Oxylipin profiling during storage of freeze-dried *Weissella paramesenteroides* LC11

Amenan A. Yao[1*], Ibourahema Coulibaly[2], Georges Lognay[3], Marie-Laure Fauconnier[4] and Philippe Thonart[1,2]

[1] Wallon Center for Industrial Biology, Microbial Technology Unit, University of Liège, Sart-Tilman B40, B-4000 Liège, Belgium
[2] Wallon Center for Industrial Biology, Bio-Industry Unit, Gembloux Agricultural University, [3] Passage des déportés 2, B-5030 Gembloux, Belgium
[3] Analytical Chemistry Unit, Gembloux Agricultural University, Passage des déportés 2, B-5030 Gembloux, Belgium
[4] Plant Biology Unit, Gembloux Agricultural University, Passage des déportés 2, B-5030 Gembloux, Belgium

Introduction

Lactic acid bacteria for food industry are commonly freeze-dried for long term-storage. Unfortunately, losses in viable cells occur during storage of dried cultures. Membrane lipid oxidation was suggested as the cause of cell death during storage. Lipids in living organisms, particularly the polyunsaturated fatty acids (PUFAs) of cell membranes, are described as being extremely susceptible to oxidative stress. Under oxidative conditions, molecular oxygen was introduced into linoleic (18:2) or linolenic (18:3) acids, leading to the formation of the hydroperoxy octadecadienoic acid (HPOD) or hydroperoxy octadecatrienoic acid (HPOT), hydroxy octadecadienoic acid (HOD) or hydroxy octadecatrienoic acid (HOT), divinyl ether containing PUFAs such as colnele (n)ic (C(n)A) acid and oxo fatty acids such as keto linole(n)ic (KO(T)D), respectively. In order to gain more knowledge about lipid oxidation during storage of dried bacteria, the accumulation of derived-oxylipins of linoleic and linolenic acids was followed.

Materials and methods

The lactic acid bacteria *W. paramesenteroides* LC11 was grown in 500 l bioreactor containing MRS medium for 18h, concentrated 20 times by centrifugation and freeze-dried. Freeze-dried powders of about 2 ±0.5 g were stored in 20 ml glass tubes over 90 days at 4 and 20°C in desiccators over the following saturated salt solutions of constant water activities (a_w): LiCL (0.11), CH_3COOK (0.23). Total oxylipins profiling during storage was analysed in three replicates using a Reversed Phase-High Performance Liquid Chromatography (RP-HPLC). The fatty acids composition and linoleic/palmitic (18:2/16:0) or linolenic/palmitic (18:3/16:0) ratio were determined by gas chromatography.

Results and discussion

Hydroperoxy PUFAs, hydroxy PUFAs, divinyl ether PUFAs and oxo PUFAs were oxylipins identified. The main oxylipins identified during storage in glass tubes with a_w = 0.11, were the unesterified hydroperoxy PUFAs (13-and 9-HPO(D)T) and hydroxy PUFAs (13-and 9-HO(D)T). During storage in glass tubes with a_w = 0.23, the unesterified hydroperoxy PUFAs (13-HPO(D)T) and hydroxy PUFAs (13- HO(D)T) as well as the esterified hydroperoxy (13-HPOD-Me), divinyl ether (can-Me) and oxo PUFAs (13/9-KOT-Me) were detected. The linoleic and linolenic acids were the main fatty acids degraded during storage. We observed four different kinetics of oxylipin appearance during storage. (i) Hydroperoxy fatty acids: they accumulate transiently during storage peaking on the 60th day. Afterwards, they might have decompose into hydroxy and oxo PUFAs. Hydroperoxy fatty acids were rapidly converted mainly into the corresponding hydroxy PUFAs. (ii) Hydroxy PUFAs: they showed the same transient accumulation as that of the hydroperoxides PUFAs. It is unclear as to what they became after degradation. Volatiles secondary degradation products (aldehydes...)?? (iii) Divinyl ether PUFAs: they accumulated transiently during storage with a maximum pointed out at the 60th day and might have decomposed into their corresponding oxo fatty acids. (iv) Oxo PUFAs: they start to accumulate from day 30. Whether they were further degraded remains unclear as they did seem to be rather stable, their amounts having stayed the same during the storage.

References

Coulibaly, I. *et al.* 2008. Survival of freeze-dried *Leuconostoc mesenteroides* and *Lactobacillus plantarum* related to their cellular fatty acids composition during storage. *Appl Biochem Biotechnol*, in press.

Laine, G. *et al.* 2006. Study of precursors responsible for off- flavor formation during storage of potato flakes. *J Agric Food Chem* 54:5445-5452.

Yao, A. *et al.* 2008. Impact of polyunsaturated fatty acid degradation on survival and acidification activity of freeze-dried *Weissella paramesenteroides* LC11 during storage. *Appl Microbiol Biotechnol* 79:1045-1052.