Institute of Brewing & Distilling

Received: 9 June 2013 Accepted: 30 June 2013 Published online in Wiley Online Library: 15 August 2013

(wileyonlinelibrary.com) DOI 10.1002/jib.83

3-OH oxylipins in *Saccharomyces cerevisiae*

It has been established that cell binding factors in yeast that contribute to flocculation in brewing occur owing to three phenomena: zymolectin binding, hydrophobic interactions and, to a lesser degree, surface charge neutralization *(1)*. Changes in cell surface hydrophobic interactions, in particular, have been known to be involved in flocculation for some time and in fact our laboratory has previously determined that two strains of brewing yeast cells reach maximum flocculation potential with a concurrent increase in cell wall hydrophobicity *(1,2)*. Furthermore, during an investigation of oxylipin distribution in brewing yeasts, it was shown that a particular hydrophobic carboxylic acid, 3-hydroxy oxylipin (3-OH oxylipin), was localized to the cell wall at flocculation onset *(3)*. Thus, the level of 3-OH oxylipins showed strong potential as a putative predictor of flocculation.

It was the general working hypothesis of our recent study that changes in cell wall hydrophobicity, which is an important contributing force to yeast cell flocculation, are due to oxylipin formation. While our laboratory has conducted extensive work to date on flocculation and measuring cell wall hydrophobicity, there have been no studies that correlate flocculation, cell wall hydrophobicity and cell wall oxylipin concentration as a function of fermentation time. Other research groups have investigated 3-OH oxylipin levels in different species of brewer's yeast, notably the Kock Laboratory, and they were able to detect two 3-OH oxylipins, in particular, 3-OH 8:0 and 3-OH 10:0 *(3,4)*. However, to our knowledge other laboratories have not investigated *Saccharomyces cerevisiae* 3-OH oxylipins nor monitored their concentration over the course of a fermentation cycle.

Our research was conducted in two main phases. In the first phase, the SMA strain of *Saccharomyces cerevisiae* was grown in yeast extract, peptone and dextrose (YEPD) broth to validate the experimental techniques and to investigate the merits of whole cell fatty acid analysis vs cell wall-associated fatty acid analysis. It was hypothesized that cell rupture and subsequent isolation of the cell wall ghosts would concentrate the cell wall-associated lipids. In the second phase, the yeast was grown by the miniature fermentation assay protocol *(5)* to more closely mimic industrial beer fermentations.

For all sample types, lipids were isolated using a hexane–isopropanol (HIP) extraction *(6)* and fatty acid methyl esters (FAME) were prepared using a standard acid-catalysed transesterification protocol. FAME were analysed by GC-MS with a FFAP column (nitroterephthalic acid-modified polyethylene glycol phase, 30 m, i.d. 0.32 mm). A mixed bacterial acid methyl esther (BAME) standard containing 3-OH 12:0 and 3-OH 14:0 was used to determine the chromatographic properties of the oxylipins, the characteristic fragments of the 3-OH structure in GC-MS, and to ultimately aid in detecting 3-OH 8:0 and 3-OH 10:0 specifically.

In both YEPD broth and the miniature fermentation assay *(5)*, flocculation and cell surface hydrophobicity increased over the course of the fermentation. It is notable that this is the third industrial lager strain to exhibit this behaviour.

Examination of the BAME standards revealed an obvious fragment of *m/z* 103 in both 3-OH 12:0 and 3-OH 14:0, representing the $CH_3COOCHOH$ fragment that was first reported by Vesonder *et al. (7)*. However, no peaks were identified with GC-MS in any yeast sample with that fragment in regular full-scan mode. When the more sensitive selective-ion monitoring for the 103 *m/z* was employed, we still failed to identify any fatty acids with the 3-OH functionality at concentrations estimated to be above 50 ng/0.5 g wet yeast.

It is the purpose of this communication to advise future researchers to avoid 3-OH oxylipin identification and/or quantification in *Saccharomyces cerevisiae* using the methods we described. We chose the HIP extraction protocol described by Göbel and Feussner *(8)* because it was used to successfully isolate oxylipins from plant material. It may be that a more a traditional chloroform and methanol extraction would give better yields. In addition, it is possible that the SMA strain has very low levels or no oxylipins at all. In future studies of 3-OH oxylipins in brewer's yeast it would be advisable to use the strains reported by Kock *et al. (3)* and Strauss *(4)*.

Greg Potter
Food Science and Technology Program, Dalhousie University, Halifax, NS B3J 2X4, Canada

Alex Speers
International Centre for Brewing and Distilling, Heriot–Watt University, Edinburgh, EH14 4AS, UK

Suzanne M. Budge
Food Science and Technology Program, Dalhousie University, Halifax, NS B3J 2X4, Canada

References

1. Speers, A. (2012) A review of yeast flocculation, in *Proceedings of the 2nd International Brewers Symposium: Yeast Flocculation, Vitality and Viability*, (Speers, R. A. Ed.), Master Brewers Association of the Americas, St Paul, MN.
2. Speers, R. A., Wan, Y.-Q., Jin, Y.-L., and Stewart, R. J. (2006) Effects of fermentation parameters and cell wall properties on yeast flocculation, *J. Inst. Brewing*, *112*, 341–342.
3. Kock, J. L. F., Venter, P., Smith, D. P., Van Wyk, P. W. J., Botes, P. J., Coetzee, D. J., Pohl, C. H., Botha, A., Ridel, K.-H., and Nigam, S. (2000) A novel oxylipin-associated 'ghosting' phenomenon in yeast flocculation, *Antonie van Leeuwenhoek*, *77*, 401–406.
4. Strauss, C. J. (2005) The role of lipids in the flocculation of *Saccharomyces cerevisiae*. Unpublished doctoral dissertation. University of the Free State, South Africa.
5. ASBC Methods of Analysis, online (2012) Yeast Method 14. Miniature Fermentation Assay. Approved 2012, rev. 2012, American Society of Brewing Chemists, St Paul, MN; doi:10.1094/ ASBC Method-Yeast 14
6. Albrecht-von-Haller Institute for Plant Sciences-Oxylipin Profiling Database (2005) HIP Extraction. Available from: http://www.oxylipins.uni-goettingen.de/methods.php?path=hip_f&caption=HIP%20Extraction
7. Vesonder, R. F., Wickerham, L. J., and Rohwedder, W. K. (1968) 3-D-Hydroxypalmitic acid: a metabolic product of the yeast NRRL Y-6954, *Can. J. Chem.*, *46*, 2628.
8. Göbel, C. and Feussner, I. (2009) Methods for analysis of oxylipins in plants, *Phytochemistry*, *70*, 1485–1503.