



General information	
PPP-number	TKI-AF-18081
Title	B-Twelve Insight, Fermented pLAnt pRotEins
Theme	Topsector Agri & food, Healthy & Safe
Implementing institute	Wageningen University & Research
Project leader research (name + e-mail address)	Jacqueline Berghout, jacqueline.berghout@wur.nl
Coordinator (on behalf of private partners)	Niko Koffeman, de Vegetarische Slager
Project-website address	Not applicable, in preparation though https://topsectoragrifood.nl/project/af-18081-fermented-plant-proteins/
Start date	01-01-2019
Final date	31-12-2021

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"/> Approved <input type="checkbox"/> Not approved
Additional comments concerning the annual report:	

Summary of the project	
Problem definition	In the past decades, the world population has grown exponentially and the level of income has increased leading to a rise in the global meat consumption. Meat is an important protein source in the human diet but to reduce impact on public health, environment and society, a reduction of meat intake by consumption of more vegetable-based protein sources is desired. Hurdles for increased meat analogue acceptance by consumers are the bite and structure, the lack of essential micronutrients and the non-meaty taste. Fermentation provides an excellent technique to meet the consumers demand for nutritional, healthy and attractive protein products that are sustainably produced.
Project goals	<p>This project aims at the development of new plant-based protein rich meat-replacers by the use of fermentation with special focus on the <i>in situ</i> production of meat associated vitamin B12 and meat-like flavours and different sources of protein rich plants or plant-based co-product streams. In the project, we will address the three challenges for fermentation as tool for vegetable-based meat analogues which are</p> <ul style="list-style-type: none"> (i) the production of meat like flavours, (ii) fortification of plant-based protein sources from co-product streams of the food and beverage industry with vitamin B12 produced by food-grade bacteria, and (iii) the use of new high protein plant sources for vitamin B12 fortified tempeh analogues. <p>Prototypes for vitamin B12-enriched tempeh-like products using legumes and other plant-based proteins will be developed along with testing of shelf life and microbiological challenge testing of developed products.</p>

Results	
Planned results 2019	The project consists of 7 work packages (WPs), described in the project plan. There is an overall planning with deliverables for the entire duration of the project, and a more detailed planning is made for each WP. A summary of the main results achieved in 2019 is given below.
Achieved results 2019	<p><u>WP1: Production of natural meat-like flavours by fermentation</u> WP1 aims at producing concentrated meat aroma's for plant-based meat replacements. Next to screening for suitable microorganisms and cultivation conditions, the use of toxic analogues has been studied to identify natural overproducing strains. The overproducers will be characterized and potentially applied to ferment high protein co-products resulting in thiamin (vitamin B1)-enriched products. In the last year of the project, meat aroma block formation will be studied. A PhD student is involved in this WP. The extraction of vitamin B1 is also part of this WP and there is a strong collaboration with WP4 on the analysis of vitamin B1 on HPLC.</p> <p><u>WP2: Development of optimized fermentation processes for lupine-based tempeh fortified with vitamin B12</u> WP2 aims to develop reliable and reproducible fermentation process for producing lupin tempeh products fortified with vitamin B12 using <i>Propionibacterium freudenreichii</i> as adjunct culture. New co-fermentations will be developed that are better adapted to industrial processing. The impact of the adapted co-fermentations on product composition, texture, anti-nutritional factors, flavor and sensory aspects will be analysed. In 2019 the main focus was on studying vitamin B12 production in lupine tempeh. Process parameter settings such as particle size, inoculum size and strain selection were varied to study the most relevant impactors on vitamin B12 production and on microorganism growth.</p> <p><u>WP3: Development of meat alternatives using alternative high protein plant-based substrates</u> This WP has the aim to optimize (co-)fermentation processes for high protein plant-based substrates. Fermentation will be optimized for strain selection, inoculum size, timing of inoculation, incubation temperature and time, oxygen supply and heat transfer. In 2019, a selection of pulses and other high-protein substrates, including co-product streams from the food and beverage industry, to enrich in vitamin B12 was made with the consortium. Screening of the substrates with several vitamin B12 producing strains was performed, under different process conditions. Initial results show enrichment of vitamin B12 for some of the selected substrates.</p> <p><u>WP4: Development of analytical assays</u> This WP aims at developing sensitive, reproducible (and fast) analytical methods to measure vitamin B12 and vitamin B1. The methods were planned to be up and running in 2019 so the other WPs could use the HPLC methods for their analyses replacing or in addition to the biological assay in use. The WP encountered some challenges, but shows promising results for the analyses. This work will continue in the first half of 2020, aiming to have a working method for vitamin B12 in the first quarter of 2020 and a working method for vitamin B1 in the second quarter of 2020.</p> <p><u>WP5: Other fermented flavours</u> This WP has the aim to explore the use of alkaline fermentation of protein-rich substrates to produce meaty flavours that can be used as an ingredient in plant-based meat analogues. No activities were scheduled for 2019.</p> <p><u>WP6: Communication and dissemination</u></p>

	<p>WP6 focuses on knowledge transfer, communication and dissemination activities to different target groups. In 2019, a project logo was designed. A webpage is not automatically created for a TKI Toeslag project. Since the project partners agree that a webpage would be desirable this will be organized. To share information with the consortium, a team website was set up. Another activity is the inventory of EU regulation with regard to the application of fermentation processes for our purpose: enriching protein-rich products in vitamin B12, and fermentation for production of vitamin B1 (as a pre-cursor for flavor). An inventory was made of the EU regulation with regard to application of fermentation processes relevant to the project. This information was shared and discussed with the project partners early January 2020</p> <p><u>WP7: Overall project management</u> The aim of this WP is to ensure alignment and coherence between the different WPs. In 2019, a kick-off meeting and two General Meetings were organized, where the progress of each WP is presented to the consortium and input is obtained from the consortium partners. Next to the General Meetings, the WPs organized one-to-one meetings with the consortium partners for progress updates and inputs and internal meetings between the two knowledge partners in the project.</p>
Planned results 2020	<p>For 2020 the activities in the project will be continued according to the GANTT chart planning. Special focus will be on:</p> <ul style="list-style-type: none"> - Fast, reproducible and sensitive methods for vitamin B12 and B1 analysis - Organizing a creative session with the consortium (WP3). - Create webpage, improve visibility of the project through scientific and popular publications.

<p>Deliverables/products in 2019 (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><u>External reports:</u> -</p>
<p><u>Articles in professional journals/magazines:</u> -</p>
<p><u>(Poster) presentations at workshops, seminars, or symposia.</u> Givaudan Inspiration Day, 11 December 2019</p>

TV/ radio / social media / newspaper:

Geen mens of dier kan zonder B12

Eddy Smid
20/04/19
VLAG, Food Microbiology
Samuel Bom

Netherlands print: De Stentor (regional), Brabants Dagblad (regional), Eindhovens Dagblad (regional), BN/De Stem (regional), De Twentsche Courant Tubantia (regional), Provinciale Zeeuwse Courant (regional), De Gelderlander (regional), Haagsche Courant (regional), Utrechts Nieuwsblad (regional), Groene Hart (regional), Rotterdams Dagblad (regional), Amersfoortse Courant (regional), Rivierenland (regional), De Dordtenaar (regional), Algemeen Dagblad (national),

Also online 22/04/2019 Dit is de reden waarom je niet zonder vitamine B12 kunt

De Stentor (regional), Brabants Dagblad (regional), Eindhovens Dagblad (regional), BN/De Stem (regional), De Twentsche Courant Tubantia (regional), Provinciale Zeeuwse Courant (regional), De Gelderlander (regional), Algemeen Dagblad (national).

Fermentations great promise

1/09/19
Wageningen World 3 2019 (National), Netherlands, Print
H. Wolkers
p. 34-39
<https://edepot.wur.nl/500115>
Eddy Smid
VLAG, Food Microbiology

Fermenteren is populair, 'maar het proces luistert nauw'

14/09/19
NOS (National), Netherlands, Television
<https://nos.nl/artikel/2301729-fermenteren-is-populair-maar-het-proces-luistert-nauw.html>
Eddy Smid
VLAG, Food Microbiology

Fermentatie: een oeroud proces vol toekomst

11/11/19
Dagblad de Limburger (Regional), Netherlands, Print
Hans van Zon
Eddy Smid
VLAG, Food Microbiology

Toch eruit voor wat lekkers - Meer eiwitten uit planten, minder uit vlees

7/11/19
De Stentor (Regional), Netherlands, Print
De Stentor
Eddy Smid
VLAG, Food Microbiology

Fermentatie: een oeroud proces met toekomst

Eddy Smid
2/11/19
VLAG, Food Microbiology
Hans van Zon

Netherlands print: Brabants Dagblad (regional), Rotterdams Dagblad (regional), De Stentor (regional), De Gelderlander (regional), Haagsche Courant (regional), Utrechts Nieuwsblad (regional), Groene Hart (regional), Amersfoortse Courant (regional), Provinciale Zeeuwse Courant (regional), Eindhovens Dagblad (regional), De

Dordtenaar (regional), Rivierenland (regional), De Twentsche Courant Tubantia (regional), BN/De Stem (regional), Algemeen Dagblad (national).

Also online:

<https://www.bd.nl/wetenschap/meer-eiwitten-uit-planten-minder-uit-vlees-oeroude-techniek-herleeft~afa7bc9e/>

<https://www.destentor.nl/wetenschap/meer-eiwitten-uit-planten-minder-uit-vlees-oeroude-techniek-herleeft~afa7bc9e/>

<https://www.gelderlander.nl/wetenschap/meer-eiwitten-uit-planten-minder-uit-vlees-oeroude-techniek-herleeft~afa7bc9e/159655319/>

<https://www.tubantia.nl/wetenschap/meer-eiwitten-uit-planten-minder-uit-vlees-oeroude-techniek-herleeft~afa7bc9e/159655319/>

<https://www.pzc.nl/wetenschap/meer-eiwitten-uit-planten-minder-uit-vlees-oeroude-techniek-herleeft~afa7bc9e/>

<https://www.ed.nl/wetenschap/meer-eiwitten-uit-planten-minder-uit-vlees-oeroude-techniek-herleeft~afa7bc9e/159655319/>

<https://www.ad.nl/koken-en-eten/meer-eiwitten-uit-planten-minder-uit-vlees-oeroude-techniek-herleeft~afa7bc9e/>

<https://www.bndestem.nl/wetenschap/meer-eiwitten-uit-planten-minder-uit-vlees-oeroude-techniek-herleeft~afa7bc9e/159655319/>

Remaining deliverables (techniques, devices, methods, etc.):

- Fast, sensitive and robust method for vitamin B12 and B1 analysis in all relevant matrices