EU cofin Project Annual Report 2019

The EU projects that receive co-finance from the top sectors must submit an annual report on their technical and financial progress. This format is to be used for reporting the technical progress.

General information	
TKI Number of the project	AF-EU-18027
Title	MOLOKO
project leader WR (name + e-mail address)	Jeroen Peters jeroen.peters@wur.nl
Address project website	EU project website: https://www.moloko-project.eu/ KennisOnline website: https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksprojecten-LNV/Expertisegebieden/kennisonline/MOLOKO.htm
Start date	January 2018
End date	June 2021

Short description/aim project (this information can be published on a website of the TKI/Topsectors)

The main objective of MOLOKO project is the manufacturing, implementation and validation of a self-managing and automatic miniaturized integrated photonic sensor to be used as process analytical instrumentation for fast response on-site monitoring of interest analytes for security and quality within milk supply chain. In particular, we aim at realizing multiplexing quantitative detection of up to 10 analytes among which food safety parameters, e.g. antibiotics (i.e. penicillin, ampicillin, cephalosporins) and toxins (i.e. mycotoxins and bacterial toxins) and food quality parameters e.g. lactoferrin and caseins by implementing a highly-integrated optoplasmonic-microfluidic sensor in the strategic checkpoints along the entire supply and value chain of milk. The MOLOKO miniaturized integrated photonic sensor is specifically designed according to milk primary production, processing and distribution end-users in order to enable and guarantee self-monitoring safety and quality standards by the use of a reliable, highly sensitive and specific, low-cost innovative self-screening photonic technology.

Planning and progress Is the project going according to plan? Are there any substantive bottlenecks? If yes, please explain with a brief description of the current situation

WFSR is work package leader for diagnostics group of the MOLOKO project. Based on the first review commission meeting, the initial short and long list for MOLOKO targets were integrated in a priority table of analytes in close collaboration with end user, the MOLOKO partners MILKLINE and PARMALAT. Especially the milk quality factors were given a much higher priority (e.g. Lactoferrin, k-casein B, β-casein A2, Alkaline Phosphatase). The definition and realization of the indirect and direct immunoassay sets to be used in the detection scheme is approaching finalization. Currently, we can assess more than 80% of the selected targets and are ready for multiplexing based on their performance in the chosen benchmark Surface Plasmon Resonance (SPR) technology. The developed assays are substantial for proving the multiplexing concept of the MOLOKO biosensor. Unfortunately, the sensitivity for the Aflatoxin M1 (AFM1) SPR immunoassay did not reach the desired MRL of 50 ppt. The same AFM1 antibody easily reached this sensitivity in commercial multiplex technology. Additionally, the development of the sulphonamides and penicillins were not successful and further development on the Biacore platform was paused to focus on the more relevant targets based on end-user partner priorities. The production of recombinant antibodies by the Technical Research Centre (VTT) in Finland, for staphylococcus enterotoxins A and B (SEA and SEB) and cephalosporins was delayed and therefore were not tested yet in benchtop SPR by

WFSR. Based on recent progress, it is expected that these tests will be performed in the first half of 2020, so the assays can still be integrated in the multiplex assay.
Sensor development is well under way. The organic photonic module has been designed and fabricated according to the defined specifications while the finalization of fabrication protocol of nanoplasmonic grating according to the MOLOKO requirements and the optical and morphological characterization of the grating response are under way. The design of the microfluidic module is progressing as planned.

Highlights and deliverables in 2019 / so far (this information can be published on a website of the TKI/Topsectors)

Within the Diagnostics Work Package of MOLOKO, successful benchtop SPR based single assays were developed for the low molecular weight milk contaminants tetracyclines, streptomycin and quinolones. These assays are now ready for implementation on the multiplex biosensor from Plasmore (iNPx) which features the same chip surface as the final MOLOKO biosensor. The development of the aflatoxin M1 assay was successful, yet unfortunately did not meet the MRL for milk (50 ppt) as set by the European Union. During the second review meeting, the commission advised to invest no further efforts in optimizing the aflatoxin M1 assay.
For the high molecular weight targets, successful SPR single assays were developed for quality (and fraud) factors lactoferrin, κ-casein (total), κ-casein B, β-casein A2 and alkaline phosphatase. These assays are also ready for further implementation on a multiplex sensor.

Number of delivered products in 2019 *(in an appendix, please provide the titles and/or description of the products or a link to the products on public websites)*

Academic articles	Reports	Articles in journals	Introductions/workshops

Titles/ description of the most important products in 2019 (5 at max) and their target group

Poster: β-Casein A1 and A2: a quality and fraud biosensor assay developed in the H2020 MOLOKO project

Periodic report: D10.1 – First Periodic Activity Report

Deliverable report: D1.1 – Specification statement for photonic, plasmonic and microfluidic modules and for production of the automatic-controlled MOLOKO sensor

Deliverable report: D6.1 – Report on selected Pabs and Mabs and their performance in the SPR biosensor Biacore

Progress presentation at the 2nd Review of H2020 project MOLOKO, Brussels 18-09-2019

Appendix: Names of the products or a link to the products on a public website including the link to the project summary on Kennisonline