



### PPP Annual Report 2019

PPP projects which are under supervision of the "Topsectoren" must report annually on the scientific content and financial progress. This form is used to report the progress of the content of the project. PPP projects that finish in 2019 should make use of a different form: "PPP-final report."

**The annual report will be published on the TKI / topsector website. Therefore, please ensure that there is no confidential information in the annual report.**

*Please, submit the report before 15 February 2020 to Hans van der Kolk*

General information	
PPP-number	AF17014
Title	CULTURED
Theme	Gezond en veilig
Implementing institute	Wageningen Food & Biobased Research TNO
Project leader research (name + e-mail address)	E.P.J. Beckers Erwin.beckers@wur.nl
Coordinator (on behalf of private partners)	Tadgh O'Sullivan (Heineken)
Project-website address	n.a.
Start date	01.01.2018
Final date	31.12.2020

### Approval by the coordinator of the consortium

The annual report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the annual report.

Assessment of the report by the coordinator on behalf of the consortium:	<input checked="" type="checkbox"/> X Approved <input type="checkbox"/> Not approved
Additional comments concerning the annual report:	None

### Summary of the project

Problem definition	
Project goals	<p>CULTURED is a multidisciplinary public-private partnership (PPP) which aims to develop novel solutions for the food industry by using natural fermentation strategies. The project will deliver high throughput systems as tools for the discovery of novel food and ingredient functionalities obtained by fermentation. In addition, this project will provide the required technological know-how and will close knowledge gaps to allow the application of fermentation technology in modern food production.</p> <p>In the end, it is foreseen that CULTURED will expand the application of fermentation in food production to include food products that are currently not standard beneficiaries of microbial intervention. In addition, targeted use of fermentation technology for specific food functionalities will be more easily implemented by the food industry. By establishing the enabling tools and technologies and by creating the toolbox required, CULTURED advances the use of fermentation technology for the discovery and development of minimally processed, wholesome, and sustainable ingredients and products. More importantly, the novel use of fermentation technology within clean</p>

	<p>labelling widens the possibilities for product development within this field.</p> <p>Bringing together fermentation and high throughput screening for specific food functionalities within the framework of clean and clear labelling advances the state of the art in many ways. Firstly, the knowledge of microorganism-food substrate combinations resulting in specific functionalities in food products will be extended. New combinations of microorganisms and substrates resulting in novel functionalities will also be identified. Secondly, high throughput screening will alleviate the constraints of process development that are based on fermentation technology. Thirdly, within CULTURED, fermentation technology will be established for food products on the basis of functionality requirements.</p>
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<b>Results</b>	
Planned results 2019	<ul style="list-style-type: none"> <li>- Finalising key indicators and (high throughput) screening method (WP1)</li> <li>- Finishing experiments on fermentability of substrates, select strains and micro-organism and define the final combinations to be screened (WP2)</li> <li>- Determine ferment functionalities (WP3). A large part of the work in the second year was dedicated to this</li> <li>- Optimised processes for dedicated approach low caloric sweetener and microbial fatty acids (WP4)</li> <li>- Finalised information on legislation and safety related to new fermented products (WP8)</li> </ul>
Achieved results 2019	<p><u>WP1 Screening method and indicators</u> Screening methods and key indicators were finalised leading to the following outcome for several functionalities:</p> <ul style="list-style-type: none"> <li>- Sweeteners: receptor based screening assay</li> <li>- Anti-microbials: pathogen growth inhibition test with qPCR</li> <li>- Umami/kokumi: chromatography determination of indicator compounds</li> <li>- Aromas: semi-automated head space compound identification</li> <li>- Texturisers: proven correlation between indicator and specific rheological properties</li> </ul> <p><u>WP 2</u> Fermentation conditions were established leading to the enhancement of specific food functionalities using predefined substrate-microorganism combinations. Approximately 50 organisms were selected for fermentation on the industrial substrates. From several of these organisms more than one single strain was included (maximised to four strains per organism). For all selected strains the envisioned functionality was defined. The selected strains (31-68 per functionality) were divided in a limited amount of groups in order to obtain a selected range of standard fermentation conditions: aerobic or anaerobic, and the preferred temperature.</p> <p>Partners submitted 98 substrates. Work was completed on their fermentability and option to be transferred with a pipet to the high throughput systems. Non-fermentable substrates were excluded and no pre-treatment was used.</p> <p>The amount of combinations of substrate and micro-organisms had to be reduced e.g. due to (time) limitation of the analytical methods.</p> <p><u>WP3 Screening of fermented substrates on functionality</u> For the screening, 96-wells plates were used, each with 71 substrates + 5 control. Prior to fermentation all plates were sterilised. Subsequently one micro-organism was added to each per plate to prevent growth differences</p>

between strains/organisms. In total 54 micro-organism were added leading to > 3000 combinations analysed on functionalities.

**Sweeteners**

A preliminary list was generated of the sweetness of ferments from selected substrate-strain combinations including positive hits from the screening.

**Anti-microbials**

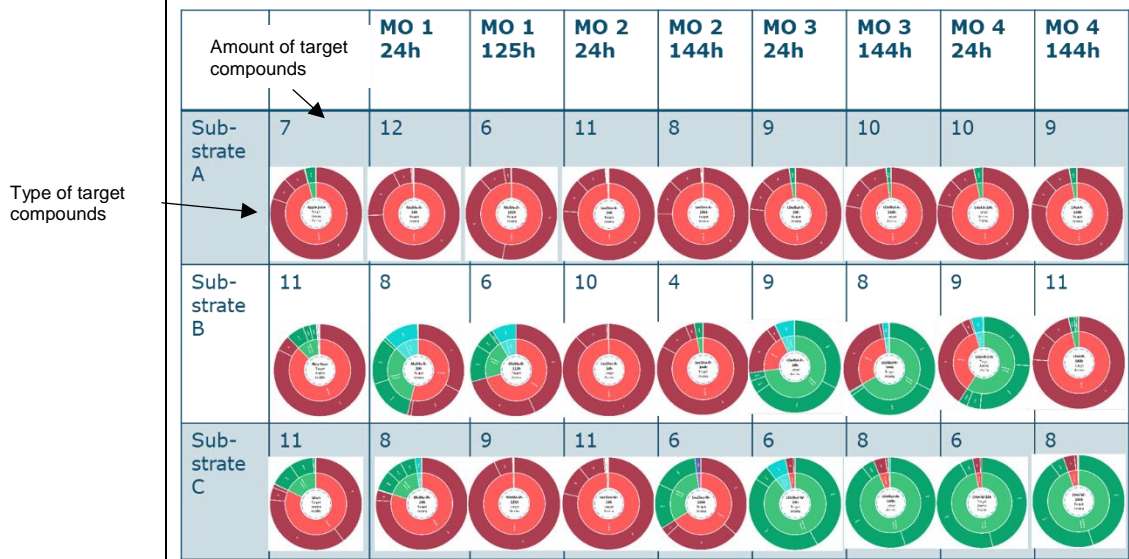
A list of selection of lead ferments (with known substrate/strain combination) containing antimicrobial functionalities was generated. Optimized fermentation conditions were determined in view of antimicrobial functionality production. Analysis of growth inhibition of all three pathogenic species in one test proved not to be viable. Screening was started using hundreds of combinations of substrates and micro-organisms.

**Kokumi and umami**

A functional analytical method for the detection of relevant umami/kokumi active compounds was finalised. A final choice was made for the production method and detailed plan how to continue.

**Aromas**

The large variety of ferments was screened on presence / absence of target aroma compounds. In samples of high interest: analysis of other aroma compounds and semi-quantification of target aroma compounds of high interest was started.



**Texturisers / gelling**

In specific terms within the project, texturizing functionalities will refer to the ability of a ferment to improve mouth feel in low-viscous beverage type products (e.g. malt beverages, soft drinks, among others) or gelling properties of high-viscous semi-solid foods (e.g. pudding-type products, among others). Determination of the indicator with the developed high throughput system (30 s/sample) were used as a function of shear rate.

**WP4**

For prioritization of promising ferments, selection criteria were made by the consortium e.g. panel testing (smell, mouth feel), temperature resistance, pH resistance. Criteria differed per functionality.

**Low caloric sweetener**

The method was finalised for fermentation of yeast strains producing the model low-caloric sweetener in lab-scale fermenters. Additionally a method

	<p>was finalised for fermentation producing the same sweetener using industrial substrates.</p> <p><i>Microbial fatty acids</i> (WP4) The method was finalised for fermentation of sugar-rich substrates using specific yeast for fatty acids production in lab-scale fermenters</p> <p><u>WP8</u> Regulatory requirements and consequences of the use of fermented functional ingredients were completed. They were also discussed in an international forum which started to work on the same topic.</p>
Planned results 2020	<ul style="list-style-type: none"> <li>• Finalised screening for all functionalities (WP3)</li> <li>• Production optimisation of best hits (WP4)</li> <li>• Incorporation in model food systems (WP6)</li> <li>• Trials in real products (WP7)</li> <li>• Economic analysis (cost effectiveness) of successful ferments, model food systems or real product (WP8)</li> <li>• Dissemination: <ul style="list-style-type: none"> <li>○ At least one article on the project in a professional journal or magazine</li> <li>○ Possibly a WUR day on food fermentation (Netherlands) in April 2020</li> <li>○ Full session of fermentation (Cultured) at Institute for Food Technology conference in June 2020 (Chicago, USA)</li> </ul> </li> </ul> <p>N.B.: WP5 (Separation and purification) will be limited to filtration to maintain a fermented product which can be labelled as such. Active ingredients will not be isolated.</p>

<p><b>Deliverables/products in 2019</b> (provide the titles and /or a brief description of the products/deliverables or a link to a website.)</p>
<p><u>Scientific articles:</u></p> <p>---</p>
<p><u>External reports:</u></p> <p>---</p>
<p><u>Articles in professional journals/magazines:</u></p> <p>---</p>
<p><u>(Poster) presentations at workshops, seminars, or symposia.</u></p> <ul style="list-style-type: none"> <li>- Workshops with all partners in May and December 2020</li> <li>- Presentation "Antimicrobiële middelen van plantaardige oorsprong" at meeting of Stichting Food Microbiology , 12 Dec 2019, Bilthoven</li> <li>- Contribute with Cultured generated knowledge in workshops on legislation of microbial food cultures at University of Copenhagen, Copenhagen 20-22 August 2019 and Danish technological University, Copenhagen, 5-6 Feb 2020</li> <li>- "Industrial Microbiology", SILS Science Day, 20 juni 2019, Amsterdam</li> <li>- "From Trash to Cash: Microbial conversion of waste streams into high-value chemicals", WCIB, Des Moines, 9 juli 2019</li> <li>- "Fermentation for production of clean label ingredients", EFFOST, 12 november 2019, Rotterdam</li> </ul>
<p><u>TV/ radio / social media / newspaper:</u></p> <p>---</p>
<p><u>Remaining deliverables (techniques, devices, methods, etc.):</u></p> <p>---</p>