THE SIXTH FRAMEWORK PROGRAMME

Publishable Activity Report

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ACRONYM : NOCHEMFOOD
TITLE : NOvel Vegetal-based Extracts Additives for CHEMical-Free FOOD

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Draft Publishable Activity Report

1. Project Execution

1. NOCHEMFOOD Objective

The NOCHEMFOOD Project (NOvel Vegetal-based Extracts Additives for CHEMical Free FOOD), aims at developing a new strategy to produce foods containing mainly natural ingredients and from which possibly chemical additives, considered harmful for human health and subjected to regulation restrictions, have been removed. In particular, the project explores the potential use of these natural preserving agents, constituted by mixtures of active molecules extracted from vegetal sources, in the sausage industry, in substitution of commonly used nitrites and nitrates. More specifically, the main scientific and technological objectives of the project are the following:

- To develop new vegetal-based extracts as additives for food, with the scope of substituting some currently used chemicals, which are subject to regulation restriction and might be harmful for human health.
- A full characterization from the chemical, biochemical and microbiological point of view of these products, with the subsequent attempt to understand their mechanism of action

- To demonstrate the effectiveness of these novel vegetal-based mixtures of compounds as preserving agents in some largely consumed foods in Europe. Target foods selected for this purposes are sausages and other food classes which will be selected and preliminarily evaluated during the course of the project.

- To develop an effective dissemination plan to fully exploit the innovation potential of these products and enhance the commercial potential of some of these typical European productions.

2. CONTRACTORS INVOLVED

NOCHEMFOOD brings together a good deal of resources from EU, combining academic and industrial expertise and stimulating the interactions between them:

BIOMA: BIOMA AgroEcology CO AG

CNR: CNR - Istituto di Scienze dell'Alimentazione (ISA) - Avellino

CTIC: Centro Tecnologico De La Indsutria Cárnica De La Rioja

CSIC: Instituto de Fermentaciones Industriales

DiSTAAM: Department of Science and Technology of Agricultural Food and Microbiology, University of Molise, Campobasso.

INNOVA: INNOVA S.p.A

SALS: Salumi Spiezia

EMB: Embutidos Dany

3. WORK PERFORMED

The research activities of NOCHEMFOOD aimed to contribute to create a novel class of food-additives based on a mixtures of substances extracted from vegetal sources. During 3 years of duration, the consortium implemented the work plan of the project. The main research lines were:

- Extraction process: sub-critical and supercritical fluid extraction, analysis of the influence of extraction conditions on the composition of the extracts
- chemical analyses to different vegetal extracts combinations and their: mutageniticity, genotoxicity, proteotoxicity, antimicrobial, antioxidant and preserving activity.

- Test of the different formulations on meat products: protocol production was defined, different meat products were prepared and analyzed
- Biochemical and microbiological analyses: meat products containing the different formulations were analyzed during ripening time and compared to results obtained with conventional products.
- Sensory and acceptability consumers tests: once the final NC formulation was defined and two types of products salami Napoli and Chorizo vela, containing NC were prepared, the tests were performed
- Other food classes: 3 other food classes, cooked ham, opathè and chorizo fresco, were prepared with the NC formulation. Production protocols were defined, biochemical and microbiological analyses were performed. Sensory panel test were performed on these 3 classes of food.

The research activities were connected to each other in a global self-evolving matrix to ensure that all elements for the creation of a novel class of "natural" food-additives based are put together. Another important aspect of the creation of a novel food-additives class is the societal acceptation. NAIMO contributed with an active communication policy to promote its research activities to various targeted audiences, such as general public, journalists, young people, etc...

4. Results achieved during the whole duration of the project

During the 4 year of duration NAIMO has achieved a considerable amount of scientific results. Highlights of scientific results are:

- BIOMA has used its competences and its industrial plant, in the extraction filed to achieve interesting results on the extraction method on vegetable sources. Highlights of interesting results are:

  The starting point for the optimization of the industrial process was represented by the observations obtained by the pilot scale. The goal was to maximize the yield of the extraction process and to obtain the best antioxidant and antimicrobial properties for each raw material. Eight different raw materials were considered (Raphanus niger, Malpighia punicifolia, Rosmarinus officinalis, Medicago composita, Spirulina pacifica, Carica papaia, Propolis, Citrus compositum) and they were extracted under different T-P conditions. Ten near-critical (SBFE) conditions were considered (T: 38 – 40 ℃; P: 68 – 73 atm) and compared to supercritical fluid extraction (SFE at the following conditions: 150 atm and 40 ℃) with or without solvents (0 %, 3% and 6 % of ethanol respectively). Table 1 lists results concerning yields and demonstrates that slight dependencies to T and P were detected.

  Table 1 – Extraction yield of the different batches.

T	P	Raphanus N.	Papaya	Propoli	Rosmarinus	Malpighia P.	Spirulina	Citrus Comp.	Medicago Comp.
38.2	68	3.35	2.60	3.45	4.00	4.10	4.50	4.50	4.00
39.2	70	3.55	2.70	3.59	4.21	4.21	4.75	4.75	4.21
37.2	68	3.27	2.61	3.35	3.91	3.97	4.31	4.31	3.91
38.7	70	3.51	2.67	3.51	4.20	4.20	4.50	4.50	4.20
37.7	68	3.30	2.69	3.40	3.93	3.95	4.32	4.32	3.93
39.7	70	3.52	2.85	3.52	4.27	4.27	4.56	4.56	4.27
40.2	70	3.57	2.92	3.57	4.55	4.65	4.89	4.89	4.55
37.2	72	3.60	2.96	3.60	4.60	4.70	4.91	4.91	4.60
37.7	72	3.62	2.99	3.62	4.65	4.72	4.93	4.95	4.67
40.0	73	3.66	3.02	3.66	4.69	4.74	4.98	5.11	4.71
Average		3.50	2.80	3.53	4.30	4.35	4.67	4.68	4.31
Dev.std.		0.14	0.16	0.10	0.30	0.32	0.26	0.28	0.31
Min		3.27	2.60	3.35	3.91	3.95	4.31	4.31	3.91
Max		3.66	3.02	3.66	4.69	4.74	4.98	5.11	4.71

  Considering the antioxidant properties, a statistical analysis was performed to determine if significant differences could be observed among different batches. In all raw materials (except rosemary) batch 5 (39.7℃ – 70 atm) provided the highest EC50 value, being the differences statistically significant.

Three different batches were considered for testing **repeatability and reproducibility** of extraction process and the antioxidant activity of the replicates was quite similar and no statistical differences among batches, in terms of antioxidant activity, were observed. Those results confirmed the repeatability and reproducibility of the extraction process (Figure 1).

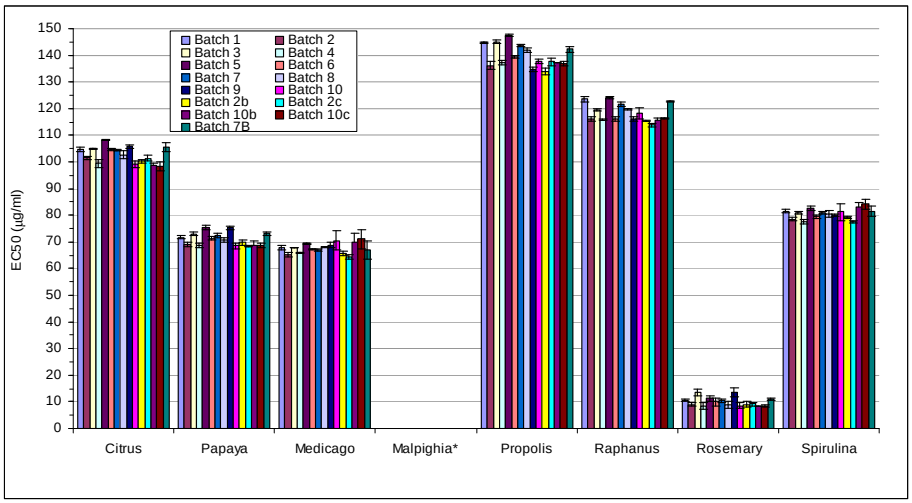

Figure 1 - Antioxidant activities measured by DPPH method (results expressed as EC50, the bigger EC50, the lower antioxidant activity)

Six different preliminary mixtures were prepared in order to estimate the capacity of different amount of each raw material to preserve meat products, instead of using conventional additives (nitrates and nitrites). The **ideal formulation (NC)** is described below in Table 2 and it was prepared using all the results coming out from experimental activities and statistical analysis. The T-P conditions were selected considering yields of extraction processes.

Table 2. Definition of the new formulation (NC)

Vegetal Source	*Extraction Conc. (g/l)*	*T (°C)*	*P (atm)*	*Formulation Comp. (mg/l)*	*Formulation Comp. (%)*
Spirulina pacifica	100	40	73	-	-
Raphanus niger	50	40	73	1	6.1
Malpighia punicifolia	250	40	73	3.5	21.2
Rosmarinus officinalis	300	40	73	1.5	9.1
Propolis	100	40	73	-	-
Medicago composita	100	40	73	4	24.2
Carica Papaya	50	40	73	3	18.2
Citrus compositum	210	40	73	3.5	21.2

The **homogeneity** of the formulation produced in an industrial plant was identified as the main critical parameter for the scale-up strategy. Homogeneity tests were carried out in order to detect T-P anomalies during the industrial process, that can affect the final composition of the final product. This test was focused on antioxidant properties that can be estimated by EC50. The antioxidant activity of two batches, produced by two different runs of the industrial plant, respectively was measured. Each batch was divided in 10 samples whose activity was measured twice, which means 20 measurements by batch. The number of samples has been decided in function of the Harmonized Protocol Procedure (M. Thompson and R. Wood, Pure Appl. Chem. 1993, 65, 2123) which gives indications for testing for “sufficient homogeneity”. The values of the antioxidant activity obtained looked pretty similar to the antioxidant activity found for the pilot scale experiment.

NOCHEMFOOD required the experimentation on two meat products: Napoli's type salami and Chorizo vela. These activities were supported by BIOMA providing the formulation to the SMEs. The project has foreseen to enlarge the range of meat products interested by novel preservatives. Therefore, BIOMA studied **other food classes** were to apply the natural preservative mixture. Since the beginning of the project the variables considered were only fermentation and uncoooking, so to complete the scenario of the variety of salami it was interesting to test the effects of other variables such as the cooking process and the use of meat mass instead of minced meat. From this point of view the other food classes identified for testing the effect of natural preservatives were: Cooked Ham (Prosciutto Cotto): entire piece, cooked, unsmoked; Pâté: cooked meat mass, made with pork liver and pork backfat mainly.

- The development of an innovative class of food-additives based on mixtures of active molecules extracted from vegetal sources by means of supercritical fluid extraction with carbon dioxide has been achieved. Due to their high antioxidant properties, these natural preserving agents have a potential use in the sausages industry as substitutes of commonly utilized nitrites and nitrates.
  A complete characterization from chemical, biochemical and microbiological point of view of such mixtures has been performed, with particular attempts to their potential mutagenic properties as well as their antimicrobial capabilities against undesirable or pathogenic micro-organisms.

  Experimental assays of direct mutagenicity on vegetal extract formulations, performed according to the protocol of Ames test, always revealed no mutagenic activity for the tested mixtures. On the other hand, interesting data regarding the antimutagenic activity of the extracts were observed. In fact, a notable capability of the vegetal formulations to inhibit *in vitro* the mutagenic action of some chemical mutagens was detected, with values reaching more than 70% of inhibition.

  The levels of antioxidant activity were also monitored by means of the radical scavenging method (DPPH) in the various vegetal formulations.

  A statistical correlation between composition of the different vegetal extracts and their antimicrobial and antioxidant activities led us to design a theoretical final formulation (NC Formulation) to be used in sausages manufacturing and, as preliminary studies, in the production of different food classes.

  A series of chemical-physical, biochemical and nutritional analyses were performed on the different samples of fermented sausages, manufactured by Spiezia factory. The experimental work was executed in parallel both on samples prepared with addition of nitrites/nitrates according to traditional procedures, and on samples treated with the NC vegetal mixture, all of them at various ripening times.

  As regard to chimical-physical properties, no significant differences were found for pH, moisture and water activity (a_w) between sausage samples treated either with nitrates/nitrites, or with the NC Formulation. On the other hand, ripening processes can be assumed equal for all sausage samples and not depending on the type of treatment.

  From biochemical point of view, the meat products prepared with the new vegetal extracts formulation revealed biochemical characteristics very similar to conventional ones. In particular, the electrophoretic patterns, obtained by means of the innovative Lab-on-a-chip capillary electrophoresis methodology, using an Experion apparatus (BioRad), showed no evident differences for the various batches.

Microbiological tests performed during all the ripening processes on *Napoli-type* and *Chorizo Vela* sausages revealed that the NC Formulation has an inhibitory effect on the growth of undesirable microorganisms, without affecting the growth of useful ones.
A very interesting result was to detect that the inhibitory effects of the NC Formulation against *Clostridium sporogenes*, previously evidenced *in vitro*, were confirmed also in *in situ* experiments.

Sensory analyses directed to define the organoleptic characteristics of differently manufactured salami revealed that both profiles of conventional samples prepared according to traditional procedures and of samples treated with the NC vegetal mixture are very similar.
Consumer acceptability showed a slight difference, not statistically significant, in the acceptance of the treated samples when compared to the conventional control products.

All reported tests, including also the evaluation of lipids oxidation processes as well as the determination of ascorbic and erythorbic acids as nutritionally relevant substances,
were performed on cooked ham, as representative of different food classes.

Interestingly, preliminary results obtained revealed very similar values both for samples conventionally prepared with addition of nitrites/nitrates, and for samples treated with the NC vegetal mixture.
Moreover, the NC mixture showed inhibitory effects against undesirable microorganisms also in cooked hams. The preliminary results are very promising in order to extend the use of vegetal extracts mixtures to other food classes.

- The group CSIC-UAM has been in charge of the chemical and statistical analysis of extracts and formulations. The chemical analysis has followed a dual way: measuring target compounds and using a metabolomic approach.

  The samples analyzed consisted on sub and supercritical extracts of: citrus, alfalfa, papaya, rosemary, propolis, acerola, spirulina and black radish. Moreover, mixtures of different extracts obtained at different extractions conditions were analyzed

  For the first purpose several methods developed in our laboratory were used (photosynthetic pigments and carnosic acid and derivatives by HPLC-DAD, volatile antimicrobial compounds by GC-MS); some other analytical protocols were adapted from literature (Total phenolic content, DPPH radical scavenging and sugars and aminoacids by GC-MS).

  In order to obtain a metabolomic profile of extracts, a method able to provide a good separation of a wide range of compounds was developed. With this method (published in the J. Chromatogr. A. http://dx.doi.org/10.1016/j.chroma.2008.02.054) water- and fat-soluble vitamins, phenolic compounds, carotenoids and chlorophylls were simultaneously detected and quantified in a single run by using an ultradeactivated C18 column.

  With the purpose of finding the main components of the extracts that contribute to their antioxidant and antimicrobial activity, statistical analysis was performed considering antioxidant activities and the vitamin-phenolic HPLC and GC profiles. Different statistical methods were used: PLS (partial least squares), MLR (Forward Stepwise Multiple Linear Regression) and Neuronal Networks models. These statistical analyses indicated that the main compounds that contribute to increase the antioxidant activity were flavonols and flavanones, while cinnamic acids contribute to decrease the antioxidant activity. Between raw materials the most antioxidant raw

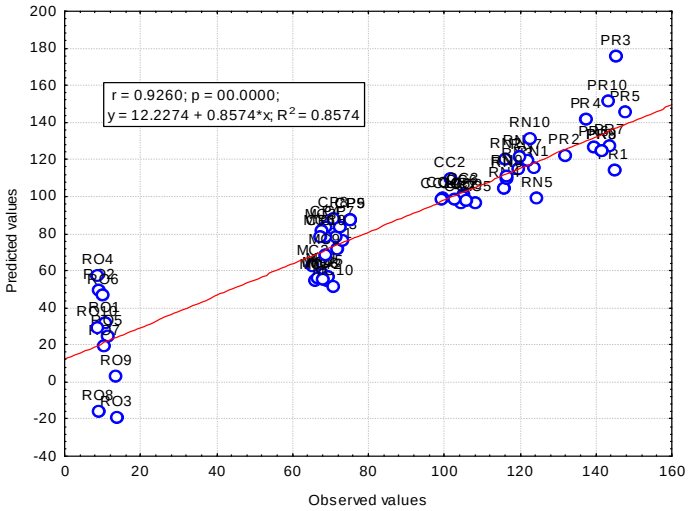

Fig. 1.- Scatterplot of predicted values for EC50 from PLS procedure vs observed values.

material was rosemary followed by alfalfa, papaya and *Spirulina*. In terms of antimicrobial activity, the most active compounds came from rosemary and citrus. Figure 1 shows how the prediction obtained with PLS adjusted to experimental data, R^2=0.9260.

The different statistical methods employed depicted different formulations (mixtures of pure extracts) to maximize the antioxidant activity, inhibit specific microorganisms or enhance the growing of desirable microorganisms. The validity of these model formulations was tested by a mixture design (modified simplex lattice 3 components, Figure 2), where the resultant antioxidant activity varied less than 3% of predicted models, depicting certain synergies and inhibitions among raw materials.

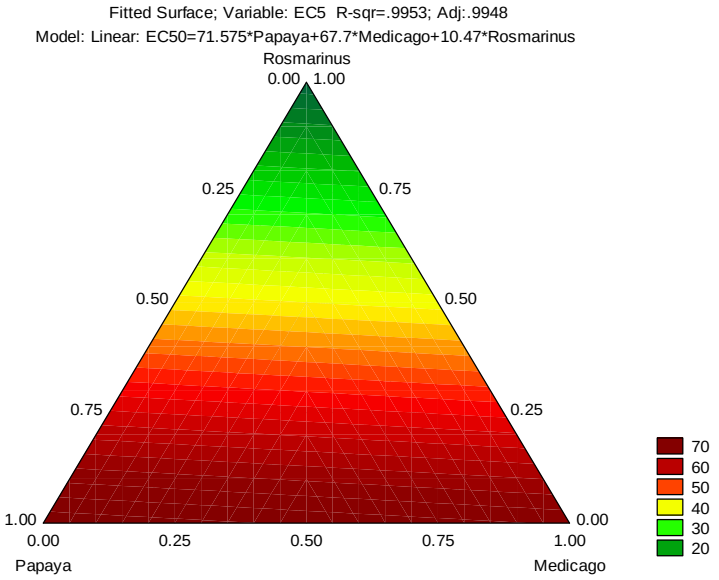

Fig. 2.- Ternary diagram illustrating the level combinations of Rosmarinus, Papaya and Alfalfa in the Modified Simplex Centroid design. The colour scale represent the fitted surface for the antioxidant activity as EC50 (the lower value, the higher antioxidant).

Furthermore, with the analysis of certain pre-formulated mixtures it was possible to obtain a formulation that could provide a high antioxidant activity, inhibit the growing of pathogenic microorganism and enhance the growth of desirable bacteria and moulds for the meat industry. This formulation need to be readapted for commercial reasons.

The resultant formulation was analyzed following the above mentioned protocols; two more methods were selected to assure its safety, namely, nitrites content by the Griess method and heavy metals content by ICP-MS. The Griess method indicated the absence of nitrites in the formulation and the ICP-MS provided levels of heavy metals 10-50 times lower than those found in common consumed raw vegetables. The rest of the analysis of the resultant formulation were according to those predicted by the model. Figure 3 shows a chromatogram obtained using the vitamin-poliphenolic-pigment method. In the first part of the chromatogram (min 2-8), the water soluble vitamins are eluted; in the middle (min 8-16) the phenolic compounds eluted, namely flavanones, flavonols, isoflavones, phenolic acids, anthocianins and phenolic diterpenes; while in the last 20 min, fat soluble vitamins and pigments should appear. In this case, no fat soluble vitamins nor pigments are detected, except a chlorophyll (min 25.5) under quantitation limit. The reddish colour of the formulation obtained is due to the different anthocianins.

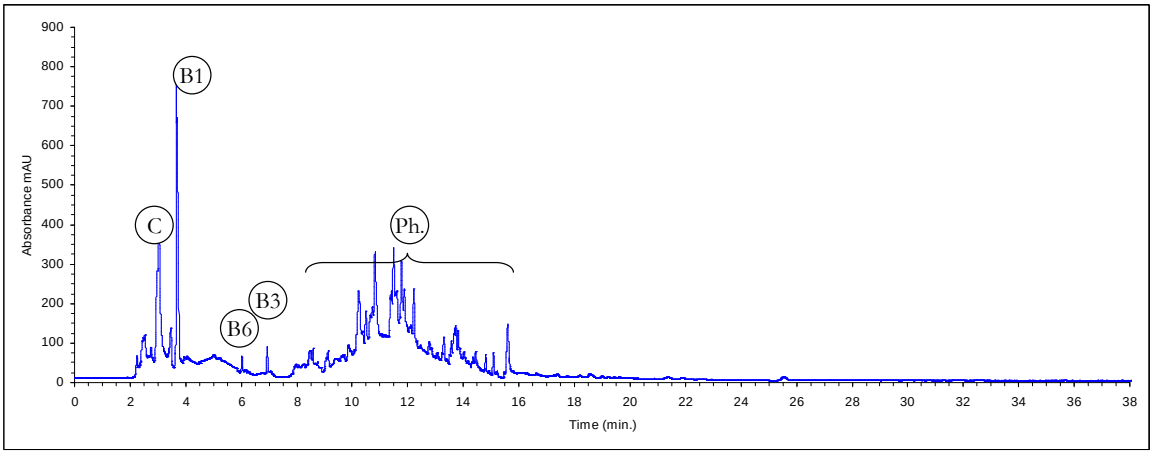

Fig. 3.- Chromatogram of formulation NC. The peaks labeled correspond to: ascorbic acid (Vit. C), thiamine (Vit. B1), pyridoxine (Vit. B6), nicotinamide (Vit. B3) and the area of phenolic compounds (Ph).

- **Development of a novel natural additive based on mixtures of active molecules extracted from vegetal sources**.

  The aim of this "natural extract" is to be used as a substitute of some currently used additives, as nitrates and nitrites. In addition to it a full characterization (from chemical, microbiological and biochemical point of view) was carried up in order to understand the mechanism of action.

- **Optimization of the addition protocol of these vegetal extracts into meat mass.**

  Different ways of addition were essayed. Diffusion of vegetal extract solution was studied in order to get a homogeneous distribution that assures complete action of the extract.

- **Isolation of new improved extracts mixtures.**

  Preliminary pilot plant experiments with "Chorizo Vela" and Salami Napoli suggest modifying some extraction conditions to get improved extracts mixtures.

- **Optimization of the concentration and proportion of extract necessary to get an acceptable batch of "Chorizo Vela" and Salami Napoli in pilot scale elaboration. Characterization of this products (from microbiological, physic-chemical and sensorial point of view) and shelf life studies.**

  After manufacturing improved extracts, new pilot plant essays allowed to get the best results. Proportion of extracts, protocol of addition to meat mass, partial replacement and total substitution of nitrites was tested in "Chorizo Vela" and Salami Napoli elaboration. This made it possible to identify the effect of extract to improve the preservation of the products.

- **Industrial scale elaboration of Salami Napoli and "Chorizo Vela". Characterizations of the industrial products and shelf life studies.**

  In accordance with the previous pilot elaboration results, industrial scale elaboration was performed. Industrial product was characterized and conclusions are similar to previous pilot essays: *Partial replacement of nitrates by the natural extract allow to get acceptable industrial products and a valuable method to reduce 80% of standard nitrite/nitrate content in "Chorizo Vela". In case of Salami Napoli, acceptable results were obtained with the complete substitution of chemical additives with vegetal extracts of NC formulation.*

- **Application of these results to industrial scale elaboration of different meat products: Cooked ham, "Pathé" and "Chorizo Fresco".**

  Optimization of elaboration processes. Complete characterization of them (from microbiological, physic-chemical and sensorial point of view) and shelf life studies were also carried up. As a result, acceptable products were obtained. It was showed that it is interesting to extend the use of these novel extracts to other class of products different than have been studied in this project.

- DISTAAM -Università degli Studi del Molise is involved in the NOCHEMFOOD project as the unit assigned to the study of antimicrobial properties of vegetal extracts and microbiological features of different kind of meats produced with the addition of vegetal extracts.

  The research that was carried out so far, produced promising results which open new horizons on the possibility to produce innovative products without chemical additives. In particular, the study aimed at characterising microbial features of bacteria involved in various steps (preparation/fermentation) of the manufacture of meat products obtained with the use of vegetal extracts instead of chemical additives (nitrate/nitrite). This aspect of the research appears of fundamental importance in order to preserve characteristics of final products due to useful microorganisms, leading to the suppression of undesirable/pathogen ones at the same time.

  Results obtained until the 24th month allowed the individuation of vegetal extracts with the best antimicrobial activities. Eight natural compounds (Carica papaya, Citrus compositum, Malpighia punicifolia, Medicago composita, Propolis, Raphanus niger, Rosmarinus officinalis, Spirulina pacifica, from Bioma ®, Swiss) were evaluated for their antimicrobial activity on useful

and undesirable microorganisms. Antimicrobial activity and effectiveness were evaluated on undesirable microorganisms and against useful ones. To individuate the best concentration that is able to produce inhibition against undesirable microorganisms, without effects on useful ones, the antimicrobial effectiveness was detected evaluating the growth of each microorganism inoculated in meat broth added of different concentrations of natural compounds (0.1%; 0.5%; 1%). The broth was prepared in order to obtain a standard medium as similar as possible to meat or meat products.

The antimicrobial action of each compound was different in intensity and specificity. *Carica papaya*, Propolis and *Raphanus niger* showed a low and limited antimicrobial activity. In particular *Carica papaya* (Fig. **1**) and *Raphanus niger* (Fig. **2**) produced a low inhibitory effect against *C. sporogenes* and *B. Thermosphacta* up to a 0.5% concentration. Propolis (Fig. **3**) evidenced a low inhibition against *C. sporogenes* and *B. thermosphacta* only when used at 1% concentration.

The three natural compounds did not affect useful bacteria. Rosmarinus officinalis (data not shown) and Citrus compositum (Fig. 4) were characterised by a large spectrum of activity and by a strong efficacy, producing an inhibitory action against all the assayed microorganisms, i.e. against both undesirable bacteria and useful ones. In fact, up to a 0.5% concentration they produced an inhibitory effect on the growth of Gram Positive Coagulase Negative Cocci. As for the undesirable microorganisms, these vegetal compounds produced the inhibition of C. sporogenes and B. thermsphacta when used up to 0.1% concentration. For all the other undesirable microorganisms, inhibitory effects were observed when the natural compounds were used up to a 0.5% concentration. The three natural compounds did not affect useful bacteria. Rosmarinus officinalis (data not shown) and Citrus compositum (Fig. 4) were characterised by a large spectrum of activity and by a strong efficacy, producing an inhibitory action against all the assayed microorganisms, i.e. against both undesirable bacteria and useful ones. In fact, up to a 0.5% concentration they produced an inhibitory effect on the growth of Gram Positive Coagulase Negative Cocci. As for the undesirable microorganisms, these vegetal compounds produced the inhibition of C. sporogenes and B. thermsphacta when used up to 0.1% concentration. For all the other undesirable microorganisms, inhibitory effects were observed when the natural compounds were used up to a 0.5% concentration.

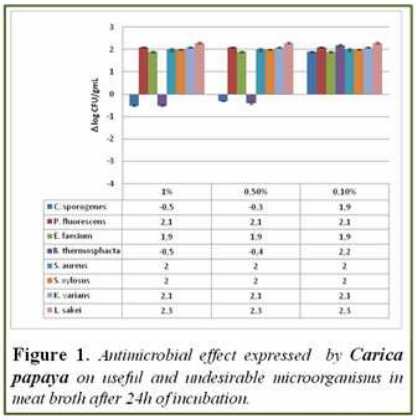

Figure 1. *Antimicrobial effect expressed by* ***Carica papaya*** *on useful and undesirable microorganisms in meat broth after 24h of incubation.*

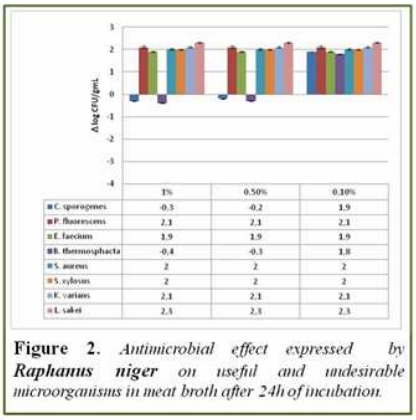

Figure 2. *Antimicrobial effect expressed by* ***Raphanus niger*** *on useful and undesirable microorganisms in meat broth after 24h of incubation.*

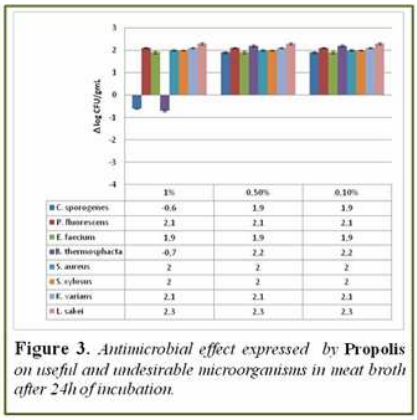

Figure 3. *Antimicrobial effect expressed by* **Propolis** *on useful and undesirable microorganisms in meat broth after 24h of incubation.*

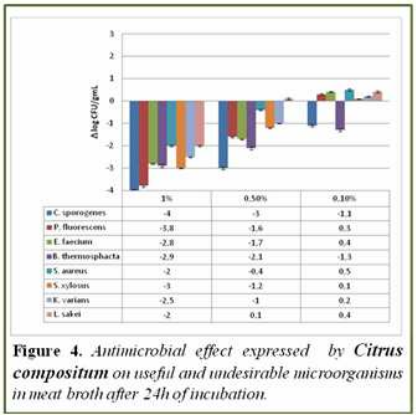

Figure 4. *Antimicrobial effect expressed by* ***Citrus compositum*** *on useful and undesirable microorganisms in meat broth after 24h of incubation.*

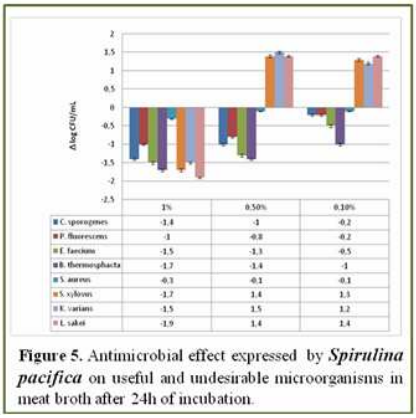

Figure 5. Antimicrobial effect expressed by ***Spirulina pacifica*** on useful and undesirable microorganisms in meat broth after 24h of incubation.

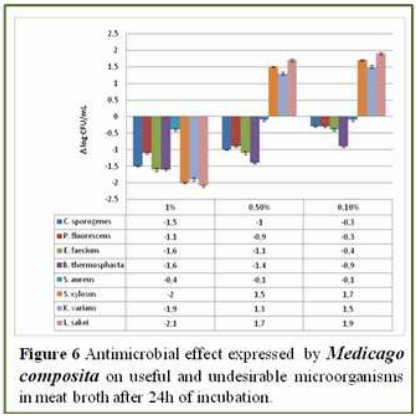

Figure 6 Antimicrobial effect expressed by ***Medicago composita*** on useful and undesirable microorganisms in meat broth after 24h of incubation.

Results obtained underline the possibility to individuate novel vegetal compounds or their mixtures alternative to nitrates and nitrites in the preparation of fermented meat products. On the bases of these results an optimal new vegetal mixture was defined: NC vegetal mixture.
The vegetal mixture used evidenced a promising antimicrobial activity, in particular against undesiderable gram positive bacteria such as *C. sporogenes* and *B. thermosphacta* (Figure 7, 8) . Moreover the results evidenced that the best NC concentration to be used in meat product preparations could be 1% or 0,5%. These concentrations allowed to strongly inhibit the growth of some undesirable microorganisms without to affect (in particular 0,5%) the growth of useful microorganisms such as Lactic acid bacteria and Micrococci-staphylococci.

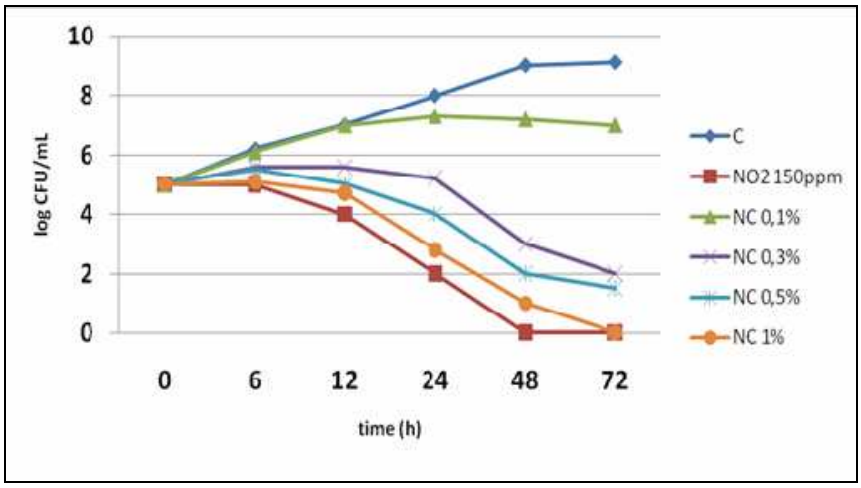

Figure 7. *C. sporogenes* growth in presence of nitrites (NO_2) or vegetal mixture (NC) at different concentrations.

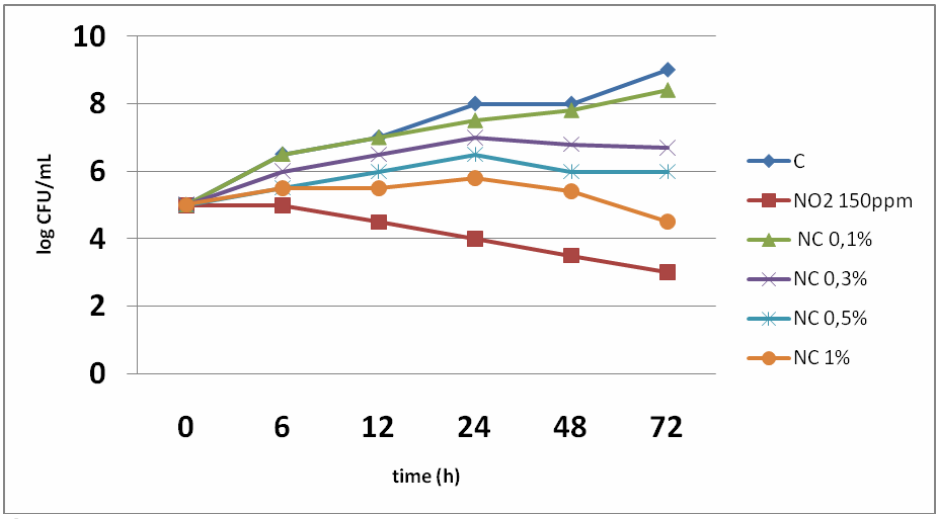

Figure 8. *B. thermosphacta* growth in presence of nitrites (NO_2) or vegetal mixture (NC) at different concentrations.

These results were confirmed *in situ* by using a pilot plant and also in large scale.

The data obtained *in sutu* evidenced that the vegetal mixture NC allowed an inhibitory effect on the growth of the main undesirable microorganisms, comparable to that obtained by using chemical additives. Moreover the results confirmed that the vegetal mixture did not produce any effects on the growth of useful microorganisms. Biomolecular analyses confirmed the data reported above: the DNA extracted directly from samples of sausages produced with the NC mixture, with nitrites and nitrates or without preservatives was subjected to DGGE and FISH analyses. The comparison of data obtained revealed very similar profiles in the case of salami produced with different (natural or chemical) preservatives, whereas the control samples was robustly different from the previous ones.

Moreover Microbiological analyses were also performed on cooked hams manufactured with conventional chemical additives or with the formulation of vegetal extracts. As expected, the results evidenced very low microbial levels of microorganisms in both batches but the NC mixture allowed an higher inhibitory effect on *Pseudomonas* spp than that produced by nitrates/nitrites. The microbiological results evidenced that the vegetal mixture NC could be used in the cooked ham manufacturing instead of nitrate and nitrite.

5. Progress against the objective

Objective Description	Status of Objective Achievement	Radical Innovation
To develop new vegetal-based extracts as additives for food, with the scope of substituting some currently used chemicals, which are subject to regulation restriction and might be harmful for human health.	A large number of vegetal extracts formulations were produced, analysed and tested on meat in order to evaluate the possibility of substituting chemical additives normally used. The subsequent tests on meat prove the feasibility of substituting nitrites and nitrates in meat processing.	SBFE (sub critical fluid extraction) proved to be an effective technique to be used with plants.
A full characterization from the chemical, biochemical and microbiological point of view of these products, with the subsequent attempt to understand their mechanism of action.	Chemical, biochemical and microbiological characterizations of all the obtained formulations were done and compared to the nitrites and nitrates behaviour. The detailed analysis of chemical additives properties allowed the design of an optimised formulation.	Chemometrical method to correlate antioxidant and antimicrobial activities with composition
To demonstrate the effectiveness of these novel vegetal-based mixtures of compounds as preserving agents in	Experiments on target food selected and on the other 3 food classes were performed and the results demonstrated the efficacy of	Optimised formulation as preserving agent for

some largely consumed foods in Europe. Target foods selected for this purposes are sausages and other food classes which will be selected and preliminarily evaluated during the course of the project.	the vegetal extracts mixture as preserving agents.	meat-based products
To develop an effective dissemination plan to fully exploit the innovation potential of these products and enhance the commercial potential of some of these typical European productions.	The exploitation framework was analysed and the exploitation plan was defined at consortium and partner level. Several conferences were attended, 2 publications were published and, a patent is being filed.	N.A.

6. Project website and logo

NOCHEMFOOD website	NOCHEMFOOD logo
www.nochemfood.eu	NOCHEMFOOD

2. Dissemination and use

Result n°1

Description of exploitable result
Extraction method applying the SBFE (sub critical fluid extraction) on vegetal sources to achieve a mixture of active molecules to be used as natural preservatives in food products
Possible market applications
Universities, Research centres, Pharmaceutical, Food industries Type of use: production of extracts and active mixtures
Stage of development
Validated method of extraction, test on homogeneity of the production of extraction and mixtures.
Type of collaboration sought or offered
Information exchange
Type of partner sought and tasks to be performed
Training and consultancy
IPR granted or published
Patent request on July 2009.
Contact details
Alessandro Capodicasa BIOMA alessandro.capodicasa@terramatica.it Tel. +41 918 401015

Result n°2

Description of exploitable result
Innovative mixture of natural antioxidant extracts to be used as preservatives in processed and fermented meats products in replacement of conventional chemical additives
Possible market applications
Immediate application in meats products industry.
Stage of development
Laboratory prototype
Type of collaboration sought or offered
Manufacturing agreement.
Type of partner sought and tasks to be performed
Meat industries for manufacturing of free chemical additives meat products.
IPR granted or published
Patent request on July 2009
Contact details
Dr. Alfonso Sada Research Scientist Istituto di Scienze dell'Alimentazione - CNR - Lab. Biotecnologie e Sicurezza Alimentare Via Roma, 64 83100 Avellino - Italy Tel. +39 0825 299304 Fax +39 0825 781585 sada@isa.cnr.it

Result nº3:

Description of exploitable result
Analytical method for simultaneous quantification of vitamins (hydrosoluble and liposoluble), phenolic compounds and photosynthetic pigments
Possible market applications
Universities, Research centres, Natural product analysis laboratories, Pharmaceutical, Food industries Type of use: quantitative and qualitative analysis of vegetables, their extracts, juices…
Stage of development
Analytical protocol validated, published and probed in vegetable extracts, fruit juices and beers
Type of collaboration sought or offered
Information exchange
Type of partner sought and tasks to be performed
Training and consultancy
IPR granted or published
Published at Journal of Chromatography A http://dx.doi.org/10.1016/j.chroma.2008.02.054
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Result nº4:

Description of exploitable result
Chemometrical method to correlate antioxidant and antimicrobial activities with composition
Possible market applications
Universities, Research centres, Natural product analysis laboratories, Pharmaceutical, Food industries Type of use: development of new antioxidant from natural origin
Stage of development
Metabolomic protocol statistically validated
Type of collaboration sought or offered
Information exchange
Type of partner sought and tasks to be performed
Training and consultancy
IPR granted or published
Submitted to be published at Journal of Agricultural and Food Chemistry
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