BE&SAVE PROJECT


CNR RESEARCH RESULTS

edited by Maria Cefola and Bernardo Pace – Institute of Science of Food Production
INSTITUTE OF INTELLIGENT SYSTEMS FOR AUTOMATION (ISSIA)

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INSTITUTE OF SCIENCE OF FOOD PROCUCTIONS (ISPA)

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INSTITUTE FOR POLYMERS, COMPOSITES AND BIOMATERIALS (IPCB)

10. FORMULATED BIOPLASTICS
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INSTITUTE OF FOOD SCIENCE (ISA)

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20. WEB ACCESSIBLE DATABASE CONTAINING SOME OF THE EXPERIMENTAL RESULTS OBTAINED IN THE FRAMEWORK OF THE PROJECT
Computer Vision System (CVS) for the non-destructive selection of vegetables quality levels

**Product description**
The CVS uses calibrated color images, regression models and machine learning algorithms to evaluate agro-alimentary products. Products with different visual appearance can be analyzed since the parameters needed to process and classify the images are set automatically. It proved successfully on nectarines, carrots, iceberg lettuce, radicchio, rocket but its adaptation and self-configuration allows the extension to other products. It can be used as laboratory tool or integrated in a production line: in both cases it provides an efficient non-destructive quality evaluation and the prediction of internal parameters such as antioxidant activity, phenols, chlorophyll, ammonia.

**Product novelty, utility and originality:**
The CVS has few innovative and original features. It adapts to new products without requiring human intervention to set parameters and configure features used to achieve its goals. It has proved successful on different products with unevenly colored surface: relevant colors are automatically identified and correlated.
to the measures of interest. It involves statistical techniques and machine learning algorithms that are used to achieve adaptation, self-configuration and effective and robust classification and prediction. It is able to provide information on the global quality level of products but also on internal parameters of interest.

Potential users:
1. **Agro-alimentary companies** to achieve continuous monitoring on products along the whole production lines
2. **Storage and transportation companies** to achieve a reliable check of product’s quality along the supply-chain
3. **GDO points** to monitor the quality of products, timely detect their degradation and assume decision to reduce waste
4. **Producers of refrigerated cells/containers** to increase the services provided by their products with a continuous monitoring of stored products
5. **Producers of refrigerators** to enhance the intelligence of their products with easy to use instrument that monitor the quality of stored products

Documents:


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2. RECOVERY OF HIGHLY PURIFIED PHENOLIC COMPOUNDS FROM VEGETABLE WASTE

Product description / Technical information:
Protocols of extraction and purification of phenolic compounds from vegetable waste have been developed. Environmental sustainability of the extraction process was ensured by the use of water or alcoholic solutions. Waste was characterized by HPLC-DAD and subjected to appropriate massive extraction; after removal of the alcoholic solvent, if present, extracts were lyophilized. The quantitative recovery of molecules of nutraceutical and/or industrial interest was achieved starting from lyophilized powders through Flash Liquid Chromatography (FLC), by scaling-up analytical chromatographic methods to preparative scale.

Product novelty, utility and originality:
Waste arising from fruit and vegetables (chicory, artichoke, citrus, etc.) was used for the extraction of phenolic compounds of nutraceutical and agro-industrial interest in several sectors, i.e. producers of food, cosmetic, herbal, pharmaceutical, plant protection and chemical products. Such compounds (chicoric acid, cynarin, naringenin and other flavonoids, etc.) were recovered from waste with high degree of chromatographic purity (>98%), good yields and high amounts (i.e. dozen of grams on laboratory scale). These features make the process highly useful for production of both already known bioactive molecules as well as compounds for new biological activity studies and applications.

Potential users:
Industrial companies working in sectors as agro-food (antioxidants, supplements, strengthened foods), cosmetic (anti-aging products), herbal (dietetics and natural medicine products), pharmaceutical (anti-inflammatory, anti-tumour), plant protection (bio-pesticides), chemical (additives, natural dyes).

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3. THE SECOND LIFE OF DOP ITALIAN CHEESES

Product description/Technical information:
The new cheese was produced mixing some DOP cheeses at the end of their commercial life with frozen unfermented curd, using the back-slopping procedure. After inoculation and brining, cheeses were ripened by autochthonous lactic acid bacteria, from different DOP cheeses, for 60 days. At the end of aging, and after all microbiological evaluation, has been demonstrated the possibility to reuse DOP Italian cheeses that are still rich in fermenting microorganisms enabling the production of a new cheese endowed with good quality with particular aromatic notes absent in similar products produced only with frozen curd.

Product novelty, utility and originality:
The large part of Italian PDO cheeses are characterized by a long shelf-life. However, portioning and packaging operations reduce cheese commercial life as it is mandatory to define an expiration date after that the cheese is intended for disposal even though it is still perfectly edible. For the first time these cheeses have been recovered to enrich an unfermented curd, often called as young curd, without pleasant organoleptic notes and having low commercial value. The new processing allows a reduction of food waste and the obtaining of a cheese with good organoleptic quality.

Potential users:
End users of this innovation are cheese maker consortia, mass retail channel sites as well as dairies.

Documents:
Patent under preparation.

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4. CRUDE ENZYMATIC EXTRACT FROM SPENT SUBSTRATE OF PLEUROTUS ERYNGII ABLE TO DEGRADe AFLATOxin B₁

Product description/Technical information:
Spent mushroom (Pleurotus eryngii) substrates, normally regarded as a waste, have been used as a source of the enzymes laccase and peroxidase. These enzymes are able to degrade several xenobiotic compounds, such as polycyclic aromatic hydrocarbons, industrial dyes and mycotoxins. From Pleurotus eryngii spent substrates, a crude extract with high laccase activity was obtained. In appropriate incubation conditions this crude extract was able to degrade more than 90% of Aflatoxin B₁, a potent carcinogenic mycotoxin, contained in the culture medium.

Product novelty, utility and originality:
The enzyme extract from spent mushroom substrates can be exploited for:
- Valorization of wastes from “cardoncello mushroom” production;
- Reduction of production cost of enzymes with biotechnological importance, such as laccase and peroxidase
- Implementation of environmental remediation programs;
- Detoxification of food contaminated with mycotoxins (Aflatoxin B₁)

Potential users:
“Cardoncello mushroom” cultivation farms;
Feed industry;
Livestock farms

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5. STRAINS OF PLEUROTUS ERYNGII FOR BIOTOXIFICATION AND BIOREMEDIATION

Scarti della filiera agroalimentare
Agri-food wastes

Mangimi detossificati per le produzioni animali
Detoxified feed for livestock

Product description/Technical information:
The *P. eryngii* isolates selected exhibit:
1) ability to degrade natural contaminants, such as mycotoxins (Aflatoxin B1);
2) yield of mushrooms produced on substrates obtained with wheat straw mixed to various agricultural wastes (corn contaminated with mycotoxins, wastes from sugar beet processing and field beans);
3) nutritional and organoleptic characteristics comparable to strains already used for commercial production of “cardoncello” mushrooms.

Product novelty, utility and originality:
The establishment of a collection of *Pleurotus eryngii* strains will allow to:
- Identify strains that are especially suitable for cultivation on substrates obtained with wastes of the food chains
- Select strains with interesting biotechnological features with regard to degrading activity of Aflatoxin B1 and other mycotoxins,
- create a database of *P. eryngii* biodiversity instrumental certification and traceability of typical products, such as the “Apulia cardoncello mushroom”.

Potential users:
“Cardoncello mushroom” cultivation farms; Mushroom substrate production plants; Feed industry; Livestock farms.

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6. SHELF LIFE MODEL FOR PERISHABLE VEGETABLE PRODUCTS

Product description

Technical information:
The model is based on Computational Fluid Dynamics (CFD) methodology, which allows the integration of the differential equations that govern the various transport phenomena. Transport phenomena represent the principles and fundamental mechanisms on which all processes in food industry are based. Through the integration of these equations, the informations on the process variables can be obtained. In the present project, the model allows to predict the shelf life depending on the operating conditions of the supply chain for fruit and vegetables (lettuce and broccolis).

Product novelty, utility and originality:

This modeling technology is widely used in other fiels such as automotive, aerospace, chemical industry. In recent decades this technology is spreading out to the food industry, in spite that few research and development groups only hold the skills to apply it to the cases at stake.

The method is highly customizable and allows to analyze, to adjust, to check the product, process and industrial plant.

Potential users:
The application area is the agri-food sector. Possible users are organizations and/or companies of manufacturing, storage, distribution and sale sectors. This technology is also accessible to small and medium-sized enterprises due to the gradual reduction of computers cost and the continuous development of efficiency and ease of use of the software.

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7. DEVELOPMENT OF MODELS FOR MINIMIZATION OF FOOD STOCKS

Product description
Technical information:
The model is based on the methodology based on agents (Agent Base Modelling and Simulation - ABMS) and is a new modeling paradigm. This is a calculation method to simulate the actions and interactions between autonomous individuals in an environment, in order to assess their effects on the whole system. In ABMS, a system is modeled as an ensemble of entities that decides (agents). Each entity individually evaluates the environment and simulation in which it is located and therefore makes decisions on the basis of given rules and the system to which it belongs, such as production, consumption or sale.

Product novelty, utility and originality:
Such modeling technology is widely used in many areas and is a new modeling paradigm. The software is used widely tested and ensures a certain stability, providing an adequate amount of documentation and tutorials. The instrument chosen, Repast, is issued free of charge with license opens source and therefore ensures the advantages of economy; its features belong to a packet that has been available for some years now, and including a number of applications developed for the environment ABMS.

Potential users:
The application area is the food industry, but this approach is applicable to all sectors of production industry. The users are organizations and/or companies of the manufacturing, storage, distribution and sale sectors.

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8. DEVELOPMENT OF INTERMEDIATE SYSTEMS TO VALORIZE THE OVERSUPPLY

**Product description**

Technical information:
The management model has been based on existing studies in the literature and the experiences of consumer goods companies. The main causes of surpluses have been identified and the extent of the phenomenon has been measured. This model aims to find solutions and possible paths providing practical examples through the following lines of action:
1. To measure the excess and to develop early warning systems.
2. To raise awareness, to engage and motivate employees.
3. To improve forecast accuracy and maximize product availability.
4. To improve product design and packaging in the direction of sustainability.
5. To carry out inspections and audits.

**Product novelty, utility and originality:**
The goal of this approach is to spread the best practices identified by defining tools to identify, monitor and manage the complex process, in order to enable companies to realize programs to reduce surpluses. The instrument chosen is a totally free and easy to use.
In order to disseminate such guidelines and best practices on the management of surplus, the following actions could be pursued:
1. to make available tools and methodologies on prevention management;
2. to enable joint projects between producers and distributors on issues of common interest;
3. to create an observatory to monitor the surplus;
4. to organize training courses in companies.

**Potential users:**
The application area is the food industry, but this approach is applicable to all sectors of production industry. The users are organizations and/or companies of the manufacturing, storage, distribution and sale sectors.

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Product description/Technical information:
Composts were produced starting from vegetable wastes of the great organized distribution properly mixed with other organic matrices to optimize the chemical and physical parameters suitable for the correct evolution of the organic matter (in particular the C/N ratio). Composts showed absence of phytotoxicity, good chemical, physical and agronomic characteristics (such as content of micro and macro elements useful to plants, absence of heavy metals and good hydrological parameters), suitable for their use as sustainable and renewable substrates for vegetable seedlings production for partial or total peat substitution.

Product novelty, utility and originality:
Composts are well adapted to specific uses as request for products with high quality standards, particularly in terms of water retention and porosity, unlike most types of compost currently on the market, which may present undesirable characteristics (particularly high presence sodium chloride, heavy metals, phytotoxic substances, high pH, high specific density) for use in polystyrene plug trays or in pots, which consequently fit only to a "generic" use (amendments in open field). The products could be used in replacement of peat, with considerable benefits in terms of environmental and economic sustainability, for the reduction of the consumption of non-renewable resources, for the recovery of organic matter and for the lowest costs of the final product.

Potential users:
Composting plants, farms, horticultural nursery operations

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**Product description/Technical information:**
We obtained poly(hydroxybutyrate)(PHB)-lignin films. PHB is a biodegradable thermoplastic polyester from bacteria. It is characterized by a glass transition temperature below room temperature and a melting point similar to polypropylene. Lignin, coming from production process of second-generation bioethanol, is a complex mixing of different molecules. We performed chemical and chemical-physical characterization. We prepared blend between PHB and lignin in an extruder machine Thermo Haake MiniLab compounder and films were obtained in melt press.

**Product novelty, utility and originality:**
PHB is a polymer with an high speed of biodegradation and high melt instability. Lignin is able to module biodegradability on composites, reducing it and increasing the duration of films, and to stabilize the melting behavior of PHB. Moreover lignin influenced positively the mechanical behavior of PHB, increasing ductility and making the compound more useful for food packaging.

**Potential users:**
Food packaging industries

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11. BIODEGRADABLE POTS

Product description/Technical information:
Pot is made by MaterBi and compost. MaterBi is a biodegradable starch based material by Novamont, it is possible to obtain film used in agriculture or as shopper. Compost, product of a bio-oxidation and humidification of several materials, was supplied by U.O.Bari. The idea was to substitute 30% of expensive MaterBi with a cheaper compost, to obtain pots for horticulture, biodegradable with different and modular biodegradability. Pots were prepared in a Brabender apparatus at 160°C and then molded in a die.

Product novelty, utility and originality:
These pots can replace traditional ones in polystyrene or other traditional plastics, more difficult to recycle. They can be use also in nursery pots technique. Moreover it is possible to insert in the mix some fertilizers or growth factor for plants.

Potential users:
Pots manufacturers

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12. DEVELOPMENT OF SIMPLE, RAPID AND REPRODUCIBLE STRATEGIES FOR THE EXTRACTION AND IDENTIFICATION OF VOLATILE ORGANIC COMPOUNDS (VOCs) IN FRESH AND READY-TO-EAT FRUIT AND VEGETABLE PRODUCTS BY HEADSPACE SOLID PHASE MICRO-EXTRACTION (SPME) COUPLED TO GC/MS.

Product description/Technical information:
Quali-quantitative analysis of VOCs profile by SPME GC/MS of fresh and ready-to-eat fruit and vegetables to assess the quality and influence of chemical, biochemical and biotechnological process carried out on the products. SPME is a pre-concentration technology which integrates sampling, extraction, concentration and sample introduction into a single solvent-free step.
SPME is based on the use of a silica fiber, coated by an adsorbent material, capable of sampling volatiles through the exposure of the fiber to the headspace of the sample. Metabolites are desorbed from the fiber directly into the injector of the GC/MS, where they are separated and identified.

Product novelty, utility and originality:
Development of rapid simple and reproducible strategies by SPME GC/MS for the analysis of the VOCs profile as a decay potential indicator of fresh and ready-to-use fruit and vegetable products.

Potential users:
Companies, Workers in the sector, National and international organizations

Documents:
2. Cozzolino R., Martignetti A., Pellicano M.P., Stocchero M., Cefola M., Pace B. and De Giulio B. Characterisation of volatile profile and sensory analysis of fresh-cut "Radicchio di Chioggia" stored in
air or modified atmosphere. Food Chemistry (2015), 192:603-611. DOI: 10.1016/j.foodchem.2015.07.045


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13. VEGETABLE WASTE EXTRACTS (BRASSICACEAE) WITH ANTIOXIDANT, ANTIMICROBIAL AND ANTI-PROLIFERATIVE ACTIVITIES.

Product description/Technical information:
A rapid and inexpensive method for the preparation of polyphenolic extracts from vegetable waste has been developed. The extracts were obtained from wastes of widespread Brassicaceae plants, at several days after harvest or from map, modified atmosphere package, at various times from the expiration date. Initially we determined the polyphenol content and antioxidant activity. We evaluated antimicrobial properties and compared to activities of fresh product extracts. Finally, we assayed the anti-proliferative activity on human tumor cell lines (by crystal violet and MTT viability colorimetric methods) and compared to extracts from fresh products.

Product novelty, utility and originality:
In nutraceutical field, dietary supplements with healthful benefits, including the ability to contribute to the prevention of diseases such as cancer, constitute a high market share. The novelty of the product lies in the origin of extracts, obtained from a material that is a problem, in terms of disposal costs and environmental impact: vegetable waste from both domestic consumption that by industry and the large distribution. The achievement of bioactive compounds makes the vegetable scraps particularly appealing and may represent, for some industrial sectors, a further source for the economy and employment.

Potential users:
para-pharmaceutical companies, large retailers.

Documents:

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14. INNOVATIVE METHOD FOR ANTI-GENOTOXIC ACTIVITY EVALUATION OF PLANT EXTRACTS.

Product description

Technical information:
Food products are generally characterized by discards with a predictable intrinsic value linked to the presence of many molecules potentially capable of exerting multiple biological activities. The objective is to verify the possibility of recovering bio-molecules from waste products for the production of nutraceuticals and/or functional foods, and yet, for potential applications in nutraceutical, pharmacological and/or cosmetic sectors. Accordingly, innovative methods have been developed aimed at the evaluation of anti-genotoxic activity of plant extracts at different stages of storage.

Product novelty, utility and originality:
We developed molecular methods for the rapid assessment in vitro of anti-genotoxic effects of plant extracts. The approaches are based on the study of the DNA induced damage by assessing the state of "relaxation" of a supercoiled plasmidic DNA after physical damage with appropriate radiation sources.

Potential users:
Companies, Workers in the Sector. Application finalized A) to gain knowledge on the nutritional characteristics of planted species / varieties; B) recovery from waste products of bioactive molecules for potential applications in nutraceutical, pharmacological and/or cosmetic fields.

Documents:

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15. DEVELOPMENT OF NEW FUNCTIONAL FOODS/INGREDIENTS, CONTAINING VEGETAL EXTRACTS AND PROBIOTICS/PREBIOTICS BY FOOD WASTE

**Product description/Technical information:**
The proposed product starts from the production of a juice giving rise from plant waste (such as rocket salad, Brassicaceae, plant products rich in anthocyanins) that serve as a basis for the growth of probiotics suitably microencapsulated in food grade matrices containing also prebiotic components. The microcapsules, after the fermentation process, are rich in antioxidant compounds, polyphenols, anthocyanins and glucosinolates; they can be represent an important component in the manufacturing of enriched foods (dairy, meat, vegetal juices, and so on). Indeed, the microcapsule allows the protection of the microorganisms during the gastrointestinal transit. The conditions of the pH in the intestine will allow the release of all the functional components of the capsule, with the benefit for the health of the consumer.

**Product novelty, utility and originality:**
In the field of nutraceuticals and of the “functional”agrofood, a high market share is represented by probiotics, capable to make beneficial effects on the consumer’s health, and to enhance some of their properties such as antioxidant or antimicrobial activity versus human pathogens. The novelty of the product is represented by the testing of a method, which allowed growing certain strains of probiotics in the presence of plant material waste. The presence in the growth medium of the plant biocomponents can induce a change in the microbial metabolism, to improve some of their probiotic properties. Microencapsulation also allows obtaining functionally important ingredients, of a sure interest to the pharmaceutical industry.

**Potential users:**
Agro food and pharmaceutics companies

**Documents:**
2. F Fratianni, S Pepe, F Cardinale, T Granese, A Cozzolino, R Coppola, F Nazzaro. *Eruca sativa* might influence the growth, survival under simulated gastrointestinal conditions and some
biological features of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains. International Journal of Molecular Sciences 15 (10), 17790-17805, 2014.


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16. PANEL TEST INTEGRATED WITH ELECTRONIC NOSE

Product description

Technical information

Quantitative Descriptive Analysis measures the sensory properties of food very precisely. Judges, trained subjects, examine the food and list all the relevant sensory properties. By using a scaling procedure, the judge estimates the intensity of each sensory attribute considered. The raw data obtained are then evaluated through statistic techniques. The electronic nose allows measuring instrumental sensory analysis of the volatile compounds of low molecular weight. The device is equipped with a series of sensors that are able to send to the computer signal variations. The data are processed by Principal Component Analysis and highlighted as homogeneous groups, the specific olfactory fingerprints.

Product novelty, utility and originality

The difficulties of working with judges are remarkable when you have to analyse a large number of samples and at the same time to obtain high reproducibility of the data. With the proposed methodology, it is possible to compare, for example, a product at various storage times and, in parallel with the panel of judges, to define the olfactory fingerprint of a product with certain aromatic properties (markers). In addition, you can specify the behaviour of unknown samples, even without the constant work of a panel. The regression method, Partial Least Square Analysis, allows the correlation of the measured values obtained by electronic nose to the olfactory descriptors defined by the panel.

Potential users

Agrifood companies. Most food companies use human panels to evaluate the sensory quality of the products, but after testing a few samples, humans lose the ability to differentiate between similar scents and must take a rest. Electronic noses have the potential to test dozens of samples a day, resulting in considerable time saving and costs.

Documents:


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17. DEVELOPMENT OF A BIOASSAY TO EXPLOIT DOMESTIC WASTE

**Product description/Technical information:**
The bioassay employs human erythrocytes and plasma as biological models to assess the protection from oxidative damage of polyphenolic extracts prepared from vegetable samples obtained from different stages of conservation (fresh, stored in a controlled atmosphere or air for different periods).

**Product novelty, utility and originality:**
Development of a rapid, simple, reproducible and low-cost strategy to determine the biological activity of biomolecules recovered from fresh and ready-to-use fruits and vegetables.

**Potential users:**
Companies, Workers in the sector, National and international organizations

**Documents:**
Idolo Tedesco, Virginia Carbone, Carmela Spagnuolo, Paola Minasi, Gian Luigi Russo Identification and quantification of flavonoids from two Southern Italy cultivars of Allium cepa L. Var. Tropea (red onion) and Montoro (copper onion) and their capacity to protect human erythrocytes from oxidative stress. J. Agric. Food Chem., 2015, 63 (21), pp 5229–5238 DOI: 10.1021/acs.jafc.5b01206

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MALDI-TOF-MS molecular profiling strategies can be applied to the evaluation of food quality and authenticity and to the monitoring of frauds. These rapid and accurate methodologies are based on the direct analysis by MALDI-TOF-MS of hydro-soluble protein/peptide extracts from food matrices. Mass spectra represent a highly specific fingerprint of the sample under analysis and allow defining unique biomolecular markers of authenticity and quality.

The developed methodology has been already applied to the analysis of several food matrices. It requires a short time for sample preparation and analysis and allow to achieve reliable, reproducible and unequivocal results. This method can be extremely valuable to control the authenticity of several food products in order to guarantee their quality and protect the Made in Italy market.

Companies, Food business operators, National and international organizations


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Product descriptionTechnical information:
Bioethanol is the product obtained by a biotechnological process in which microorganisms of plant origin (yeast belonging to the genus Saccharomyces) are capable to ferment food waste starch (potatoes not edible). First the substrate is treated to hydrolyze the starch and to obtain fermentable sugars. Under controlled conditions inoculated with experimental starter to perform the fermentation for ethanol production.

Product novelty, utility and originality:
The political and social changes that are observed more frequently in the world highlight the demand for greater availability of renewable fuels and at the same time the search for fuel sources, which have less negative effects on the climate, the environment and human health. The task of the research is to study new alternatives or reassess energy sources and production processes. The availability of micro-organisms able to adapt their capacity to ferment fermentable substrates made from food waste of plant origin, may offer the opportunity to produce biofuels at minimal cost and no less important, less polluting fossil fuel. This could be interesting and competitive market keeping in mind the disastrous effects that are causing food waste for disposal and management, the environment and economy of the country.

Potential users:
The bioethanol production from natural sources such as agricultural and food waste could offer new opportunities or the possibility of converting the production activities of small and medium industries. In particular, industries located in areas where the availability of waste is greater, reducing the costs of supply.

Documents:
2. F. Boscaino, A. Sorrentino, E. Ionata, F. La Cara, M.G. Volpe. 2014. Evaluation of autochthonous selected yeasts from grapes and cellar in winemaking of Aglianico vine. Bioprospect J. V.24, No. 3; 66-70

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20. WEB ACCESSIBLE DATABASE CONTAINING SOME OF THE EXPERIMENTAL RESULTS OBTAINED IN THE FRAMEWORK OF THE PROJECT.

Product description/Technical information:
A database, able of storing the results obtained by ISA’s researchers on products of interest for the project, was designed. The DB was structured and implemented using PostgreSQL as DBMS, and has been populated with actual data. In order to make the data readily available, it has been developed a web application that allows to navigate data starting from the product, from the type of analysis or from the group that carried out the analysis itself. The application was developed in JAVA with the STRUTS framework, and then JSP has been for the visualization level.

Product novelty, utility and originality:
The product is useful in the dissemination of the results of the project. It could be applicable in other projects, similar for the experimental activity performed (biochemistry, microbiology, biology, etc.)

Potential users:
Companies, Workers in the sector, National and international organizations.

Documents:
Database’s ER project.

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