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Monitoring Lactic Acid Fermentation in Media Containing Dandelion (*Taraxacum officinale*) by FTIR Spectroscopy

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Abstract

Fourier-transform infrared (FTIR) spectroscopy is considered to be a comprehensive and sensitive method for detection of molecular changes in cells and media. In the present study, FTIR spectroscopy was employed as an easy, rapid and reliable technique to evaluate the lactic fermentation of *Lactobacillus casei* on a model de Man, Rogosa and Sharpe (MRS) medium with or without the addition of dandelion extract (DE). Dandelion, due to its high content in fructans, can be used as an additional carbon source in lactic fermentation. Lactic fermentation in a dandelion extract, using the FTIR fingerprint as a qualitative and semi-quantitative assay for lactic acid production was monitored. Specific bands of carbohydrates in the fingerprint region (1200-900 cm^{-1}) and their shifts indicated the hydrolysis and metabolism during fermentation. The band at 1336 cm^{-1} may be considered a sensitive marker for the identification of *L. casei* during fermentation, while the dandelion extract showed a unique characteristic peak at 1436 cm^{-1}. The results proved that the species were detectable and that significant spectral differences existed between fermented samples in media with or without dandelion addition. Representative peaks of bacteria and dandelion appeared in the spectra of a mixture of bacteria and dandelion. The peaks were evident in the samples taken using the model MRS media from the beginning of fermentation as opposed to at the end of fermentation.

Keywords: alternative nutrients, bacteria, biomarkers, hydrolysation, infrared

Introduction

Detection and identification of microorganisms by spectroscopy are of great value because of the sensitivity, rapidity, low cost and simplicity of the techniques. Furthermore, spectroscopic techniques provide a wealth of qualitative and quantitative information about a given sample. The infrared spectrum of any compound is known to give a unique 'finger print' (Naumann *et al*., 1991).

Lactic acid bacteria are among the most important microorganisms used in the production of fermented foods. Traditionally, lactic acid fermentation has been characterised based on the morphology of the bacteria and from chemical tests. However, phenotypic procedures are time consuming, involve the use of a large variety of methods, and the results at the strain level are often inconclusive (Lefier *et al*., 2000). An alternative method, apart from being fast and at least relatively inexpensive, must meet the following criteria: simple, reliable, uniform, and highly specific (i.e., differentiation/identification of bacteria at the species or subspecies level) (Naumann *et al*., 1991). Fourier transform infrared (FTIR) spectroscopy has been recognised by many researchers (Amiel *et al*., 2000; Lefier *et al*., 2000; Leopold *et al*., 2011a, 2011b; Naumann *et al*., 1991) as a method fulfilling these requirements.

Dandelions are one of the most common and recognisable weeds and are found in almost every part of the world (Koo *et al*., 2004; Yarnell and Abascal, 2009). The *Taraxacum* spp. includes the species *Taraxacum japonicum*, *Taraxacum mongolicum* and *Taraxacum officinale*, although the differences in the composition of each of these species remain vague (Jeon *et al*., 2008). This plant has been used in Traditional Chinese Medicine (TCM) and traditional Native American Medicine for its medicinal activity for the treatment of diseases ranging from diarrhea and digestive diseases to hepatitis and cancer (Jeon *et al*., 2008; Yarnell and Abascal, 2009). More specifically, they have traditionally been used for treatment of breast cancer and leukaemia (Sweeney *et al*., 2005). Dandelions are perennial weeds composed of a variety of chemical compounds that are thought to act alone or in combination the medicinal activity of dandelions is derived from a variety of chemical components that act alone or in combination (Schutz *et al*., 2006a). The chemical composition of dandelion extracts has been evaluated, and some of the important components of the extract include sesquiterpene lactones and phenylpropanoids, which are believed to have anti-inflammatory, anti-oxidative and anticancer properties leading to the array of observed effects of dandelion extracts (Yarnell and Abascal, 2009). Other components have not been fully characterised, and therefore, their activities remain unknown (Schutz *et al*., 2006b). There are limited scientific studies investigating the anticancer activity of dandelion extracts and very little is known about the mechanism of action; reports on the effect of dandelion extracts and the production of cytokines

have remained ambiguous to date (Kim *et al*., 2000; Koo *et al*., 2004).

In addition to starch, fructans are the most abundant nonstructural polysaccharides found in a wide range of plants. Inulin is a polydispersed fructan consisting mainly of β (2, 1) fructoysl-fructose links that are terminated by a sucrose residue (De Leenheer, 1996). It serves as a storage polysaccharide in many members of Liliaceae, Amaryllidaceae, Gramineae, Asteraceae etc. and is accumulated in the underground roots and tubers of several plants including Jerusalem artichoke (*Helianthus tuberosus*), chicory (*Cichorium intibus*), dahlia (*Dahlia pinnata*), and dandelion (*Taraxacum officinale*) (Gupta and Kaur, 1997; Trojanova *et al*., 2004). Dandelion (*T. officinale* syn. *T. officinale* ssp. *vulgare*) is a flowering plant of the family Asteraceae. It is a biennial herbaceous plant native to temperate areas, and it contains large amounts of inulin (12-15%) and oligofructans in its tap roots (Schutz *et al*., 2006a; Van Loo *et al*., 1995). Due to their high content of fructans, dandelions can be used as an additional carbon sources in lactic fermentation.

This paper describes and characterises the lactic acid fermentation of *L. casei* on MRS model media with and without the addition of dandelion extract, using FTIR spectroscopy to identify specific markers during the fermentation processes.

Materials and methods

Inoculums preparation

Lactobacillus casei was obtained from THT SA Science Park of the University of Gembloux, Belgium. A vial of freeze dried probiotic bacteria, was inoculated into 5 ml of MRS (de Man, Rogosa, Sharpe) broth (Merck, Germany) and incubated at 37°C for 24 h.

Dandelion preparation

Dandelion roots were purchased from local farmers and were ground in the lab using a grinder. The powder obtained was added to the MRS media at a concentration of 10% (v/w).

Tab. 1. Abbreviations used for trial samples

Trials	Abbreviation
Dandelion in MRS media	1
Dandelion in MRS media at the beginning of fermentation processes of *L. casei*	2
MRS media at the beginning of fermentation processes of *L. casei*	3
Dandelion in MRS media at the end of fermentation processes of *L. casei*	4
L. casei suspension	5
MRS model media at the end of fermentation processes of *L. casei*	6
Dandelion in water	7
MRS model media	8

Fermentation process

Fermentations were carried in a 250 mL laboratory flask at 37°C on a shaker set to 200 rpm. The control medium was MRS broth (Merck, Germany). The flask was sterilised at 121°C for 15 minutes. Aliquots of the fermentation liquid were taken every 2 h to fingerprint specific biomarkers during fermentation. The dandelion root powder was purchased from a shop and is presently available on the market. The codifications of fermentation trials are shown in Tab. 1.

FTIR Analysis

FTIR spectra using attenuated total reflectance (ATR) and an internal reflection accessory made of composite zinc selenide (ZnSe) and diamond crystals were obtained on a Schimatzu IR Prestige- 21 spectrometer. Each spectrum was registered from 4000 to 500 cm^{-1}. The FTIR spectra were recorded for all samples in parallel with controls. Three spectra were acquired for each trial variant at room temperature. Each spectrum was composed of an average of 128 separate scans. The measuring time was approximately 9 minutes per sample (n=3), depending on the number of scans per spectrum. Accordingly, as the average number of scans increased, the measuring time increased.

Results and discussion

FTIR spectra of different conditions of fermentation

Fig. 1 shows the general FTIR spectra of analysed samples and presents four absorption areas: 900-1200 cm^{-1} (F), 1200-1500 cm^{-1} (II), 1500-1800 cm^{-1} (III), 2800-3000 cm^{-1} (IV). Samples obtained from different conditions of fermentation were examined by FTIR spectroscopy to find specific spectroscopic biomarkers for rapid identification and discrimination between *L. casei* fermentation on MRS model media and MRS media with dandelion.

Results presented in Fig. 1 show the FTIR spectra of the *L. casei* strain in different fermentation conditions. Despite the general similarity between the spectra of these fermentation conditions, there is a unique spectrum for each one with specific differences compared to the others. These results provide a preliminary indication for possible spectral parameters for identification of *L. casei* and dandelion. These results are in agreement with previously published results that showed a unique 'fingerprint' for fermentation condition out of a large number of bacterial strains (Maquelin *et al*., 2003).

According to Chiş *et al.* (2011), specific markers were identified for carbohydrates. In Fig. 2, the fingerprint region (900-1200 cm^{-1}) includes characteristic glucose bands at 994, 1032, 1079, 1150 cm^{-1}, and the highest intensity is near 1032 cm^{-1}, a primary characteristic of this compound. In the case of fructose, specific marker are located at 966, 1068, 1089 cm^{-1}.

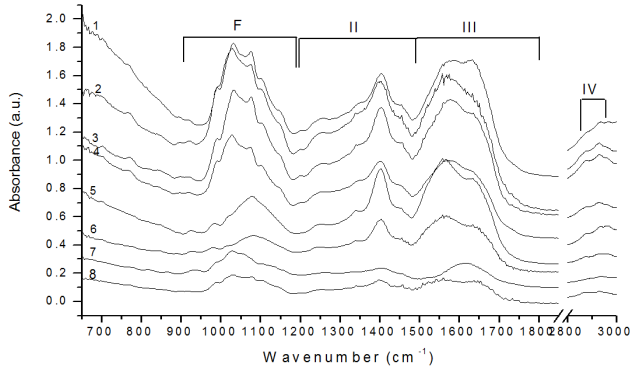

Fig. 1. The general FTIR spectra (3000-650 cm^{-1}) indicate four specific regions for samples 1-8. F- fingerprint region (900-1200 cm^{-1}), II (1200-1500 cm^{-1}), III (1500-1800 cm^{-1}), IV (2800-3000 cm^{-1}). The sample abbreviations were displayed in Tab. 1

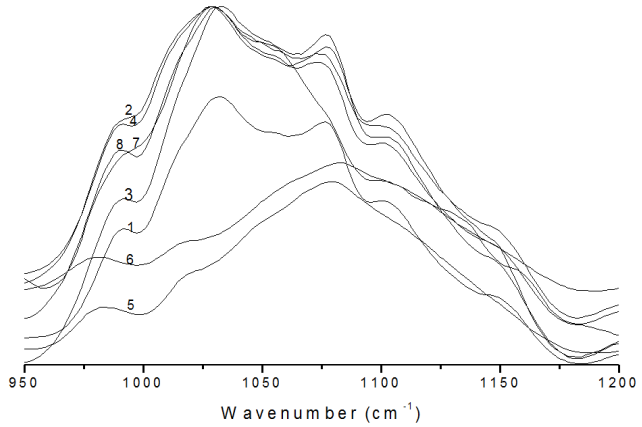

Fig. 2. FTIR spectra (950-1200 cm^{-1}) for fingerprint region` of samples 1-8. The sample abbreviations were displayed in Tab. 1

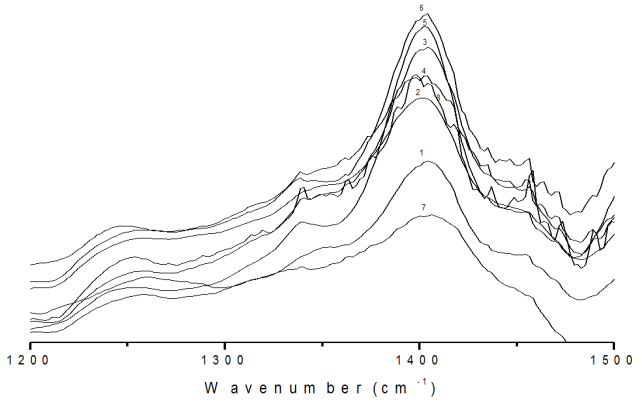

Fig. 3. FTIR spectra (1200-1500 cm^{-1}) for region II specific for carbonyl groups in samples 1-8. The sample abbreviations were displayed in Tab. 1

Bands of inulin that represent the prebiotic substrate for lactic acid fermentation of *L. casei* are present in the fingerprint region; characteristic bands with maximum absorption at 979, 1125, 1178 cm^{-1} are present. In Fig. 2, samples taken at the end of fermentation from MRS media containing dandelion (Sample 4) exhibit sharp peaks at 995, 1073, 1103, 1147 cm^{-1} compared with media taken at the beginning of fermentation (Sample 2). In samples without added dandelion, taken at the end of fermentation (Sample 6), the specific peaks located at 991, 1032, 1104, 1147 cm^{-1} are missing, but these peaks are present in samples that were taken at the beginning of fermentation (Sample 3). The 1080 cm^{-1} signal is present in all fermented samples, suggesting that they are a specific biomarker for *L. casei*.

By comparing all the spectra, it is possible to point to several spectral peaks that could be considered unique for bacteria and dandelion. For *L. casei* spectra (Sample 5), the dominant band at 1554 cm^{-1} was attributed to protein amide I and II bands (Dukor *et al*., 2001). The shoulder at approximately 1650 cm^{-1} was attributed to lipid C=O stretching vibrations (Dukor *et al*., 2001). The band at 1465 cm^{-1} was assigned to the CH_2 bending mode of the cell lipids. The band at 1401 cm^{-1} represents asymmetric CH_3 bending modes of ethyl moieties in proteins (Wong *et al*., 1993; Stuart, 1997). The band at 1356 cm^{-1} represents the C-O stretching vibration of COO^- (Maquelin *et al*., 2001) and is assigned to lipids (Wong *et al*., 1993), and the band at 1347 cm^{-1} represents the C-H bending mode of CH_2 (Brandenburg and Seydel, 2001). According to previous studies (Dukor *et al*., 2001), the remaining IR bands were assigned as follows: the peak at 1082 cm^{-1} was attributed to PO_2^- asymmetric and symmetric stretching vibrations of phospholipids. The peak at 1044 cm^{-1} resulted from the overlap of several bands, including absorption due to the vibration modes of CH_2OH and the C-O stretching vibration coupled to the C-O bending mode of cell carbohydrates (Yang *et al*., 1995). All samples containing *L. casei* have a clear and significant peak at 1336 cm^{-1}, which is missing in samples with dandelion (Fig. 3).

All tested samples that contain dandelion have a unique peak at 1436 cm^{-1}that is missing in samples with bacteria without added dandelion (Fig. 3). The band located at 1396 cm^{-1} represents groups of proteins and lipids. Furthermore, all samples containing bacteria show a shoulder peak at 1456 cm^{-1}. This band, as mentioned above, was attributed to proteins (Wong *et al*., 1993). Specific markers from cell wall fatty acids were located at 2840 and 2950 cm^{-1}. All the medicinal activity of dandelions is derived from a variety of chemical components that act alone or in combination bacterial samples show a sharp peak at 2964 cm^{-1}, whereas all samples without *L. casei* show only a moderate shoulder at this area.

Conclusions

The present study examined the potential of FTIR spectroscopy for easy and rapid discrimination between different stages of lactic acid fermentation on model MRS media and MRS with dandelion. Specific bands of carbohydrates in the fingerprint region (1200-900 cm^{-1}) and their shifts indicated the hydrolysis and metabolism during fermentation. According to the FTIR absorption peaks for glucose (at 1032 cm^{-1}), fructose (at 1068 cm^{-1})

and inulin (at 1125 cm^{-1}) that appeared during fermentation, were identified changes in the shape and intensity of these peaks in the region 1200-900 cm^{-1} compared to control. The band at 1336 cm^{-1} may be considered a sensitive marker for the identification of *L. casei* during fermentation, while the dandelion extract showed a unique characteristic peak at 1436 cm^{-1}. These preliminary results offer interesting information and FTIR spectral indications for lactic fermentation of plant substrates. Developing specific biomarkers by FTIR spectroscopy could be very important for future investigations of *L. casei*-mediated fermentation.

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