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Detection of Mold-Damaged Chestnuts by Near-Infrared Spectroscopy

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ABSTRACT

Mold infection is a significant postharvest problem for processors of chestnuts (*Castanea sativa*, Miller). Fungal disease causes direct loss of product or reduced value due to the lower-quality grade of the chestnut lot. In most cases, fungal infection is not detectable using traditional sorting techniques. In this study, the feasibility of using Near-Infrared (NIR) spectroscopy to detect hidden mold infection in chestnut was demonstrated. Using a genetic algorithm for feature selection (from 2 to 6 wavelengths) in combination with image analysis grading and Linear Discriminant Analysis (LDA), Quadratic Discriminant Analysis (QDA) or *k*-Nearest-Neighbors (*k*NN) routines, classification error rates as low as 2.42% false negative, 2.34% false positive, and 2.38% total error were achieved, with an Area Under the ROC Curve (AUC) value of 0.997 and a Wilk's λ of 0.363 ($P < 0.001$). A Savitzky–Golay first derivative spectral pretreatment with 33 smoothing points was used. The optimal features corresponded to *Abs*[1118 nm], *Abs*[1200 nm], *Abs*[1626 nm], and *Abs*[1844 nm].

The results represent an important step toward the development of a sorting system based on multispectral NIR bands, with the potential to rapidly detect and remove chestnuts contaminated by fungi and reduce the incidence of hidden mold in chestnut lots.

1. Introduction

Chestnut (*Castanea sativa*, Miller) is a seasonal fruit found in many parts of the world, although the majority of commercial production is in Europe and Asia. The highly perishable nature of the nut creates significant postharvest challenges for industry. Chestnut quality depends on external factors such as color, shape, size, surface blemishes and surface mold, as well as on internal factors including physiological disorders and freshness. The predominant factors which reduce fresh-chestnut quality are microbiological internal decays, which are primarily related to mold, rot, and insect damage. The worldwide mold agents in chestnut are *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp., *Phomopsis castanea*, *Acrospeira mirabilis* and *Sclerotinia pseudotuberosa* (syn. *Ciboria batschiana*, *Sclerotinia batschiana*; anamorphic from *Rhacodiella Castanea*, syn. *Myrioconium Castanea*) (Donis-González et al., 2012). Fungal growth starts in the larval cavity and the nut may become infected before harvesting. Some molds infect at various stages during fruit development, while others grow during storage (Jermini et al., 2006; Sieber et al., 2007). Insect feeding and respiration activity promote favorable conditions for fungal growth (Pasikatan and Dowell, 2001). Although damage increases with the amount of mold, even a very small infection compromises fruit quality. Thus, fungal contamination of chestnuts can lead to substantial economic losses for farmers, traders and food handlers. In fact, fungal disease can cause direct loss of product or reduced value due to the lower-quality grade of the lot. Moreover, the presence of fungal infection is a food safety issue due to the potential for subsequent mycotoxin development (Tallada et al., 2011). Severity of mold infections vary depending on many factors such as: relative humidity, temperature, precipitation and other weather factors; biotic and abiotic constraints; cultivar; and storage condition. Under the right conditions, the incidence of damage can be substantial (Moscetti et al., 2014). During a wet harvest season, mold damage can affect over 30% of the total harvest.

Moldy chestnut is rarely detectable until the fruit is cut open, and reliable detection of mold at early growing stages has not yet been accomplished. Consequently, various pre-storage treatments

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have been adopted to reduce the likelihood of mold contamination, such as warm and cold baths (or 'water curing') (Botondi et al., 2009; Cecchini et al., 2011). In addition, a salt solution flotation system is generally used to separate lower density fruit generally associated with microbial decay or infestation. However, the flotation method has low sensitivity, since density differences might be caused by differences between cultivars, variability among individual chestnuts, void spaces, or thick shell or pellicle embedded in the sound kernel. Consequently, the flotation system tends to discard excessive amounts of sound product. Moreover, it is slow and cumbersome (Donis-González et al., 2012).

Improved mold control strategies are required for commercial chestnuts (Sieber et al., 2007). Removal of diseased fruit prior to storage would be most desirable. Unfortunately, no satisfactory method has been reported for the rapid detection of mold in postharvest chestnuts. However, online non-destructive grading and sorting lines have been implemented for the inspection of internal fruit quality parameters including those based on electrical properties, Near-Infrared (NIR) spectroscopy (Saranwong et al., 2011), sound/noise/vibration (Liljedahl and Abbott, 1994), ultrasound, nuclear magnetic resonance (Zhang and McCarthy, 2013), X-ray (Haff and Toyofuku, 2008; Donis-González et al., 2012), volatile emission and others (Butz et al., 2005).

NIR spectroscopy has a number of desirable qualities including minimal sample preparation and a wide range of applications. NIR spectra can provide useful information about the interior of a sample as it can penetrate more than 9 mm with proper sample preparation and specific wavelength region (i.e. 800–1100 nm) (Moscetti et al., 2014). NIR spectroscopy can be applied in a variety of configurations, including transmission, reflection and interactance modes. NIR spectroscopy has been used to demonstrate the potential for rapid detection of internal disorders (Pasikatan and Dowell, 2001; Guthrie et al., 2004; Wang et al., 2010, 2011; Saranwong et al., 2011; Moscetti et al., 2013, 2014) and fungal contamination (Davies et al., 1987; Kiskó, 1998; Dowell et al., 1999; Delwiche, 2003; Wang et al., 2004; Berardo et al., 2005; Börjesson et al., 2007; Shenderey et al., 2010; Delwiche et al., 2011; Tallada et al., 2011) in various food commodities and seeds. However, online inspection systems for mold contamination of fresh produce, including chestnuts, are still not in common use. NIR techniques have the potential to benefit the fresh food market by reducing the risk of buying poor quality products and consequently allowing compliance with consumer demands for uniform high quality products (Butz et al., 2005).

The objective of the present study was to investigate the detection of internal mold-damaged chestnut by using NIR spectroscopy and identifying combinations of features (based on absorbance of light in the NIR band from 1100 to 2300 nm) having optimal discriminatory ability and testing different classification methods.

2. Materials and methods

2.1. Sample preparation

Twenty kg (1344 fruit) of chestnuts (*C. sativa* Miller cv. Marrone Fiorentino) were manually harvested from a local orchard (Central Italy) early in October, 2012, and immediately stored at 26 ± 0.5 °C (laboratory room conditions) for 24 h to allow for temperature and moisture equilibrium prior to NIR spectroscopy. Following the NIR measurement, the fruit were dissected to classify the chestnuts in terms of absence of infections (sound product) and presence of infections (unsound product). Unsound samples were graded via digital imaging analysis. The severity of fungal damage was classified according to the following criteria: Grade #1 (slightly infected: from 2% to 30% kernel discoloration), Grade #2 (medium infected: from 31% to 70% kernel discoloration) and Grade #3 (severely infected: from 71% to 100% kernel discoloration).

2.2. NIR spectral acquisition

A Luminar 5030 Acousto-Optic Tunable Filter-Near Infrared (AOTF-NIR) Miniature 'Hand-held' Analyzer (Brimrose Corp., Baltimore, USA) was used to perform the measurement. The instrument was allowed to warm up for at least an hour to reach a stable state. The instrument was equipped with reflectance post-dispersive optics, a pre-aligned dual beam lamp assembly, and an indium gallium arsenide (InGaAs) array (range 1100–2300 nm, 2 nm resolution) with an integrating time of 60 ms. Each acquired spectrum was the average of 10 scans. The scanning of chestnuts was randomly sequenced. The reference spectrum was automatically measured by the instrument as described by Cayuela and Weiland, (2010). For each sample, spectra were acquired for each of three different scanning positions as indicated in Fig. 1a, using the configuration shown in Fig. 1b. Diffuse reflectance spectra were acquired using SNAP! 2.04 software (Brimrose Corp.). Reflectance spectral data were transformed into absorbance ($A = \log R^{-1}$) using R 2.15.2 statistics software. Immediately after the spectral acquisition, chestnuts were dissected to visually determine presence or absence

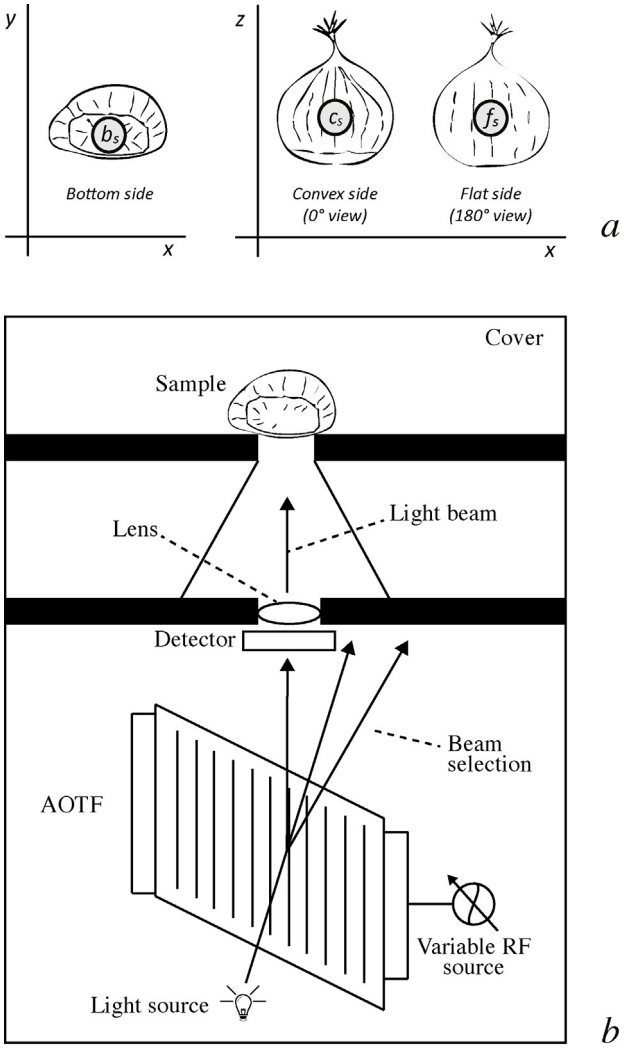

Fig. 1. Side view of the three points of spectra acquisition (a). Experimental setup for acquiring NIR spectra (b).