detect fungal infection in wheat (Jirsa & Polišenská, 2014; Singh, Jayas, Paliwal, & White, 2012) and maize (Tallada et al., 2011). A summary of the different non-destructive techniques that have been used in the study of fungal infection in cereal grains is given in Table 1. Singh et al. (2012) extracted a total of 179 features (colour and textural) from the colour images of fungal infected and healthy wheat kernels. Two-way classification algorithms were developed using the top ten selected features of the 179 colour and textural features. The top ten features were selected due to their high discrimination capability using the STEPDISC (step-wise discrimination analysis) procedure in SAS (Version 9.1, SAS Institute Inc., Cary, NC, USA). Healthy kernels were correctly classified with 94.3, 90.3, and 89.3% accuracy by linear discriminant analysis (LDA), quadratic discriminant analysis (QDA) and Mahalanobis discriminant classifiers, respectively (Singh et al., 2012).

The performance of colour imaging to discriminate maize kernels infected by eight fungal species at different levels of infection was evaluated by Tallada et al. (2011). Colour images were used to develop linear and non-linear prediction models using LDA and multi-layer perceptron (MLP) neural networks. Higher levels of infection had better classification accuracies of 81 to 89%. Colour imaging was not able to classify mould species well. The use of principal component analysis (PCA) on image data did not improve the classification results. The LDA models performed as well as the MLP models, with or without the use of PCA (Tallada et al., 2011). One likely constraint in the use of colour imaging is the limited electromagnetic range in which optical data can be obtained, and physical discolouration in the kernels is the only source of variation. Therefore, this technique could possibly work well at a rather advanced stage of infection (Tallada et al., 2011).

3. Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS)

Photoacoustic spectroscopy (PAS) is a non-destructive technique that directly measures energy absorbed by the sample rather than what is transmitted or reflected (Ryczkowski, 2010). PAS operating in a Fourier transform mid-infrared (FTIR) system, is based on the photoacoustic effect caused when a modulated infrared beam from the spectrophotometer impinges on a sample surface in a sealed cell purged with inert helium (Ryczkowski, 2010; Sivakesava & Irudayaraj, 2000). Helium is commonly used because of its superior thermoacoustic coupling properties (Jiang & Palmer, 1997). The light absorbed, increases the temperature of the sample and the heat migrates to the gas/sample interface and produces a pressure wave in proportion to the absorbance by the sample. The resultant pressure signal is then detected by a sensitive microphone and converted into a wavenumber versus absorbance intensity spectrum (Anderson et al., 2013). A schematic diagram of a PAS cell is shown in Fig. 2. The shape of the photoacoustic spectrum is independent of the morphology of the sample under investigation (Ryczkowski, 2010). Among the key advantages of FTIR-PAS is the depth profiling capability for non-destructive evaluation of successive layers below the sample surface (Sivakesava & Irudayaraj, 2000). FTIR-PAS in food related research is limited, however it has found application in food characterisation (Irudayaraj, Sivakesava, Kamath, & Yang, 2001), microorganism detection (Irudayaraj, Yang, & Sakhamuri, 2002), classification of rapeseed varieties (Lu, Du, Yu, & Zhou, 2014), analysis of potato chips (Sivakesava & Irudayaraj, 2000), pea seeds (Letzelter, Wilson, Jones, & Sinnaeve, 1995) and coffee (Gordillo-Delgado, Marín, Cortés-Hernández, Mejía-Morales, & García-Salcedo, 2012).