Prolonged Shelf Life in Fermented Milk Products Investigated by Bioassay-Guided Chemometrics

Vinther, Joachim Møllesøe; Garrigues, Christel; Nyberg, Nils; Jäger, Anna; Franzyk, Henrik; Stærk, Dan

Publication date:
2014

Document version
Early version, also known as pre-print

Citation for published version (APA):
Prolonged Shelf Life in Fermented Milk Products Investigated by Bioassay-Guided Chemometrics

Joachim M. Vinther, Christel Garrigues, Nils Nyberg, Anna K. Jäger, Henrik Franzyk, and Dan Stærk

1 Natural Products Research, Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen
2 Discovery, Cultures & Enzymes Division, Chr. Hansen A/S

Yeast-growth bioassay and bioassay-guided NMR-based metabolomics

A yeast-growth bioassay for evaluation of the antimicrobial effect of aqueous extracts of fermented milk products has been developed and used in a bioassay-guided NMR-based metabolomics study. The bioassay revealed significant differences between the reference samples and the samples containing bioprotective compounds. The NMR study, however, revealed only minor concentration changes. It is therefore concluded that the antimicrobial compound is potent at sub-mM concentrations. Further studies using other analytical techniques are being conducted.

Introduction

Specific strains of lactic acid bacteria have been found to prolong shelf life without significantly changing taste or texture of fermented milk products if present during fermentation. The hypothesis is that the bacteria produce small amounts of antimicrobial compounds. The present study intends to investigate which antimicrobial compounds are optimised: microplate assay of 200

Sample preparation

• choice of contaminant (yeast species)
• yeast harvested at different phase of growth
• growth temperature
• growth medium
• incubation time
• initial yeast concentration

Development of assay

The yeast-growth bioassay was developed as a microplate assay of 200 μl aqueous extracts of the fermented milk products with yeast. Aimed for is the largest difference between BioP and reference samples, the following parameters were optimised:

Results

Bioassay

The bioassay shows a clear and reproducible differentiation between BioP and reference samples as exemplified in figure 2. Two BioP extracts (red curves) were found to have a significantly lower antimicrobial activity probably ascribed to the sample preparation or inhomogeneities in the samples.

Principal Component Analysis

An overall analysis resulted in the data shown in the top row figure 3 and did not point to a differentiating factor between BioP and reference samples: only a minor concentration difference is found in the BioP samples with low antimicrobial efficacy. BioP samples with low antimicrobial activity are in all cases seen to group with the other BioP samples.

Discussion

With the significant differentiation of the samples in the bioassay, it is surprisingly small differences which are found in the NMR data. The data does not show any new compounds in the BioP samples and the differences in concentrations are clearly too small to explain the large and consistent biological differences. Moreover, the BioP samples with low activity (red) group with the active BioP samples. As such, it is most likely that the antimicrobial activity must be ascribed to a compound present at a sub-mM concentration not visible in the proton NMR data.

Conclusions and future work

A well functioning yeast-growth bioassay which clearly distinguishes between fermented milk samples with and without bioprotective lactic acid bacteria has been developed. The bioassay was successfully applied in the 24-samples metabolomics study. The PCA of the NMR spectra of the samples revealed only minor unspecific concentration differences around 5(H) = 3.5 attributed to sugars. However, sub-spectral PCA revealed differences between BioP and reference samples regarding acetic acid, aceton and an unidentified compound with δ(H) = 8.35. All compounds showed a slightly higher concentration in the BioP samples. These trends, however, do not explain the very distinctive difference observed in the bioassay and we therefore conclude that the antimicrobial compound must be present at a sub-mM concentration and therefore not visible in the NMR data.

With this conclusion, the further analytical work will rely primarily on the use of MS-based techniques.

Acknowledgements

NMR equipment used in this work was purchased via grant #10-085264 from The Danish Research Council for Independent Research | Nature and Universe.


NATURAL PRODUCTS RESEARCH & CHR. HANSEN A/S
UNIVERSITY OF COPENHAGEN

Figure 1: Results from microplate-based yeast growth assay (Microplates after incubation. Cultures 2.5 BioP samples, 6 reference samples, 10 & 11 sterile and growth tests.)

Figure 2: Growth curves obtained for the samples in the metabonomics study (Growth monitored by the developed bioassay as optical density at 630 nm (OD630) as a function of incubation time. Green: BioP samples, Blue: Reference samples; Red: BioP samples with app. low antimicrobial activity.)

Figure 3: Score and loading plots from the proton NMR data (Green: BioP samples, Blue: Reference samples, Red: BioP samples with low biological activity. First row: All spectral region. Second and third row: Sub-spectral regions showing some degree of separation. The loadings are depicted in colour code on the spectra (blue-yellow-red) and as time.)

Figure 4: Principal component analysis (Loadings 1)

Figure 5: Principal component analysis (Loadings 2)