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PHYTOME FINAL REPORT

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Executive summary

The PHYTOME project aims to develop innovative meat products in which the food additive nitrite has been replaced by natural compounds originating from fruits and vegetables. These biologically active compounds, also referred to as phytochemicals, are known to contribute to improved gut health and are added to the meat as natural extracts. EU legislation strikes a balance between the risk of the formation of carcinogenic N-nitroso compounds (NOC) due to the presence of nitrite and the protective effects of nitrite against the presence of pathogenic bacteria.

In 6 different types of meat products, carefully selected combinations of natural antioxidants and other biologically active compounds occurring in vegetables, herbs and fruits have been added during meat processing. Some of these compounds possess an antimicrobial activity allowing them to replace nitrite, whereas others possess a natural red colour that may contribute to the desired appearance of the products. Also, some of these compounds are known to protect colonic cells against damaging effects of cancer causing compounds. The PHYTOME project has developed new technologies to introduce the natural extracts during processing into 6 different types of meat products. A wide range of natural extracts rich in bioactive molecules was found suitable for meat product fortification. In vitro evaluation of radical scavenging capacity showed that rather large differences in products from different suppliers may occur. Reduction or even elimination of nitrite was addressed according to meat categories, and the lowest nitrite addition in cooked meats was 25 mg/kg. In dry-cured meats, elimination of nitrite was found affordable hence recommended for further evaluation at the industrial scale manufacturing stage. The overall amount of phytochemicals was considerably higher in minced meats (2 g/kg of total polyphenols), as compared to whole cuts (cooked and dried hams). In dried hams, incorporation was enhanced using brine vacuum impregnation in the salting stage with positive results (around 1.5 g/kg). Nitrite could be eliminated in dried hams and in dry sausages made according to Southern European style. In the remaining meat items, the level was reduced to a minimum level of 25 mg/kg (cooked ham, cooked sausages and northern style dry sausages) or 75 mg/kg (dried hams, brine injection).

The health promoting effects of these products have been evaluated in a human dietary intervention study with healthy volunteers. Participants consumed during consecutive periods of 2 weeks, maximally 300 grams of conventionally processed meat products, white meat (as reference), and processed meat products with added natural extracts. Faecal excretion of NOC was significantly increased after consumption of processed meat as compared to white meat consumption, but this increase was effectively reduced by adding natural extracts to the meat products. Faecal genotoxicity measurements demonstrated that consumption of meat products increases the induction of DNA strand breaks in colonic cells as compared to white meat. However, DNA strand breaks were not higher when the PHYTOME meat products were consumed. Also DNA-adduct levels were higher after consumption of conventional products as compared to white meat, but not after consuming the PHYTOME meat products. Both the results on faecal genotoxicity and DNA-adducts suggest a phytochemical-induced preventive effect. Gene expression analysis and DNA methylation profiles established in colonic biopsies provided molecular mechanisms which may mechanistically support a preventive effect of the PHYTOME meat products. Elaborate consumer research has demonstrated that European stakeholders and consumers were generally positive about the idea to replace nitrite in meat products by natural compounds. Consumers indicated that the new meat products should possess equal sensory characteristics and improve healthiness as compared to currently available meat products. Four different consumer segments were identified: “enthusiasts”, “accepters”, “half-hearted” and “uninterested”. As attitude emerged as the main driver for purchase intention, “accepters” and “half-hearted” are identified as the primary target segments of interests for marketing communications. After sensory evaluation, the differences between the conventional and the PHYTOME meat products were found to be acceptable among consumers, and information on health risks was the most interesting information for consumers. The outcome of the PHYTOME project was communicated using various means, including the website www.phytome.eu.

A summary description of project context and objectives

With regard to meat consumption, consumers are increasingly influenced by messages in the media that consumption of nitrite preserved meat products contributes to human cancer risk. According to various reports of the World Cancer Research Fund (WCRF), there is convincing evidence that the consumption of red and particularly processed meat is associated with cancer risk. More recently, the International Agency on Research on Cancer even classified processed meat as carcinogenic to humans (Group 1 carcinogen), based on sufficient evidence in humans that the consumption of processed meat causes colorectal cancer. Aside from the formation of food preparation-related heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs), it has been proposed that endogenously formed N-nitroso compounds (NOCs) are responsible for the link between red meat consumption and colorectal cancer risk. Meat is a source of NOC precursors in the form of amines and amides and haem protein present in red meat is thought to catalyze endogenous nitrosation. As most NOCs have mutagenic and genotoxic properties, which explain their carcinogenic effect in test animals, they may also contribute to CRC development in humans. On the other hand, meat and meat products form a conventional part of the human diet and also contribute to the health of consumers in view of the supply of essential amino acids, iron, zinc, selenium, vitamin B6 and B12 and vitamin D. From this perspective, reduction of meat consumption may also have a negative impact on the nutritional status of consumers.

The aim of the PHYTOME project is to develop new meat processing technologies, resulting in innovative products that have no or strongly reduced nitrite levels and that have been shown to contribute to improved gut health. The new meat products are enriched with carefully selected biologically active compounds, so called phytochemicals, present in various natural plant extracts. Specific phytochemicals possess antimicrobial activity that may allow replacement of nitrite without hampering microbiological safety. Moreover, there is a growing body of evidence that these biologically active compounds in the diet, particularly coming from the consumption of fruits and vegetables, reduce the risk of developing colon cancer and improve human health in general. It is generally accepted that specific biologically active compounds in plants and herbs, like vitamin C, tocopheroles, flavonoids, carotenoids, glycol alkaloids and others have a cancer preventive effect. Different types of biologically active compounds may exert their beneficial action via different mechanisms, including effects at the level of formation and kinetics of carcinogenic compounds in the colon, and at the level of cellular protection. More specifically, some polyphenols and ascorbic acid are known inhibitors of the endogenous nitrosation process through which the NOCs are formed that are held responsible for the meat-cancer association. Due to synergistic interactions, combinations of different classes of phytochemicals may be more effective than single compounds.

Therefore, the PHYTOME project aims to deliver optimized food processing techniques to introduce phytochemicals into a range of meat products and that will guarantee microbiological safety and good sensory quality. These new technologies will be transferred to the other end-users to demonstrate their applicability in industrial settings after finalization of the project.

The new meat products have been evaluated in a human dietary intervention study to establish their effect on cancer risk markers in colonic tissues using the newest genomics techniques available.
Elaborate consumer studies have been performed to evaluate the response to the newly developed products. Both consumer acceptance and the willingness to buy this new type of products were tested, and crucial information to support marketing strategies has been obtained.

The European and national SME associations have an excellent network to disseminate the results of the PHYTOME project across the entire EU meat processing sector, stimulating the exploitation of the identified growth market for ‘light and healthy’ meat products.

A description of the main S&T results/foregrounds

Consumption of meat products in most EU countries is stagnating and consumers are becoming more critical with regard to health and safety aspects of foods in general, and therefore also regarding meat products. As a consequence, the market segment indicated as 'light and healthy' is indeed the main segment in the market for meat products showing considerable growth during the last 10-15 years. Therefore, strengthening the innovation capacity of EU meat processing industry in this particular market segment, by bringing new technology based products on the market, would enable a better exploitation of this specific growth market. Consumer research commissioned by the meat processing industry, has however shown that consumer perception is negatively influenced by messages in the media that unhealthy diets are (among other things) characterised by high meat consumption, and this image of meat products is further influenced by the potential health risks associated with the addition of preservatives such as nitrite, indicated as an E-number on the label.

On the other hand, nitrite is added for good reasons: it is important to control pathogenic microbes, to control oxidation and rancidity and to ensure an appealing pink meat colour, which is also desired by the consumers. Therefore, the problem that the meat processing industry needs to solve is to find innovative technological solutions that allow for the reduction or replacement of nitrite without hampering microbiological food safety and losing sensory quality, particularly taste and colour. The successful introduction of such new meat processing technologies would enable further expansion of the market for healthy meat products.

Additionally, the ongoing discussion on regulation of nitrite in meat products and on the European standard for added nitrite forces the meat industry into a more proactive role in the risk-benefit evaluation of nitrite in meat and the search for better alternatives. These issues affect the whole meat processing industry and therefore the European and national SME associations are eager to find a solution for all their members.

The aim of the PHYTOME project is to develop new meat processing technologies, resulting in innovative meat products that have low or no nitrite and that have been shown to contribute to improved human health. This will be achieved by introducing carefully selected mixtures of biologically active compounds originating from natural plant extracts. The active compounds, referred to as phytochemicals, are found in a wide range of vegetables and fruits and that are known to have beneficial health effects. These compounds also possess antimicrobial activity and may therefore contribute to microbiological safety of the product. Most importantly, they are known to reduce the formation of NOC in the human body when consumed simultaneously with meat products. Phytochemicals are also known to protect the gut from the induction of for instance oxidative genetic damage by other dietary factors and thus adverse health effects.

By developing and evaluating an innovative concept for healthy meat products, the PHYTOME project will deliver optimized food processing techniques to introduce phytochemicals into a range of meat products, and that guarantees their stability during various ways of processing. The new products will be evaluated for microbiological safety and used in a well-controlled human dietary intervention study to establish their impact on human health. As consumers are becoming more aware of healthy alternatives in their diet, it will be evaluated how they respond to the newly developed products. After designing communication strategies, prototype products will be produced along with specific label texts. Both consumer acceptance and the willingness to buy this new type of products will be tested.

To demonstrate the applicability of the concept in the final industrial setting, all outcomes of the project, including the selection of compounds, the new processing technologies, the established human health impact and marketing strategies, will be applied in a business case in which the SME partners will produce a variety of new technology based products for evaluation.

As the newly developed meat processing technologies will have to be transferred from research laboratory conditions to industry, the meat processing SME associations play a key role in the dissemination and implementation of knowledge. The European and national SME associations have an excellent network for disseminating the results of the RTD work across their members, relevant regulatory authorities such as the EFSA, across the entire meat processing sector, and to consumer organizations.

The project consists of 3 separate clusters of activities and 8 different work packages that all have very strong interactions. The coherence between the various elements is illustrated in figure 1.

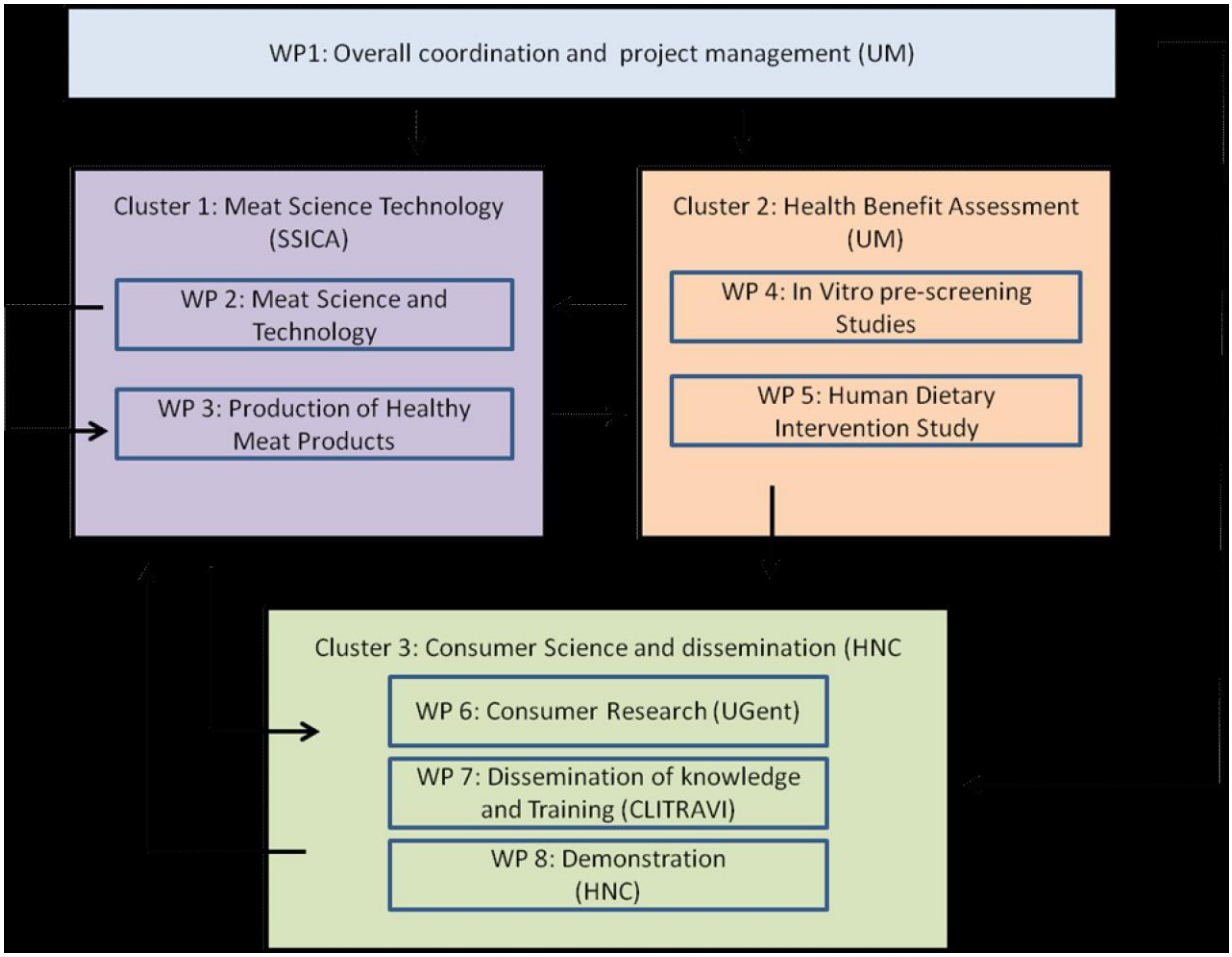


Figure 1: Project Flow Diagram

Cluster 1 Meat Science and Technology
This cluster includes all activities related to "Meat Science" and the development of new technologies, as well as the application of these new technologies by the participating end-users and the production of meat products that have been used for evaluation in cluster 2.

Cluster 2: Health benefit assessment
The second cluster is focussed on the Health Benefit Assessment of the phytochemicals and extracts to be used in meat processing as well as the phytochemical enriched meat products after human consumption. Cluster 2 provided the input on selection, suitability and affectivity of phytochemicals and natural extracts that have been used in the meat technology cluster. Cluster 1 produced and delivered a range of new meat products for the health impact assessment in the human study in cluster 2. Typical health messages derived from the human study in cluster 2 have been evaluated in the consumer research in cluster 3.

Cluster 3: Consumer sciences
This cluster combines the Consumer Sciences, Training and Dissemination activities of the SME-AGs, focussing both on the transfer of knowledge within the project, particularly with regard to the implementation of new meat processing technology from SSICA to the end-users in the project, and dissemination of the final outcome the project to the SME-AG members and the general public.
As a business case, a series of demonstration activities have been done to evaluate the applicability of the new technologies in industrial settings and to establish sensory quality, microbiological safety and consumer perception of the products produced by the end-users.

In the following pages, the list of main achievements of the PHYTOME project is reported with a brief description of main activities. More details are reported in the referenced Deliverable documents. Deliverables and Milestone description are also reported.

WP1 - COORDINATION AND MANAGEMENT
WP LEADER: COORDINATOR UM

Duration Planned: 36 months; Actual: 36 months

Partner	Main Tasks
COORDINATOR UM	***Communication with the European Commission*** Partner 1 - Coordinator - has been the intermediary for any communication between the EC and partners during the duration of the project. ***Administration of the financial contribution*** The Community financial contribution was carefully managed regarding its allocation between partners and activities taken by the consortium. All payments were made without delay and records have been kept in order to determine at any time of what portion of the Community financing has been paid to each beneficiary. The Commission was informed of the distribution of the contribution. The cost claim process was carried out through the FORCE system. The planning of resources has been done so as to accommodate a realistic distribution of resources, based on the ongoing project situation. ***Communication and Reporting*** Communication within the consortium has been ensured through the normal means such as electronic mail, teleconferences and in-person meetings. Technical documentation generated by the Project have been exchanged by using the internal Partners system of the PHYTOME website and made available to partners. A mailing list service has been developed. ***Legal and administrative issues*** The Project Management Team (PMT) has established the rules for the access and exploitation of the background of the individual partners and of foreground. ***Definition of the Advisory Board*** An independent Scientific Advisory Board (SAB) has been set up, identified per their expertise and have been asked to contribute to specific activities and meetings. ***Organization of project meetings*** A plan of meetings has been agreed among partners at project start. Partner 1 took care of the logistics and organisation of the project meetings, ensuring that all partners took part according to their role and

	responsibilities. The Project Management Team supported the Project Coordinator in the definition of the agenda and in the collection of presentation by the partners. Minutes have been collected, distributed to partners. Also teleconferences have been held. ***Harmonisation of procedures and documentation*** Common operational procedures, written guidelines, operational instructions and common templates have been produced at the beginning of the project. Common templates and forms have been made available through the website. An internal deliverable review process has been put in place in order to ensure compliance with planned milestones and the overall quality of the outputs that are produced. ***Knowledge management*** Matters related to Confidentiality and IPR handling have been defined in Consortium Agreement and monitored during the reporting period.

Results Achieved

Deliverable N.	Due Date	Actual Date	Deliverable Description
D1.1	M01	M02	PHYTOME Press Release
D1.2	M02	M02	Consortium Agreement PHYTOME
D1.3	M25	M26	Report on Exploitation plan of IP

Additional comments: None

WP2 - INVESTIGATION OF MEAT MODEL SYSTEMS
WP LEADER: P1 UM

Duration Planned: 9 months; Actual: 9 months

Partner	Main Tasks
P1 UM P6 VDBILT P7 LUSETTI P10 SSICA	According to the overall goal of the PHYTOME project, the WP2 aimed at studying and developing innovative formulations and technological procedures for new meat products to a) be enriched in natural extracts as a source of bioactive polyphenolic substances with antioxidant and chemoprotective properties, and b) contain no or reduced levels of added nitrite in order to limit the endogenous formation of *N*-nitrosocompounds (NOCs). To this end, the workpackage was based on a coordinated approach from the Consortium members, in particular from UM and URead as scientific partners, and HNC and VDBILT and Lusetti as end-users. They played a role in several testing procedures and were involved in the choice of natural extracts and pigments to be incorporated in meat products, as well as in the assessment of the stability of the bioactive substances, the development of revised technologies allowing the fortification of the meat products still warranting their sensory qualities. The technological and experimental trials were aiming to: - Provide a list of natural substances rich in phytochemicals and red pigments known to be biologically active and/or able to impart red colour to a meat matrix; - Develop the most optimal procedures for supplementation of selected

	natural extracts and pigments to meat model systems, designed to represent major meat categories; - Releasing technical guidelines for the manufacturing of meat products with selected phytochemicals and natural pigments and with reduced or no nitrite (WP3), meeting the needs of the human intervention studies (WP5). The issue of polyphenol fortification and concurrent nitrite reduction was carried out using several meat model systems, prepared to fit and reflect the major processing techniques currently used on a larger scale. Manufacturing guidelines were ultimately obtained from experimental trials based on real meat matrices including cooked and dried sausages and cooked and dried loins. **Natural extracts** A selected range of natural extracts rich in bioactive molecules were shown suitable for meat product fortification. They comprised Resveratrol and Quercetin and Green Tea as a source of Epigallocatechin Gallate; additional polyphenol sources were Oreganon, White Grape and Rosemary extracts. Acerola extract was identified as the natural source of ascorbic acid. All the extracts are listed in table 1, according to their polyphenol composition and the maximum level of addition in a meat matrix, still compatible with good sensory characteristics. Because plant polyphenol fortification could adversely impact some basic traits of meat products, such as colour, it was concluded that adding red natural pigments (i.e. Red beet extract, Carmine and Curcumin) alone or in combination with low nitrite doses was a means to achieve a satisfying colour in meat model systems, both dry cured or cooked. Natural extracts could also pose major problems for their incorporation into the meat, owing to the low solubility and poor diffusion of the polyphenol substances, especially in whole meats. Using dried loins, it was observed that brine immersion combined with pulse vacuum improved the absorption and the diffusion of polyphenols in the meat. **Nitrite elimination or reduction** As an additional step in meat product innovation, nitrite reduction and nitrite elimination were investigated and results compared with those obtained following full-nitrite formulations. Therefore, meat products were prepared with the lowest nitrite level ensuring safety and sensory requirements. Results showed that elimination or even reduction of nitrites could be addressed according to meat categories. Final formulations had to be tailored on individual meat class properties, after evaluation of functions and roles of meat technologies as adopted by meat industry. According to experimental results, the lowest nitrite addition in cooked meats was 25 mg/kg. In dry-cured meats, elimination of nitrite was found affordable hence recommended for further evaluation at the industrial scale manufacturing stage (WP3). **Technical procedures of innovative meat products** At the end of this work package the end-users (industrial partners) were provided with guidelines for the inclusion of relevant plant polyphenols in meat products, to be used with the pertinent amount of nitrite. It was concluded that fortification with appropriately selected extracts enables the preparation of a broad range of meat products with satisfactory sensory

	characteristics and a final level of total polyphenols ranging from 0.7 g/kg in cooked whole loins to 2 g/kg in dried loins, cooked and dried sausages

Results Achieved

Deliverable N.	Due Date	Actual Date	Deliverable Description
D2.1	M05	M05	A description of technical procedures
D2.2	M09	M16	Report on the phytochemical-nitrite and pigment combinations

Milestone N.	Due Date	Actual Date	Milestone Description
MS2.1	M09	M16	Decision on the procedures to be applied for manufacturing of meat products

Additional comments: None

WP3 - MEAT PROCESSING
WP LEADER: P6 VDBILT

Duration Planned: 26 months; Actual: 26 months

Partner	Main Tasks
P6 VDBILT P7 LUSETTI P8 VDBORRE	The main aim of WP3 was to complete the manufacturing stage of meat products that, according to the scheduled tasks, were to be prepared at industrial level and made available for the human intervention (WP5) and consumer studies (WP6). Therefore, the innovative formulations and the modified processing techniques, designed to promote the phytochemical incorporation, were independently applied by the SMEs (Vanden Borre, Van De Bilt and Lusetti), with SSICA as supervisor. Meat products, including cooked ham, cooked sausage, dry cured ham, and dry fermented sausages (northern and southern style) were prepared by the SMEs following recipes and recommendations contained in D3.1 'Formulations and recommendations for manufacturing meat products', and MS7.1, 'Meat processing techniques transferred to end-users'. Main results: - **Uptake of phytochemicals** As expected, the overall amounts of phytochemicals, including polyphenols and ascorbic acid (all from natural sources), were largely greater in minced meats (cooked or dried), around 2 g/kg of total polyphenols, than in whole cuts (hams, cooked and dried) [Figure1]. In deboned dried hams, absorption was successfully stimulated using brine vacuum impregnation in the salting stage (around 1.5 g/kg)) [Figure 1]. Results from the cold storage test showed that total polyphenols were fairly stable throughout the storage at -2°C, regardless of nitrite addition. Ascorbic acid was likewise at constant concentrations under the same conditions in both dried ham classes. In contrast, ascorbic acid levels declined in all minced meats, especially where nitrite was at low levels (range of depletion rate 10-25%). Accordingly, sausages made with standard nitrite levels exhibited a negligible loss of ascorbate throughout

	their storage time. - **Nitrite reduction** The additive could be eliminated in dried hams and in the sausages made according to South Europe style. In the remaining meat items, the molecule was reduced to a minimum level of either 25 mg/kg (cooked ham, cooked sausages and northern style dry sausages) or 75 mg/kg (dried hams, brine injection). - **Microbiology** Each individual product used in human intervention studies was submitted to microbiological analysis. Results showed the absence of these major pathogens: *Listeria monocytogenes, Salmonella spp., Sulphite reducing bacteria, and Staphylococcus aureus*. *Bacillus cereus* were negative in cooked hams. - **Colour and other sensory properties** Colour was susceptible to changes in reformulated products, with major differences observed in lowest- vs full-nitrite items. Main downsides were pale or brownish colour, or spotted surface, the latter mainly affecting whole cuts [Figure 2]. In selected cases, adding an extra pigment proved effective, helping offset differences with the control meat product. - Oxidation. According to chemical measurements (TBARs), there was no occurrence of oxidation. Rancidity, when perceived, was at extremely low levels, even where nitrite was removed, reflecting the ability of polyphenols and ascorbic acid to prevent oxidative reactions. The incorporation of polyphenols as a means to improve the health profile of meat products could be successfully achieved by adapting existing technologies and reformulating current recipes to include adequate amounts of plant extracts. Depending on the meat item, and with the aid of natural red pigments, the inclusion of polyphenols was paralleled by a significant reduction of added nitrites, with no or limited impact on colour and overall acceptability.

Results Achieved

Deliverable N.	Due Date	Actual Date	Deliverable Description
D3.1	M19	M20	Report manufacturing technologies
D3.2	M32	M20	Report data analyses results

Milestone N.	Due Date	Actual Date	Milestone Description
MS3.1	M12	M13	Industrial-scale manufacturing

Additional comments: None

WP4 - IN VITRO PRE-SCREENING STUDIES
WP LEADER: P1 UM

Duration Planned: 36 months; Actual: 36 months

Partner	Main Tasks
P1 UM	In order to establish the actual phytochemical content of the natural extracts to be used in the production of processed meat products, and to identify the extracts showing the strongest radical scavenging and oxidative damage preventive effects, a pre-selection of potentially relevant extracts had to be made. In accordance with the description of work, a literature search on bioactive compounds that inhibit endogenous nitrosation and activate cellular defence systems was performed by UM to establish the most effective compounds. Using this information, a search was made for commercially available natural extracts containing these phytochemicals. The phytochemical content of these products was established by SSICA (total polyphenols) and UREAD using LC-MS analysis, whereas the radical scavenging potential and preventive effect was measured by UM, using Electron Spin Resonance Spectroscopy (ESR) and alkaline single cell gel electrophoresis, or so called COMET assay. Although quite persistent technical problems occurred during the first phase of the project related to the cell culturing, the data of the cell-free system and the chemical analysis could be used to select the preferred natural extracts for further use. Therefore, the key objectives have been met after the first reporting period. The remaining work in this workpackage therefore related to the operationalization of the COMET assay as this test was also needed in WP 5 for the assessment of the faecal water genotoxicity test. A final shortlist of natural extracts was created containing 24 substances, some of them from different companies. These 24 substances were further evaluated for their technical and sensory properties by WP2 and their phytochemical content was established by UREAD using GC-MS analysis. - Evaluation of the radical scavenging capacity of these natural extracts showed that both onion and, to a lesser extent, carmine were inducing radical formation, making them not suitable for further application as replacers of nitrite in processed meat products; - Green tea, cranberry, jasmine, grape seed and acerola showed to have the highest antioxidant capacity in this cell-free system. These are therefore from the perspective of antioxidant potential the most promising candidates; - There were only small differences between tea and acerola brands and the dose-response curves were similar; however, big differences were observed between various rosemary brands. The Vivox version of rosemary appears to have a much lower capacity to scavenge radicals; - The outcome of the chemical analyses in combination with the radical scavenging potential was used to support the selection of extracts for further use in WP2 and 3.

Results Achieved

Deliverable N.	Due Date	Actual Date	Deliverable Description
D4.1	M08	M16	Report selection of phytochemicals
D4.2	M12	M17	Report protective effects natural extracts

Milestone N.	Due Date	Actual Date	Milestone Description
MS4.1	M06	M09	Advising of WP2 on phytochemicals to use in manufacturing of meat products

Additional comments: None

WP5 - HUMAN DIETARY INTERVENTION STUDY
WP LEADER: P1 UM

Duration Planned: 36 months; Actual: 36 months

Partner	Main Tasks
P1 UM P8 VDBORRE P9 UREAD P12 NHRF	This main task of WP 5 is to evaluate the impact of replacing or reducing the added nitrite levels in processed meat products by natural compounds on the formation of NOCs, DNA damage and gene expression changes as well as changes in the regulation of gene expression in humans. The consumption of processed meat products has been associated with colorectal cancer risk, and particularly the presence of nitrite as preservative has been suggested as one of the potential factors behind this association. WP2 and 3 have successfully produced a range of meat products in which nitrite has been replaced or reduced by adding natural plant extracts that are rich in antioxidants and other bioactive compounds that can take over the functionalities of nitrite. Nitrite is added to meat to control pathogenic microbes, oxidation and rancidity and to ensure an appealing pink meat colour. However, endogenous nitrosation catalysed by haem proteins induce the formation of carcinogenic N-nitroso compounds (NOCs), which are responsible for the link between red meat consumption and colorectal cancer (CRC) risk. The new meat products, with nitrite replaced by natural compounds, were evaluated in a human dietary intervention study to establish their effect on cancer risk markers in colonic tissues using evaluation of NOC levels, DNA damage analysis and by establishing changes in whole genome gene expression and DNA methylation. Overall, the data from the human dietary intervention study support the rationale behind the PHYTOME concept, that the introduction of natural extracts in combination with reduced amounts of added nitrite in processed meat products, results in the reduced formation of N-nitroso compounds and adverse reactions in the human large intestine. Molecular mechanisms have been identified which may mechanistically support this finding. The fact that the presence natural phytochemicals without the necessity of further nitrite reduction also has a reducing effect on NOC exposure levels, illustrates the importance of a well-balanced diet in colon cancer prevention. Furthermore, high drinking water nitrate was found to stimulate endogenous nitrosation. It is therefore crucial to take dietary intake of phytochemicals

	from fruits and vegetables, as well as drinking water nitrate into consideration when evaluating the risk of colon cancer risk associated with meat consumption.

Results Achieved

Deliverable N.	Due Date	Actual Date	Deliverable Description
D5.1	M03	M03	Written proposal to the Medical Ethics Committee
D5.2	M12	M17	Ethical clearance intervention study
D5.3	M36	M38	Report on potential health benefits

Milestone N.	Due Date	Actual Date	Milestone Description
MS5.1	M36	M38	Establishment of the potential health benefits of the consumption of the new type of meat products

Additional comments: None

WP6 - CONSUMER RESEARCH
WP LEADER: P6 VDBILT

Duration Planned: 36 months; Actual: 36 months

Partner	Main Tasks
P6 VDBILT P8 VDBORRE P11 UGent	Regarding the processed meat products with added natural compounds and reduced nitrite (PHYTOME meat products), three consumer studies have been conducted: **1. Qualitative Focus Group Discussions** *Exploration of consumer and stakeholder opinions* (n = 54 in BE, NL, GE and IT) - Reactions on the idea of nitrite replacement with phytochemicals were generally positive; - Despite low levels of awareness, nitrite had a negative health image among consumers, while natural extracts (phytochemicals) were perceived as natural and healthy; - Nitrite-replacing agents should fit with the meat matrix and be free of controversy or risk; - Sensory characteristics and proven healthiness were key attributes for acceptance; - Matching perceptions with facts about ingredients emerged as communication challenge. The work performed in Task 6.1 has been written up in the format of a scientific journal paper, which has been published in the scientific journal Food Control as: Hung, Y., Verbeke, W., & de Kok, T. M. (2016). Stakeholder and consumer reactions towards innovative processed meat products: Insights from a qualitative study about nitrite reduction and phytochemical addition. Food Control, 690-698.

	2. Quantitative Consumer Online Survey *Consumer attitude and purchase intention (n = 2057 in BE, NL, GE and IT)* - Half of the consumers had heard of nitrite being used in processed meat products; - The majority had fair knowledge about the function of nitrite; - Attitudes and purchase intentions towards the PHYTOME meat products were generally favourable; - Four consumer segments were identified: "enthusiasts", "accepters", "half-hearted" and "uninterested"; - As attitude emerged as the main driver for purchase intention, "accepters" and "half-hearted" were identified as the primary target segments of interests for marketing communications. **3. Experimental Consumer Study** *Consumer acceptance, appeal and willingness to pay (n = 221 in BE and n = 107 in NL)* - Sensory evaluation indicated apparent differences between the conventional and PHYTOME meat products and the PHYTOME meat products were proven to be acceptable among consumers; - Appearance of the conventional meat products was preferred, but consumers' preference shifted to the PHYTOME meat products after tasting; - Overall liking of the PHYTOME cooked sausage and of the cooked ham after sensory evaluation were important determinants of consumers' willingness-to-pay; - Consumers were not willing to pay more than the reference market price of similar conventional products for the PHYTOME meat products; - Reduced health risk was the most interesting information for consumers regarding the new meat products; and food labelling was the most frequently used medium to obtain information.

Results Achieved

Deliverable N.	Due Date	Actual Date	Deliverable Description
D6.1	M12	M12	Exploration of consumer and stakeholder opinions on reducing nitrite in meat product
D6.2	M24	M29	Report on consumer attitudes towards meat products that contribute to better gut health

Milestone N.	Due Date	Actual Date	Milestone Description
MS6.1	M36	M36	Consumer acceptance of different meat products is established

Additional comments: None

WP7 - DISSEMINATION OF KNOWLEDGE AND TRAINING
WP LEADER: P2 CLITRAVI

Duration Planned: 36 months; Actual: 36 months

Partner	Main Tasks
P12 CLITRAVI P3 VNV P4 FENAVIAN P5 HNC P7 LUSETTI P8 VDBORRE P10 SSICA P11 UGent	WP7 aims at setting and implementing a strategy for SME-AGs to disseminate the knowledge and experiences of the new processing techniques to other members of the associations as well as to share the results of the PHYTOME Project with external stakeholders. The PHYTOME project required a coordinated dissemination approach in order to stimulate the introduction of new processing technologies in the EU meat processing industry and to speed up the process of finding consumer acceptance. The dissemination strategy is focused on three different levels: 1. Within the consortium to facilitate the transfer of technological knowledge and knowhow from RTD to SME's; 2. Dissemination of knowledge to members of the SME-AG, to disseminate technological innovations widely within the meat processing industry, and 3. Informing stakeholders and consumers on the outcome and impact of the project. As part of the work in WP7, the consortium organized a training workshop in Parma (Italy) on 19 September 2013 for the transfer of knowledge on technological procedures from the RTD performers to SME's and SME associations. A workshop aimed at transferring knowledge on health impacts and consumers' attitudes to SMEs project partners and at discussing the outline of consumer studies and potential marketing strategies for the PHYTOME meat products has been organized in Maastricht in September 2014. Another workshop has been organized in Parma in June 2015 to discuss the outline of the Dissemination and Transfer of Knowledge strategy of the PHYTOME project. To disseminate technological innovations widely within the meat processing industry, the consortium organized specific sessions on the PHYTOME project at general assembly meetings for members of the national associations as well as for members of the European association (CLITRAVI). CLITRAVI members have been regularly informed on the progress of the PHYTOME Project as at every meeting of the Nutrition and Health working group of CLITRAVI (i.e. three times per year). An update on the PHYTOME project has been provided and the Project coordinator has attended some of those meetings to present the state of play. The website (www.phytome.eu) launched to disseminate the results on all different aspects of the project to CLITRAVI members was regularly updated. Newsletters have been produced and posted on the website and digitally distributed to the association members. Slides and outcome of the several meetings and workshops have been regularly uploaded on the website to allow an effective dissemination. A flyer in which the overall outcome is presented has been distributed at the end of the Project. To spread the results among the external stakeholders, in August 2015 (from 23rd to 28th August 2015) the first outcomes of the PHYTOME project were presented at the 61st International Congress of Meat Science and Technology (ICoMST) in Clermont-Ferrand. Separate meetings had been

	organized by the national partners. The final PHYTOME Project Dissemination Meeting was successfully organized on Monday, 16 November 2015 in the Crowne Plaza hotel in Brussels. There were about 65 participants from the meat manufacturing industry, the food ingredients industry, the retail sector as well national and European authorities. After the formal end of the meeting, the participants were invited to taste a traditional cooked ham and sausage versus a PHYTOME based cooked ham and a PHYTOME cooked sausage. A press release has been uploaded on the website. The PHYTOME Project has been mentioned by Commissioner for Research, Innovation and Science in an official reply to a written question from a member of the European Parliament, as well as by several media.

Results Achieved

Deliverable N.	Due Date	Actual Date	Deliverable Description
D7.1	M03	M04	Operational PHYTHOME website
D7.2	M15	M17	Interim plan for the use and Dissemination of Knowledge
D7.3	M36	M37	Project flyer
D7.4	M36	-	Video Clip
D7.5	M36	M36	Final Plan Use and Dissemination of Knowledge

Milestone N.	Due Date	Actual Date	Milestone Description
MS7.1	M24	M27	Meat processing techniques transferred to end-users

Additional comments: In the "Technical Annex 1 - Description of Work" on page 16-17 a Deliverable Report "D7.4 Video clip" has been scheduled. However, the Executive Board decided not to produce a video clip based on the following argument: the variety of different target groups (SME-AG's and end users, regulatory experts, consumer organisations, retail platforms health advice platforms etc.) makes a single video clip of limited added value to the final brochure that will be produced as a Deliverable Report D7.3.

WP8 - DEMONSTRATION
WP LEADER: P2 CLITRAVI

Duration Planned: 36 months; Actual: 36 months

Partner	Main Tasks
P2 CLITRAVI P5 HNC P7 LUSETTI P8 VDBORRE P10 SSICA	The main task of this Workpackage was to demonstrate that the transfer of knowledge on meat processing procedures and technologies can be successfully applied by end-users and to demonstrate that the products produced by the end-users satisfy the required quality criteria. In the first meetings with participants of associations and SME's involved in the project it was suggested that effective communication and implementation would be easier in case we could realize meat concepts without any E numbers and full elimination of nitrite this is not the primary objective of the project). Having this in mind Partner HNC participated in the qualitative stakeholders studies, in order to advice on urgency and priority of this additional demand. The high priority for good sensory quality lead to the

	decision that full elimination of nitrite in all products would not be feasible or necessary. We decided to do additional meat model studies at partner SSICA. We decided to carry out additional pilot studies at the SME's, before starting the work on WP3, resulting in batches of meat for the human intervention study. This resulted in adaptations of the meat process for those products where phytochemicals were injected. Also we changed some phytochemical products because of their negative effect on distribution of product and colour when injecting. We also learned from this exercise that it is important to work on a communication plan and effective ways of claiming/ communicating health benefits is earlier stages. Potential strategies, as proposed by WP6 after completion of the quantitative consumer survey, have been discussed wiith stakeholders before starting the final demonstration. Processed meat products were produced by the SME partners using the selected phytochemicals and procedures proposed by WP2 and WP4. These products have been used for further quality control and in the human dietary intervention study.

Results Achieved

Deliverable N.	Due Date	Actual Date	Deliverable Description
D8.1	M36	M38	Report on safety new products
D8.2	M36	M38	Report on consumer evaluation

Milestone N.	Due Date	Actual Date	Milestone Description
MS8.1	M33	M36	Availability of meat products for further evaluation

Additional comments: None

2 The potential impact

2.1 Potential impact

The results of the PHYTOME project have a clear and demonstrable beneficial impact for the SME members of the SME-AGs participating in the consortium. The knowledge obtained regarding natural extracts that can be used in meat processing and the optimized and validated procedures to be applied during the production process can immediately be applied in practice. This has been demonstrated during the last phase of the project, where the participating end-users have adapted the developed technologies for the production of phytochemical enriched products that are being evaluated in house for sensory effects and microbiological safety. Market research has demonstrated that the segment of innovative and light products is growing faster than for traditional products, indicating the potency of further growth. The availability of healthier alternatives to traditional meat products that are increasingly imported from outside of Europe gives the EU SME-AG members a clear competitive advantage.

Although the discussion on standards for nitrite levels in meat products is ongoing for several decades, only little advancements have been made in finding good and safe alternatives. On the other hand, the scientific basis for standard setting for safe nitrite levels is rather weak and does not take carcinogenic risk into consideration. As a consequence, there is a strong and ongoing scientific debate on safety regulation of nitrite (as well as nitrate). The outcome of the PHYTOME project makes a major contribution to the risk-benefit evaluation of nitrite in meat products, as it is unique in its approach to evaluate the effect of processed meat products with different levels of added nitrite. The benefit for the SME members lies in the fact that a better substantiated nitrite standard may end this ongoing discussion on the safety of added nitrite, and that demonstration that the combination of phytochemicals and nitrite levels at or below the standard are safe indeed. In order to guarantee the impact of the project results on EU policy making, dissemination activities towards policy makers at European and National level have been the main focus of WP7.

The PHYTOME project brings new products to the market that has a quantified and validated impact on human exposure and on human health risks. This health impact does not depend on individual decisions to change unhealthy and unbalanced dietary habits, which in practice are difficult to realize, but is achieved by combining different dietary ingredients into a more balanced product. This implies that meat products produced according to the new concept can be part of health promotion campaigns that aim to stimulate more balanced dietary habits. Successful implementation of the new technologies in the European meat processing industry, accompanied by a good marketing and promotion strategy that are also main outcomes of the project, will contribute to the reduction of chronic disease, improved quality of life and an overall healthier population.

2.2 Main dissemination activities

The PHYTOME project required a coordinated dissemination approach in order to stimulate the introduction of new processing technologies in the EU meat processing industry and to speed up the process of finding consumer acceptance. This required an adequate dissemination strategy at three different levels: 1) within the consortium to facilitate the transfer of technological knowledge and knowhow from RTD to end-users; 2) with members of the SME-AG, to disseminate technological innovations widely within the meat processing industry, and 3) with stakeholders and consumers.
The PHYTOME project has act correspondingly, through its active dissemination strategy, according to the EU FP7 Grant Agreement which required project participants to communicate and engage with actors beyond the research community. The dissemination and training work package has been organised as a separate Workpackage as an element of the coordinating activities, and under the guidance the Steering Committee consisting of SME-AG members, and strongly collaborating with the other PHTYOME partners and the international community of stakeholders. The dissemination plan

has considered adequate messages about the objectives of the project and its societal and economic impact. The tools that have been used included web-based communication, press releases, brochures, booklets, multimedia material, etc. The 'dissemination material' has been regularly updated to provide the latest version of the project status and objectives.

In the PHYTOME project, the new technologies have been developed and evaluated for the major meat products with the largest market share. Now the concept can be further developed and tailored to other product types. It is intended that the SME-AG's will continue to stimulate the exploitation of the project results by continuing to demonstrate the economic benefits to their SME members.

The cooperation between the SME-AG at an international level, coming from different member states and including the European Association CLITRAVI, resulted in a concerted and proactive action to improve the quality and consumer perception of meat products in Europe. Being in the position to steer the direction of the research, the SME-AG are now able to ensure that their member SME's will benefit maximally from the outcome of the project and will be able to serve both national and international markets. As a result of this combined international effort, the meat processing industry will be less vulnerable to changes in consumer demands for healthier products.

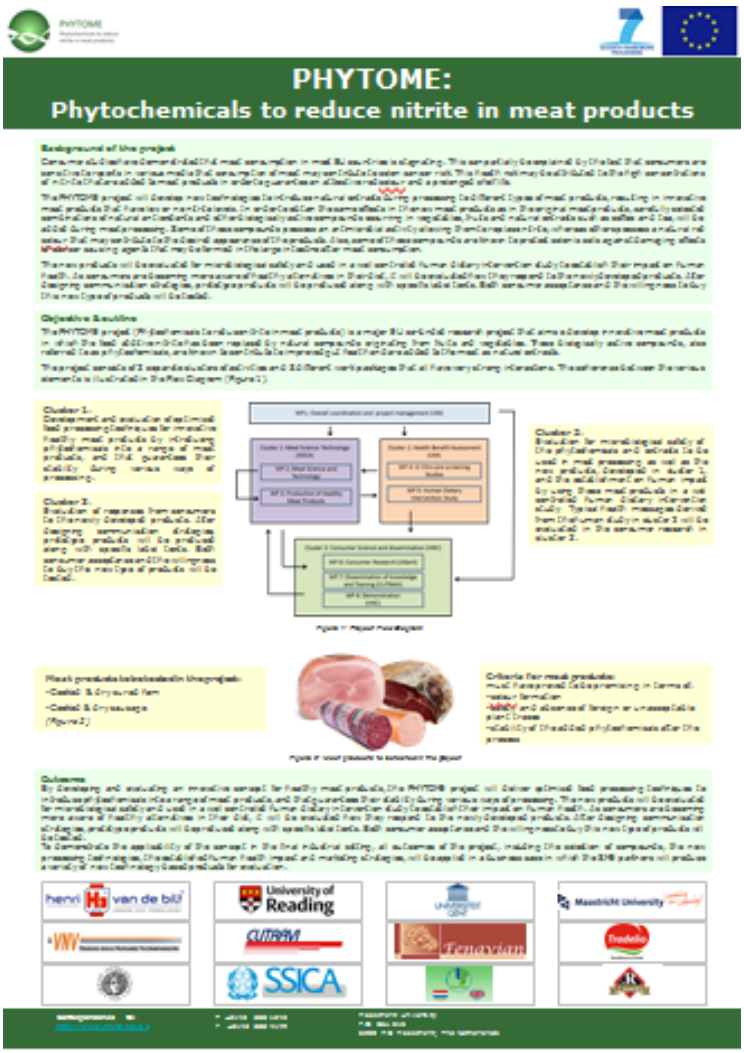
2.3 Dissemination material

The PHYTOME project logo has been used in all dissemination material, distributed during scientific meetings, conferences at national, European and international level.

The multiplicity of scientific, industrial, and regulatory stakeholders to the PHYTOME project constitutes a complex, multi-layered environment for disseminating the project's results. In this respect, important stakeholders to address were the EU's regulatory authorities such as the European Food Safety Authority (EFSA) and relevant industrial trade organizations, the International Life Sciences Institute (ILSI) and particularly specific ILSI-expert groups were targeted. Furthermore, the reconciling of the communications goals of the consortium and those of the EU also entails addressing a very broad range of recipients. Because of the major interest of the general public concerning food safety there is an equal responsibility to show citizens and non-governmental organizations the outcome of EU funded research.

The outcome of the PHYTOME project has made a significant contribution to the risk-benefit evaluation of nitrite in meat products, as it is unique in its approach to evaluate the effect of processed meat products with different levels of added nitrite. The benefit for the SME members lies in the fact that a better substantiated nitrite standard may end this discussion on the safety of added nitrite, and that the results of the project may demonstrate that the combination of phytochemicals and nitrite levels at or below the standard are safe indeed.

PHYTOME Poster: a project poster highlighs the structure of the PHYTOME project in collaboration with the entire consortium. Together with the rest of the dissemination material, this poster has been made available to partners and others through to PHYTOME project website.



Press release

A press release in English has been produced and used to approach journalists and journals to raise the awareness of the PHYTOME project to a wider audience, not limited to that belonging in the scientific and technologic fields: http://www.phytome.eu/v2/mediacenter.html

2.4 Address of the project public website, as well as relevant contact details

The address of the project website is: www.phytome.eu

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