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. This congress takes place in a different country every year, alternating between the 5 continents. There were more than 400 meat scientists and meat technologists and stakeholders from more than 40 countries. At such a forum one can share knowledge and discuss scientific and technical advances in the meat sector. Organized in half-day sessions, the Congress addressed a whole range of issues linked to meat consumption, production, technology, and marketing.

In conjunction with Session 10 entitled “Meat, Nutrition and Health” two abstracts (both attached to this Newsletter) were presented:

One abstract entitled “Health benefits of meat products with reduced nitrite levels: the PHYTOME Project” was presented by the Universities of Reading and Maastricht. The preliminary conclusions of the health impact assessment was that the addition of natural extracts and the reduction of added nitrite may indeed reduce the formation of N-nitroso compounds. As these compounds are known to induce genetic damage in the colon and are found to modify molecular processes that are involved in cancer formation, the PHYTOME meat products may reduce health risks associated with such exposures. These finding are fully in line with the research hypothesis of the project.

Another abstract was entitled “Bioactive natural polyphenols in reformulated meat products. Effects on quality traits” was represented by the Parma based “Stazione Sperimentale Conserve Alimentari” Here the technical procedures to introduce natural extracts to replace nitrite were presented. The resulting meat products retained considerable amounts of the added polyphenols while exhibiting acceptable sensory properties and a regular shelf life behaviour. Adding natural red pigments was found to be a useful aid in nitrite reduction, helping to limit the negative effects of reduced nitrite levels on colour development.

Finally, in session 11, on consumer attitudes and meat consumption, a third abstract was presented: “Consumer attitude and purchase intention towards processed meat products with natural compounds and reduced nitrite” was presented by the University of Ghent. Here the consumer attitude and purchase intention towards meat products with added natural compounds and reduced nitrite content (wNCrN) was presented as major outcome of the Consumer Sciences workpackage of the PHYTOME project. Cross-sectional data were collected in Belgium, The Netherlands, Italy and Germany (n=2057). It was found that consumers generally expressed favourable attitudes and purchase intentions. Based on these two criteria, four consumer segments were identified: “enthusiasts”, “accepters”, “half-hearted” and “uninterested”.

At the 5 October 2015 CLITRAVI Nutrition and Health working group meeting, Prof. Theo De Kok, Project Coordinator of the PHYTOME Project, gave the state of play on the Project. Among others, he told CLITRAVI members that incorporation of polyphenols as a means to improve the health profile of meat products can be successfully achieved by adapting existing technologies and reformulating current recipes to include adequate amounts of plant extracts.

At that meeting a discussion also took place on a draft PHYTOME Flyer. As a number of members had not seen the draft, CLITRAVI members asked for more time to comment on the flyer.
BIOACTIVE NATURAL POLYPHENOLS IN REFORMULATED MEAT PRODUCTS. EFFECTS ON QUALITY TRAITS.
Saccani G., Barbieri Gp., Bergamaschi M., Blasi C., Franceschini M., Parolari G.
Stazione Sperimentale Conserve Alimentari, Viale Tanara 31A, 43121Parma (Italy)

Abstract – Consumption of meat products has been challenged for their potential role in the onset of chronic diseases. In this study, a broad range of popular meat derivatives including dry and cooked sausages, and dry and cooked hams were reformulated and their processing techniques revised to enable the inclusion of polyphenol-rich plant extracts and a concurrent reduction or even elimination of added nitrates. The resulting meat products retained considerable amounts of the added polyphenols while exhibiting acceptable sensory properties and a regular shelf life behaviour. Adding natural red pigments was an aid in nitrite reduction, helping to limit the negative effects on colour. However, differences were found between the family of mince (cooked and dried sausages) and whole (cooked and dried hams) meats, with the latter exhibiting worse colour traits when lower nitrite levels were used in combination with plant extracts.

Key Words – Meat products, nitrite, plant extracts

I. INTRODUCTION

In the last decade, several studies have been focused on the potential health risks linked to meat preservatives such as nitrite and salt, and the possible relationship between processed meat and cancer [1]. Therefore, innovative meat products and technologies have been investigated in order to protect and even promote consumer’s health. To achieve this objective, the potential role of biologically active polyphenols as meat ingredients has been suggested [2]. Plant-derived polyphenols such as flavonoids, flavones, anthocyanins, proved able to exert a chemomodulatory effect through a variety of physiological processes. Amongst them, the promotion of a chemoprotective activity against chronic diseases such as colon cancer has been postulated [3-4]. Many naturally occurring herbs, fruits and vegetables are packed with bioactive substances and a variety of commercially available plant extracts have been manufactured to deliver a broad range of stable polyphenols for use as ingredients in foods, including meat products. From a technical point of view, incorporating polyphenol-based extracts or their crude sources in the meat matrix is a complex task, for their potentially adverse impacts on colour, texture, flavour and overall acceptability. Whether a powdered solid or an aqueous or oil extract, polyphenols can pose major problems in terms of compatibility, especially when they are intended for inclusion in whole muscles or large meat cuts such as hams or shoulders. The scope of this study was to investigate the effect of natural extract supplementation in six major meat products, with special emphasis on their quality and sensory traits. To this aim meat products were reformulated and processed using two possible levels of nitrates and compared to the standard production. The meat items were chosen to represent some of the most popular meat products available throughout Europe, i.e. cooked ham (brine injected, BI), cooked Bologna sausage, Northern- and Southern-style dry-fermented sausages, and boneless dry-cured ham (brine injected, BI and brine vacuum impregnated, BVI) [5].

II. MATERIALS AND METHODS

For each meat item, three batches were manufactured:
(1) Control, with a standard ingoing amount of preservative (sodium nitrite or a mix of sodium nitrite/sodium nitrate), (2) std-NO2, standard preservative and a blend of polyphenols, and (3) low-NO2, without nitrite (Southern-style sausage and dried ham BVI), or 75mg/Kg (dried ham BI) or 25mg/Kg (cooked ham, cooked Bologna and Northern-style dry sausages).

Natural red pigments, allowed by current regulations, were added in low-NO2, except in dried ham. The amounts of polyphenols, added in
each meat product by means of plant extracts, were identified in preliminary trials (Table 1).

Table 1. Added amounts of polyphenols in the meat products

<table>
<thead>
<tr>
<th>Product</th>
<th>Polyphenols (g/Kg of meat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bologna sausages</td>
<td>2.5</td>
</tr>
<tr>
<td>Dry sausages (NS)</td>
<td>2.0</td>
</tr>
<tr>
<td>Dry sausages (SS)</td>
<td>2.0</td>
</tr>
<tr>
<td>Dried ham (BI) - 10% of injection</td>
<td>5.7</td>
</tr>
<tr>
<td>Cooked ham (BI) - 10% of injection</td>
<td>6.6</td>
</tr>
<tr>
<td>Dried ham (BVI) - ratio brine:meat (1:3)</td>
<td>33</td>
</tr>
</tbody>
</table>

Major chemical components, total polyphenol content, ascorbic acid and lipid oxidation index were assessed for all meat formulations (N.3 samples for each formulation). Moisture, fat, protein and ash were determined according to the Official Methods 950.46, 991.36, 981.10, and 920.153, respectively [6]. The concentration of total phenolic compounds in meat samples was determined by the Folin-Ciocalteu method, following the extraction procedure reported by Rituparna et al. [7]; results were expressed in g of gallic acid equivalents (GAE) per kg of meat sample. Ascorbic acid was evaluated according to the procedure reported by Valls et al. [8]; results were expressed as mg ascorbic acid per kg of meat product. Sensory qualities of meat products were assessed by a trained panel of 8 members, using a quantitative descriptive analysis method [9], to evaluate the effect of natural polyphenols supplementation on the final sensory traits of each meat item. Panelists independently evaluated color, odor, taste and overall acceptability of whole and sliced meat products, focusing on foreign flavor or taste possibly generated by plant extract supplementation. Each sensory attribute was scored on a non-structured 0-9 intensity scale, where 0 and 9 identify the lowest and the highest attribute intensity, respectively.

Data were analyzed using the ANOVA procedure of SPSS package ver. 21; significant differences between the formulations for each meat item were investigated by the LSD procedure.

III. RESULTS AND DISCUSSION

The polyphenol concentrations in meat products supplemented with plant extracts are graphically reported in Fig. 1.

Figure 1. Concentration of total polyphenols in the meat products manufactured with natural polyphenols and two categories of sodium nitrite addition.

Values (in gallic acid equivalents) show that the available contents are dependent on the added plant extract amounts and the processing technique. In minced meats, thermal treatment results in a loss of the polyphenols compared with their fermented counterparts, where the plant substances are stable or even increased as a consequence of meat dehydration. As a tendency, all the minced meat items exhibit lower gallic acid equivalents in samples prepared with standard than with reduced nitrite addition. This is particularly the case with cooked Bologna sausages, whose polyphenol concentrations differ statistically, (P<0.05) according to the added nitrites. Data in Fig. 1 document a much lower uptake of plant substances in the family of whole meat products, whether cooked or dried. However, when dried hams are considered, those treated by vacuum impregnation are clearly shown to absorb more polyphenols than their conventionally brine-injected peers. Residual amounts of ascorbic acid in the finished meats are reported in Table 2. Values reflect the combined effect of the processing technique and added nitrates, with lower ascorbate losses in those items where nitrite depletion was greater. This observation is in agreement with the known nitrite reactivity, leading to rapid consumption of ascorbic acid when the two substances are allowed to interact. This was likely the case in both cooked...
meats, as well as in northern-style dry sausages and in BVI dried hams, where nitrite was possibly lost as result of the process, when added at the lower concentration level. Being quickly wasted, there was no enough nitrite left to react with ascorbic acid, which thus persisted at higher levels in low- than in standard nitrite added meats. There is no obvious reason why the same phenomenon did not occur in southern style sausage and in brine injected dried hams, whose residual ascorbic contents were greater in standard nitrite addition. It can be supposed that in these products the ascorbate was more intensively involved as an antioxidant, replacing the wasted nitrates.

Table 2. Content of ascorbic acid in meat products manufactured with a supplementation of natural polyphenols at two different amounts of sodium nitrite.

<table>
<thead>
<tr>
<th></th>
<th>Ascorbic acid [mg/kg]*</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Std-NO2</td>
<td>Low-NO2</td>
</tr>
<tr>
<td>Bologna sausages</td>
<td>302±6</td>
<td>441±27</td>
</tr>
<tr>
<td>Dry sausages (NS)</td>
<td>365±28</td>
<td>425±20</td>
</tr>
<tr>
<td>Dry sausages (SS)</td>
<td>510±8</td>
<td>433±32</td>
</tr>
<tr>
<td>Dried ham (BI)</td>
<td>180±40</td>
<td>61±22</td>
</tr>
<tr>
<td>Dried ham (BVI)</td>
<td>38±2</td>
<td>431±71</td>
</tr>
<tr>
<td>Cooked ham (BI)</td>
<td>306±26</td>
<td>405±10</td>
</tr>
</tbody>
</table>

*Ascorbic acid differs (p<0.01) between std-NO2 and low-NO2.

Proximate composition data (Fig. 2) show that all products, excluding Bologna sausages, are a considerable source of protein, with dried meats ranging between 20-30%. Fat is relatively large in minced products, especially southern style dry sausages, whose 30% of fat essentially reflects the major shrinkage, hence concentration, resulting from dehydration. Nevertheless, the plant extract content is such that even a small portion will deliver a substantial amount of polyphenols with a relatively limited caloric intake.

As a rule, adding polyphenol-rich extracts affected selected sensory properties of reformulated meat products, including colour and flavour. This was the case with dried and cooked sausages and dried hams, where differences were found in the sensory attributes as assessed by the panelists. As shown in Fig. 3-4, where the sensory scores are graphically reported, dry (northern) and Bologna sausages received higher ratings when the low-nitrite formulation was compared with its standard counterpart, an outcome likely due to the natural red pigments added in the former (low-NO2) recipe.

Figure 2. Protein and fat content of meat products.

In whole dried meats (hams), colour assessment (Fig. 5) resulted in greater scores being assigned to standard-nitrite samples, whereas hams made with lower nitrite were impaired by brown spots and grey discoloration, two major drawbacks probably tied to the combined effect of polyphenols and low nitrite. Herbal or foreign notes could be perceived by some panelists tasting the most heavily polyphenol-enriched samples. In spite of this, the attribute proving most influential in terms of acceptance was colour, the trait found the primary driver for overall liking of reformulated meat products in this study.

Figure 3. Sensory analysis of dry sausages (NS): mean values (n=3) for each sensory descriptor by process formulation. For each descriptor the relevant
significance is reported (**p<0.01, *p<0.05, n.s. = not significant)

![Figure 4. Sensory analysis of Bologna sausages: mean values (n=3) for each sensory descriptor by process formulation.]

![Figure 5. Sensory analysis of dried hams (BVI): mean values (n=3) for each sensory descriptor by process formulation.]

**IV. CONCLUSION**

Incorporation of polyphenols as a means to improve the health profile of meat products can 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 can be paralleled by a significant reduction of added nitrates, with no or limited impact on colour and overall acceptability.

**ACKNOWLEDGEMENTS**

The authors acknowledge the financial support from the European Commission. This paper reports on results from work package 3 of the SME Associations project PHYTOME financed under the 7th Framework programme (EU-FP7 grant agreement no. 315683), entitled ‘Phytochemicals to reduce nitrite in meat products’.

**REFERENCES**


Abstract – This study investigates consumer attitude and purchase intention towards meat products with added natural compounds and reduced nitrite content (wNCrN). Cross-sectional data were collected in Belgium, The Netherlands, Italy and Germany (n=2057). Consumers generally expressed favourable attitudes and purchase intentions. Based on these two criteria, four consumer segments were identified: “enthusiasts”, “accepters”, “half-hearted” and “uninterested”. Purchase intention associated positively with: attitude towards processed meat products wNCrN; preference for natural over chemical additives in food including meat; perceived harmfulness of chemical additives; risk importance; attitude towards innovation in food; general health interest; educational level; and consumption frequency of processed meat products. Consumers from Italy and Germany had a lower level of purchase intention compared to the ones from Belgium. These results enable the planning of product development and marketing communication strategies tailored for specific target segments.

Key Words – Additives, Health, Marketing

I. INTRODUCTION

A causal relationship between intake of processed meat and risk of colorectal cancer was recently pointed out in a scientific report wherein experts recommend consumers to limit red meat and avoid processed meat consumption [1]. Alongside the consecutive waves of meat safety scares [2], the recent debates about the health consequences of processed meat products have made its production and consumption a controversial issue [3]. Owing to the trend of increased consumer demand for convenience, processed meat products remain important in the human diet despite these negative publicities [4]. Therefore, the development of improved meat products based on scientific knowledge is topical and offering potential benefits for both public health and processed meat marketing.

Product reformulation is a way to improve processed meat products whereby the content of potentially unhealthy ingredients and nutrients is reduced [5]. Nitrite is one of the preservatives commonly present in processed meat products, which was found to be associated with possible carcinogenic risks in epidemiological studies [6]. Meanwhile, natural compounds present in fruits and vegetables (phytochemicals) were proposed to be a suitable candidate for partially or completely replacing nitrite. These natural compounds are bioactive and known to have health-promoting efficacy [7], to exhibit strong antimicrobial activities [8], and to possess substantial anti-carcinogenic and anti-mutagenic properties [9]. Adding natural compounds to processed meat products can potentially exert protective effects on the human gut by reducing the amount of carcinogenic N-nitroso compounds formed and prevent the induction of oxidative genetic damage [7, 9]. This concept of new meat products with natural compounds and reduced nitrite (henceforth, "new meat products wNCrN") can be a promising solution to improve both the actual healthiness and the health image of processed meat products.

However, consumer acceptance cannot be taken for granted as they often express uncertainty about the health impact of new or functional food products [10, 11], e.g. due to the different perceptions regarding the derived health benefits [12]. Consumer perception of the new meat products wNCrN is an important determinant of their future consumption which can largely influence the meat industry’s competitiveness [13]. Hence, this study aimed to investigate consumer attitude and purchase intention for new meat products wNCrN, to identify market segments based on interest and to elucidate how this purchase intention is associated with various personal characteristics.
Several personal characteristics were selected based on literature, including five consumer characteristics such as attitudes towards the new meat products wNCrN, attitudes towards meat products with chemical additives, involvement in meat products (i.e. the perceived personal interest or importance evoked by processed meat products in relation to consumers’ enduring or situation-specific goals) [14], domain specific innovativeness (i.e. predisposition to buy new food products) [15], general health interest (i.e. orientations towards the healthiness of food and dieting behaviour) [16]; as well as a series of socio-demographics such as age, gender, educational level, and country of origin.

II. MATERIALS AND METHODS

Study design and sampling

Data were collected in December 2014 through a cross-sectional quantitative online survey with samples representative for age, gender and region in four European countries: Belgium (BE, n = 532), The Netherlands (NL, n = 501), Italy (IT, n = 502) and Germany (DE, n = 522). These countries were selected based on the importance of processed meat products and their national consumption levels. The total sample includes 2057 participants aged between 18 and 75 years.

Questionnaire and scaling

Attitudes towards meat products with chemical additives consisted of two distinct factors: (1) Preference for natural over chemical additives in food including meat (5 items, e.g. "Replacing chemical food additives with green tea extract makes meat products healthier") and (2) Perceived harmfulness of chemical additives (3 items, e.g. "Meat products containing chemical food additives are harmful to human health").

Involvement in meat products was measured based on four factors: Pleasure value (6 items, e.g. "Meat is very important to me"), Symbolic value (3 items, e.g. "My choice of meat gives other people an image of me"), Risk importance (3 items, e.g. "I would find a bad choice of meat terrible") and Risk probability (2 items, e.g. "I never know if I make the right choice of meat") [17]. Attitudes towards innovation in food were measured by 6 items of the domain specific innovativeness scale (DSI-scale) (e.g. "I buy new foods before other people do") [15]. General health interest was measured by 8 items (e.g. "The healthiness of food has little impact on my food choices") [16].

Consumer attitude towards the new meat products wNCrN was measured by asking their agreement with the statement “New meat products with natural compounds and reduced nitrite are healthy / of high quality / safe / nutritious / tasty”. Purchase intention towards the new meat products wNCrN was measured by means of three items (e.g. “I plan to try these new meat products with natural extracts instead of nitrite in the future”) [18]. Prior to answering the questions related to attitude and purchase intention, participants were introduced to the concept of the new meat products wNCrN: “Imagine there are some new meat products, in which natural compounds originating from fruits and vegetables (henceforth, named “natural compounds”) are added, and in this way, the food additive nitrite can be partially replaced.” All the items mentioned were measured on a five-point interval scale. Socio-demographics were assessed at the end of the questionnaire.

Statistical analyses

Data were analysed with SPSS Statistics 22.0. First, Cronbach’s alpha coefficients were computed to assess the internal consistency of the scales. Second, factor analysis was performed to determine the relationships among items measuring the same concepts. Third, consumer segmentation based on consumers’ attitude and purchase intention towards the new meat products wNCrN was done through cluster analysis. Lastly, multivariate regression analysis was performed to explain consumers’ purchase intention by means of various personal characteristics. The robust bootstrap method was used to account for issues of non-normality and heteroscedasticity.

III. RESULTS AND DISCUSSION

Consumers’ attitude and purchase intention towards the new meat products wNCrN were generally favourable (μ = 3.39 ± 0.71 and μ = 3.56 ± 0.79 on five-point scales, respectively); and
significantly and positively correlated (Spearman's 
 rho = 0.486, p-value <0.001). Based on the two 
 segmentation criteria, i.e. attitude and purchase 
 intention, a four-cluster solution was determined 
 as the optimal number of clusters. Segment 1 
 “Enthusiasts” accounted for 39.3% of the sample and 
 included consumers who reported the highest 
 mean score for attitude and purchase intention. 
 Participants from Belgium accounted for the 
 largest proportion of “Enthusiasts”. Segment 2 
 “Accepters” included 11.9% of the sample, in 
 which the participants had a strongly positive 
 attitude but moderate level of purchase intention. 
 Participants from Germany accounted for the 
 largest proportion of “Accepters”. Segment 3 
 “Half-hearted” (42.3% of the sample) included 
 participants who had moderate levels of attitude 
 and purchase intention. Segment 4 “Uninterested” 
 (6.5% of the sample) referred to consumers with 
 low levels of attitude and purchase intention. 
 Participants from Italy accounted for the largest 
 proportion of the “Uninterested” (Figure 1).

Moving along the groups from “Uninterested” to 
 “Half-hearted”, then to “Accepters” and 
 “Enthusiasts” indicates an increase in consumer 
 interests in the new meat products wNCrN. Based 
 on the segmentation, consumer interest was higher 
 among participants with a higher educational level, 
 better perceived financial situation and among 
 people having more frequent health check-ups.

A linear regression model was estimated to 
 explain the relationship between purchase 
 intention towards the new meat products wNCrN 
 and personal characteristics. The resulting model 
 accounted for 31.7% of the variance in the 
 reported purchase intention (Table 1).

Table 1 Linear regression model for reported purchase 
 intention towards the new meat products wNCrN

<table>
<thead>
<tr>
<th>Variables entered</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attitude towards the new meat products wNCrN</td>
<td>0.395**</td>
</tr>
<tr>
<td>Preference for natural over chemical additives in food including meat</td>
<td>0.135**</td>
</tr>
<tr>
<td>Italy (dummy)</td>
<td>-0.121**</td>
</tr>
<tr>
<td>Perceived harmfulness of chemical additives</td>
<td>0.118**</td>
</tr>
<tr>
<td>Risk importance</td>
<td>0.113**</td>
</tr>
<tr>
<td>Germany (dummy)</td>
<td>-0.111**</td>
</tr>
<tr>
<td>Attitude towards innovation in food (DSI score)</td>
<td>0.086**</td>
</tr>
<tr>
<td>General health interest</td>
<td>0.066*</td>
</tr>
<tr>
<td>Education level</td>
<td>0.046*</td>
</tr>
<tr>
<td>Processed meat product consumption frequency</td>
<td>0.044*</td>
</tr>
</tbody>
</table>

** p ≤ 0.001; *p < 0.05 based on robust bootstrap method.
β: standardized coefficient

Attitude towards the new meat products wNCrN was the main driver for European consumers’ purchase intention. Consumers who had a more positive attitude had a higher level of purchase intention. The standardized regression coefficient (β) indicates that the effect of attitude was almost three times greater than the second factor in the model. Other consumer characteristics having positive effects on purchase intention were: higher levels of preference for natural over chemical additives in food including meat, perceived harmfulness of chemical additives, risk importance, attitude towards innovation in food (DSI score), general health interest and processed meat product consumption frequency (in decreasing order of importance). These results were largely consistent with expectations. The level of purchase intention was higher with higher educational level. Compared to consumers in Belgium, consumers from Italy and Germany reported a lower level of purchase intention. Other socio-demographics variables did not have significant effects when simultaneously accounting for the aforementioned effects.

IV. CONCLUSION

The processed meat market segmentation yielded four consumer segments, which differ significantly in personal characteristics. Communication can thus be tailored according to the target segments. As attitude is the main driver for purchase
intention, “Accepters” and “Half-hearted” can be primary targets of interests. Since “Accepters” have positive attitudes, marketing and communication efforts stimulating trial (e.g. product sampling) can enhance their future purchase intention. For “Half-hearted”, their attitude can be made more positive through providing more extended information such as advertisements or product labelling that highlights the benefits of the new processed meat products.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from the European Commission. This research is the result from work package 6 of the SME Associations project PHYTOME financed under the 7th Framework programme (EU-FP7 grant agreement no. 315683), investigating the replacement of nitrite in processed meat products by natural compounds.

REFERENCES

HEALTH BENEFITS OF MEAT PRODUCTS WITH REDUCED NITRITE LEVELS: THE PHYTOME PROJECT

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Abstract – The European PHYTOME project (Phytochemicals to reduce nitrite in meat products), 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 (or phytochemicals), are known to contribute to improved gut health and are added to the meat as natural extracts. A range of products with reduced nitrite levels have been produced and the health promoting effects of these products are currently being evaluated in a human dietary intervention study with 80 healthy volunteers. After consumption of a fully controlled diet with either relatively high amounts of the traditional meat products or products produced following the new concept, faeces and colonic material are collected and investigated for markers of colorectal cancer risk. Preliminary data from a feasibility study (n=20) show that increased consumption of traditional processed meat products increase the capacity of faecal water to induce genetic damage to colonic cells, but does not increase faecal levels of N-nitroso compounds (NOC), a class of compounds hypothesized to explain the link between meat consumption and cancer risk. Faecal NOC levels were however increased by the combination of increased drinking water nitrate and meat consumption.

Key Words – Health promotion, Phytochemicals, nitrite reduction.

I. INTRODUCTION

Trend analyses indicate that the consumption of meat products in most EU countries is stagnating, with the exception of the market segment ‘light and healthy’. However, consumer perception is increasingly influenced by messages in the media that consumption of nitrite preserved meat products contributes to human cancer risk. The World Cancer Research Fund (WCRF) has classified the evidence for a causal relationship between intake of processed meat and risk of colorectal cancer as convincing and has recommended consumers to limit red meat and avoid processed meat consumption [1]. The proposed mechanism to explain this association involves the formation of N-nitroso compounds (NOC) in the intestinal tract which is stimulated by the combination of nitrite and haem iron, both factors known to be present in processed meat (Vermeer et al., 1998; Cross et al., 2003). 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 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. Some of these bioactive natural compounds possess antimicrobial activity that may allow replacement of nitrite without hampering microbiological safety [4]. Phytochemicals are also known to protect the gut from the induction of genetic damage and adverse health effects [5-7]. The industrial partners in the PHYTOME consortium have produced a range of meat products in which nitrite has been replaced or reduced by natural extracts containing a complex mixture of bioactive ingredients. In order to evaluate the positive impact on human health, a human dietary intervention study has been designed focussing on cancer risk markers in
colonic tissues using the newest genomics techniques available. Here we present the design and first outcomes of this health impact evaluation. The final meat products resulting from the meat sciences studies as well as the outcome of the health benefit assessment studies will be used for elaborate consumer studies to establish the response to the newly developed products.

II. MATERIALS AND METHODS

This human intervention study has a parallel design with only healthy volunteers, divided over 2 groups. Subjects receive a completely controlled diet with 2 different types of meat products, with or without the addition of natural compounds, with either normal levels, or low levels of nitrite, separated by a wash-out period were only white meat is consumed. As drinking water nitrate may be converted to nitrite in the body, also intake of drinking water nitrate is controlled and the effect on relevant endpoints is evaluated. The meat products include a combination cooked ham, cooked sausage, dried sausage and dry cured ham, the latter two both produced according to northern and southern style. These products are provided by SSICA (The Experimental Station for the Food Preserving Industry, Parma, Italy), Vanden Borre (Oudenaarde, Belgium) and Henri van de Bilt (Beuningen, Netherlands), all partners of the PHYTOME consortium.

After each of the three intervention periods of 15 days where participants consume 300 grams meat per day (corrected for body weight), blood, urine, saliva, mouth wash and faeces are sampled. Previous intervention studies on NOC formation indicate that a period of 15 days for each part of the study is sufficient to detect changes in the markers that are measured [8]. Additionally, colonic biopsies are collected during an endoscopic examination. In order to establish the impact of natural compounds in processed meat products on the formation of NOC in humans faecal and urinary level of NOC is measured as Total Apparent Nitroso Compounds (ATNC) and used as an indicator of colonic exposure to endogenous nitrosation products [9]. Also the genotoxic potential of faecal water is assessed by means of the COMET assay for DNA breakage. Additionally, DNA damage, gene expression profiles and DNA methylation will be measured in colonic tissues, which can be interpreted as an indicator of potential cancer risk.

All data analyses are done group wise to examine the overall effects of nitrite levels in meat. This is done for each intervention period (normal meat products and meat products enriched with natural compounds) and the wash-out period (white meat) serves as a control.

III. RESULTS AND DISCUSSION

Preliminary results from a pilot study population (n=20) to test the feasibility of the study design show that increased meat consumption has a stimulating effect on the genotoxicity of faecal water. The combined intake of red meat products with increased drinking water nitrate was not significantly influencing genotoxicity scores as compared to the base line measurements (Table 1). On the other hand, there was no statistically significant difference in N-nitroso compounds between baseline samples and after the intervention (t-test). In contrast to this, nitroso compounds were significantly higher with high nitrate drinking water (p=0.0001 as compared to meat intervention, p=0.0002 when compared with baseline levels). This difference was more pronounced in participants with a red meat diet than the white meat diet (Table 1). In participants with a red meat diet, faecal water nitroso compounds were higher after consumption of high nitrate water as compared to participants on a white meat diet, although this difference was only marginally significant (p=0.07). The impact of replacement of nitrite by natural bioactive compounds was not established in this pilot study.

Elaborate analyses of all markers in the main study, which is still ongoing, will reveal differences in transcriptomic and epigenomic markers after consumption of meat products enriched with natural compounds. These markers can be interpreted as an indicator of reduced cancer risk.

Furthermore, correlating gene expression changes to changes in genotoxic endpoints (DNA damage, reduction in N-Nitroso compounds) will provide insight in the
molecular processes involved in cancer risk reduction. The identification of molecular pathways that are crucial in the carcinogenic process will demonstrate a causal association between dietary changes and markers of carcinogenic risk. The outcome of these studies will be used in further consumer acceptance studies.

Table 1 Fecal water genotoxicity (%) and ATNC (μM of NaNO₂ eqv) levels after 1 week high meat diet following 1 week high meat diet combined with high nitrate drinking water consumption

<table>
<thead>
<tr>
<th>Genotoxicity</th>
<th>Baseline</th>
<th>Meat</th>
<th>Drinking water</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>100 ± 0</td>
<td>175.1 ± 103.4</td>
<td>157.7 ± 127.9</td>
</tr>
<tr>
<td>White Meat</td>
<td>100 ± 0</td>
<td>191.4 ± 129.4</td>
<td>187.2 ± 190.2</td>
</tr>
<tr>
<td>Red Meat</td>
<td>100 ± 0</td>
<td>163.6 ± 86.6</td>
<td>138.0 ± 70.2</td>
</tr>
<tr>
<td>ATNC</td>
<td>Baseline</td>
<td>Meat</td>
<td>Drinking water</td>
</tr>
<tr>
<td>All participants</td>
<td>14.7± 9.2</td>
<td>15.5± 10.4</td>
<td>35.63 ± 21.10</td>
</tr>
<tr>
<td>White Meat</td>
<td>12.7± 9.4</td>
<td>14.5± 11.0</td>
<td>28.2 ± 18.6</td>
</tr>
<tr>
<td>Red Meat</td>
<td>17.4± 8.7</td>
<td>17.0± 10.1</td>
<td>45.9 ± 21.0</td>
</tr>
</tbody>
</table>

IV. CONCLUSION

A range of new processed meat products has been produced by the PHYTOMIE consortium for evaluation of expected beneficial health effects. A human dietary intervention study has been designed to establish markers of exposure and molecular responses in human colonic tissue. The first results have demonstrated that the intake of traditional meat products can stimulate the capacity of the colonic content to induce genetic damage which could ultimately result and cancer development, but also that the evaluation of drinking water nitrate as an alternative source of nitrite may have a strong influence on the colonic exposure to potentially carcinogenic NOC. Further evaluation of the gene expression responses to the intervention and the comparison between traditional and new products will provide insight in the beneficial effects of the (partial) replacement of nitrite by natural extracts and which molecular mechanisms are involved in these effects.

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REFERENCES
