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Metabolic profiling of yeast culture using gas chromatography coupled with orthogonal acceleration accurate mass time-of-flight mass spectrometry: Application to biomarker discovery

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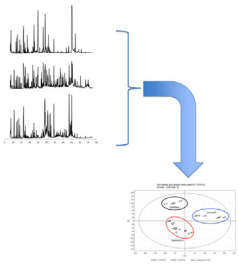
HIGHLIGHTS

- Method described for microwave-assisted derivitization of extracts from commercial yeast cultures.
- Procedure for GC–time of flight mass spectrometry analysis of extraction solutions.
- Factor analysis characterization of extracted solutions, leading to identification of important groups of metabolites.
- Identification of potential biomarkers linking yeast culture to application as feedstock supplement.

GRAPHICAL ABSTRACT



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ABSTRACT

Yeast and yeast cultures are frequently used as additives in diets of dairy cows. Beneficial effects from the inclusion of yeast culture in diets for dairy mammals have been reported, and the aim of this study was to develop a comprehensive analytical method for the accurate mass identification of the 'global' metabolites in order to differentiate a variety of yeasts at varying growth stages (Diamond V XP, Yea-Sacc and Levucell). Microwave-assisted derivatization for metabolic profiling is demonstrated through the analysis of differing yeast samples developed for cattle feed, which include a wide range of metabolites of interest covering a large range of compound classes. Accurate identification of the components was undertaken using GC-oa-ToFMS (gas chromatography-orthogonal acceleration-time-of-flight mass spectrometry), followed by principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) for data reduction and biomarker discovery. Semi-quantification (fold changes in relative peak areas) was reported for metabolites identified as possible discriminative biomarkers (p-value <0.05, fold change >2), including D-ribose (four fold decrease), myo-inositol (five fold increase), L-phenylalanine (three fold increase), glucopyranoside (two fold increase), fructose (three fold increase) and threitol (three fold increase) respectively.

1. Introduction

The microorganism yeast has played a significant role in human culture over thousands of years, due to its application in both traditional and modern biotechnology for the production of foods and

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beverages, and more recently enzymes, fine chemicals and pharmaceutical reagents [1,2]. There are many yeast-based products available, each having major chemical and functional differences which produce unique products with their own unique properties. Yeast has also been fed to animals for more than a hundred years, either in the form of yeast fermented mash produced on the farm (yeast by-products from breweries or distilleries), or as commercial yeast products specifically produced for animal feed.

Today, the practice of adding antibiotics to livestock diets to enhance production efficiency has declined. As a result there has been an increase in research aimed at developing alternatives with particular emphasis on the potential use of natural feed additives, one of which is yeast [3]. Yeast products are fed to high producing dairy cows on more than half of the dairy farms in the USA [4]. Yeast cultures consist of a complex mixture of the products of yeast fermentation, residual yeast cells (*Saccharomyces cerevisiae*) and culture media. Active dry yeasts (ADY) are also used; these products consist of purified dried cells, which have a viability of $15–25 \times 10^9$ colony forming units (CFU) per gram. Three physical forms of ADY are commercially available: tunnel-dried yeast, fluid-bed-dried yeast and rotoluver-dried.

Several yeast products on the market have nuances in their manufacturing process that may have an influence on performance; however, very few studies have been conducted to compare yeast culture in the same experimental environment [5]. For example, Diamond V XP, Yea-Sacc and Levucell yeast cultures have been the subject of extensive research. These studies found that Diamond V XP in particular improved dry matter intake, milk production [6–8] and feed conversion efficiency [9], which has a positive effect on herd profitability even though changes in production or feed intake may be very slight. Kamande et al. conducted a study on the efficacy of dietary supplementation with yeast culture for grazing cows and for calves [3]. The study involved a review on the effects of Diamond V XP yeast on dairy cows and calves. The trials were carried out in Victoria, Australia and it was found that XP supplementation increased milk production of Friesian cows by $2.2\,L\,d^{-1}$, while milk fat and protein content did not differ. In vivo and in vitro studies indicate that yeast products differ in terms of their efficacy. Alshaikh et al. compared the effects of Diamond V XP and Yea-Sacc, a live yeast product, on the performance of mid-lactation dairy cows [10]. The trial found both yeast products to increase production efficiency but Diamond V XP had the greatest effect. Furthermore Bernard et al. conducted a field trial in Florida in which 5472 Holstein cows were fed XP or Yea-Sacc. Diamond V XP increased milk production to a greater extent than Yea-Sacc did; these results concur with those of Alshaikh et al.

Analysis of metabolites in yeast is important as some metabolites present in the yeasts can have a positive impact on stimulating bacterial growth in the digestive tract and optimizing feed intake to animals. In recent years yeast culture has been subjected to metabolite profiling as an aid to clarify the potential effects and gain further understanding of quality assurance. Due to their complex metabolism, yeast organisms are known to produce a broad range of metabolites of different classes (e.g. fatty acids, sugar, amino acids, etc.) at very different concentrations. The composition of the metabolome can vary greatly, depending on the organism analyzed; *S. cerevisiae*, is the most prominent yeast used and has an estimated 600 metabolites [11]. In order to obtain precise qualitative and quantitative results to broaden the understanding of yeast, these metabolites have to be profiled accurately, with accurate identifications a necessity. For many years yeast has been at the forefront of research in modern genetics, molecular biology and cell biology, yet relatively little attention has been paid to its chemical composition and to sample preparation procedures. The analysis of metabolite profiles in a yeast culture can be performed using a wide range of analytical techniques. Most yeast metabolite analyses are undertaken using gas chromatographic (GC) methods due to the sensitivity and low detection limits, selectivity (separation factor) and small sample amounts required. GC–mass spectrometry (MS) can be used to analyze a wide range of derivatized non-volatile and volatile metabolites. An alternative to GC–MS is direct infusion (DI)-MS which can be used to identify the many metabolites rapidly in samples in order to obtain a general metabolic profile of the yeast. Developments in atmospheric pressure ionization allow reliable clean mass spectra to be obtained without the need for a chromatographic separation. This can be achieved in a short time period by directly infusing a biological sample into the MS, without the need for derivatization which adds to the sample preparation time [12–15]. Additionally, there are many alternative and complementary techniques which could be used in the metabolic profiling of complex samples, such as: GC–MS/MS (and QQQ) (gas chromatography with tandem mass spectrometry), GC × GC-ToFMS (comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry), LC-ToFMS (liquid chromatography with time-of-flight mass spectrometry), LC–MS/MS (Q/TOF, QQQ, Orbitrap, etc.) (liquid chromatography with tandem mass spectrometry), LC × LC-ToFMS (comprehensive two-dimensional liquid chromatography with time-of-flight mass spectrometry) and others.

Table 1
Summary of yeast extracts analyzed.

Sample type	Number of samples
Diamond VXP June 2007	4
Diamond VXP July 2007	5
Diamond VXP August 2007	4
Levucell	4
Yea-Sacc	6
Total	23

In this study an analytical method for the accurate mass identification of the 'global' metabolites in order to reliably differentiate different yeasts at different growth stages (Diamond V XP, Yea-Sacc and Levucell) is investigated. Identification and subsequent quantification of the components was undertaken using GC-accurate mass-ToFMS followed by principal component analysis with the main aim being to study different yeast cultures for cattle feed using an accurate mass methodology for possible metabolite biomarker discovery.

2. Methods

2.1. Samples

Twenty-three samples of commercial yeast culture were obtained and maintained at 4 °C until required for analysis. The sample types included: Diamond V XP yeast culture, cultivated in three different months (June, July, August of 2007) at different temperature condition, Levucell SC 20 and Levucell SC (OU) and Yea-Sacc yeast cultures (Table 1).

The main ingredient in the yeast cultures includes *S. cerevisiae* yeast which is grown on a media of ground yellow corn, hominy feed, corn gluten feed, wheat middlings, rye middlings, malt, corn syrup and cane.

2.2. Reference compounds, extraction and microwave derivatization parameters

2.2.1. Reference compounds

The compounds and reagents were all of analytical grade except where otherwise stated. Isoleucine, proline, propionic acid, alanine, serine, methionine, glucose and malic acid were purchased