Deliverable 5.1

WG5 Technical roadmap - key food applications and standardized procedures

Scope

Identification of key food applications and requirements they pose on plasma technologies.

Definition of standardized procedures to be adopted across the laboratories.

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1) Methodology

The present document is the result of a year-long discussion between WG5 members, whose opinions were collected in the following events:

- Management Committee meeting 1 (6-7/10/2020)
- WG5 first year meeting (28-29/1/2021)
- Training school (17-19/3/2021)
- Workshop on Plasma Applications for Smart and Sustainable Agriculture (2-3/9/2021)
- Management Committee meeting 2 (6/10/2021)
- Round table on plasma and berries (13/10/2021)

WG5 members also provided their inputs by replying to questionnaires proposed before the WG5 first year meeting (Questionnaire 1) and in preparation of the present document (Questionnaire 2). Questionnaires presented the following questions:

Questionnaire 1:

Q1) What areas of food processing require low temperature plasma (LTP) technologies and why?

Q2) Referring to the indicated food processing areas (Q1): what are the largest knowledge gaps and technical barriers to increase LTP technology maturity with the aim of translating into commercial products?

Q3) Referring to the indicated food processing areas (Q1): do you foresee regulatory issues in relation to LTP technology introduction as commercial products?

Q4) What is your opinion on the identification of key parameters and the adoption of standard procedures to evaluate LTP treatment efficacy? Please provide examples of suitable performance indicators and procedures

Q5) What is your opinion on the identification of key parameters and the adoption of standard procedures to evaluate the safety and quality of plasma treated products? Please provide examples of suitable performance indicators and procedures

Q6) Although not only, researchers are the current core of WG5 members. Who is also needed? Please provide names of institutions/companies/regulatory agencies/consumers associations, if you can.

Q7) Specifically, can you name companies that have LTP products under development or already on the market for food processing applications. Please name company and application.

Questionnaire 2:

Q1) In your opinion, what are the key food applications (applications with the potential for being transferred to the production environment) of plasma technology and what makes plasma competitive with respect to conventional technologies for these applications?

Q2) Referring to the key food applications identified in Q1, what requirements do they pose on plasma technologies (e.g. operation gases, process times, type of plasma treatment)?

Q3) Referring to the key food applications identified in Q1, please provide performance indicator and procedures that you believe suitable to be adopted as standards to evaluate the treatment efficacy

Q4) Referring to the key food applications identified in Q1, please provide performance indicator and procedures that you believe suitable to be adopted as standards to evaluate the safety and quality of plasma treated products

Q5) Are you aware of companies that have low temperature plasma products under development or already on the market for food processing applications? Please name company and application.

Q6) Referring to the questionnaires submitted in preparation of the WG5 first meeting: do you have any comments on their outcomes?

Q7) Did you perform any dissemination activity (publications, presentations, …) on WG5 related subjects during 2021? Please indicate here the dissemination activities you performed

2) Overview of the state of the art

Different aspects of the state of the art on the applications of plasma processes and technologies in food industry were covered by review papers published in the last year; a list of selected reviews is here reported:

- A review of microbial decontamination of cereals by non-thermal plasma, Foods 10(12), 2021 (link)
- Cold plasma processing on fruits and fruit juices: A review on the effects of plasma on nutritional quality, Processes 9(12), 2021 (link)
- Consequences of non-thermal cold plasma treatment on meat and dairy lipids – A review, Future Foods 4, 2021 (link)
- Application of cold plasma and ozone technology for decontamination of Escherichia coli in foods - A review, Food Control 130, 2021 (link)
- Recent progress in the application of plasma-activated water (PAW) for food decontamination, Current Opinion in Food Science 42, 2021 (link)
- Cold plasma an emerging nonthermal technology for milk and milk products: A review, International Journal of Dairy Technology 74(4), 2021 (link)
- Cold plasma: Microbial inactivation and effects on quality attributes of fresh and minimally processed fruits and Ready-To-Eat vegetables, Trends in Food Science and Technology 116, 2021 (link)
- Inactivation of viruses using nonthermal plasma in viral suspensions and foodstuff: A short review of recent studies, Journal of Food Safety 41, 2021 (link)
- Application of nonthermal processing technologies in extracting and modifying polysaccharides: A critical review, Comprehensive Reviews in Food Science and Food Safety 20(5), 2021 (link)
- Impact of cold plasma on the biomolecules and organoleptic properties of foods: A review, Journal of Food Science 86(9), 2021 (link)
- Chemical changes of food constituents during cold plasma processing: A review, Food Research International 147, 2021 (link)

- Application of cold plasma technology in the food industry and its combination with other emerging technologies, Trends in Food Science and Technology 114, 2021 (link)
- Cold plasma technology for fruit based beverages: A review, Trends in Food Science and Technology, 114, 2021 (link)
- Pros and cons of cold plasma technology as an alternative non-thermal processing technology in seafood industry, Trends in Food Science and Technology 111, 2021 (link)

The relevant state of the art was also analysed in the context of a virtual mobility grant (grantee: Milan Vukic) that provided a bibliometric analysis of the last 20 years of research in the field.

The annual scientific production of the field, reported in Figure 1 for the years 2000-2021, is in constant growth; the slight decrease in 2021 is due to considering in the analysis only papers published before the 10th of October 2021. This growth can be explained in terms of i) broadening of the scientific community, which saw an exponential increase of researchers active in this field, and ii) a rising interest for new technologies that provide minimally processed food products to consumers. The natural consequence of this interest, demonstrated by a co-word analysis whose results are reported in Figure 2, is that 'nonthermal processing of food' and 'cold atmospheric plasma' were identified as motor themes of the field. As a final point evidenced by the bibliometric analysis, all the core sources (the most influencing scientific journals, determined in accordance with the Bradford's Law and shown in Figure 3) belong to the food science and technology area.

As a final note on the state of the art of the industrial developments of the technology, there are currently four companies active in the field: IMPEL Group (Croatia), Plasmaleap (Ireland), AlmaPlasma (Italy) and Tayra (Spain).

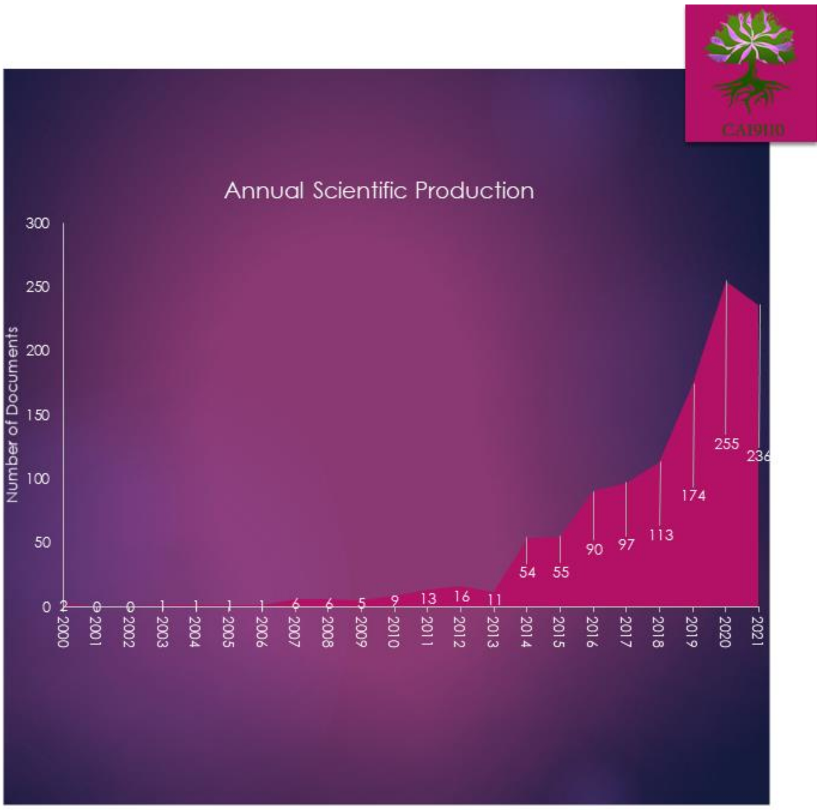

Figure 1: Annual scientific production regarding the applications of plasma processes and technologies in food industry

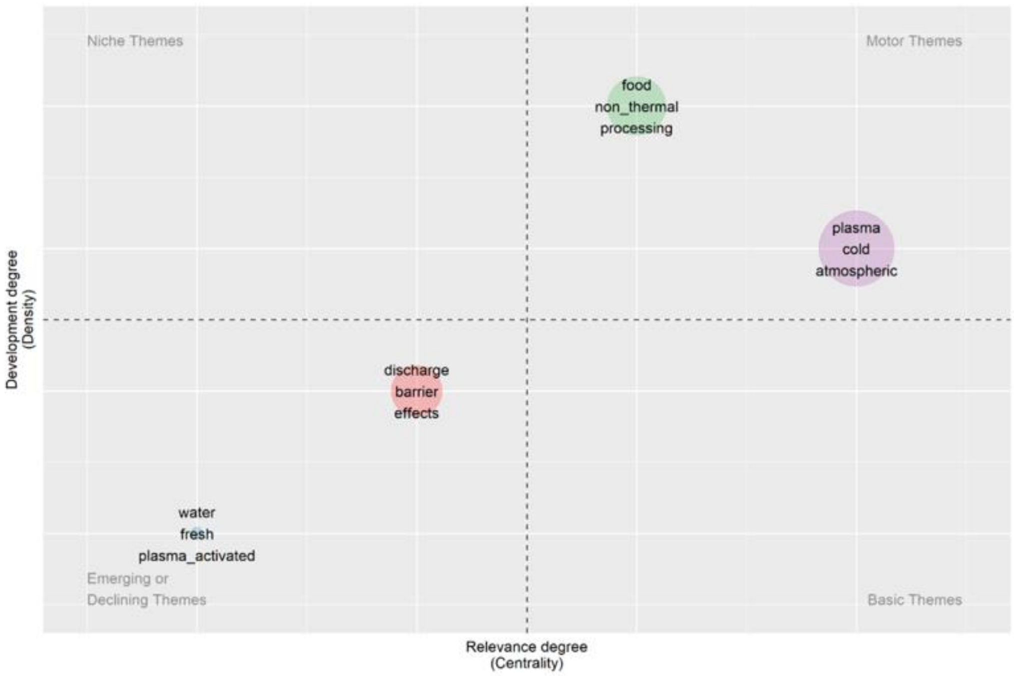

Figure 2: Conceptual structure of cold plasma applications in food production framework using a word co-occurrence network to map and cluster terms extracted from keywords of the papers considered in the bibliometric analysis

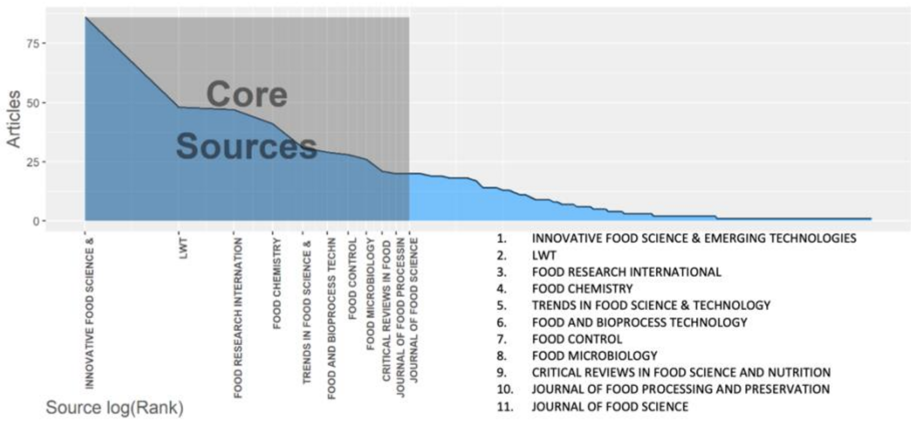

Figure 3: Core sources classified in accordance with the Bradford's Law

3) Discussion on key food applications and standardized procedures

Key food applications

The term 'key food applications' is here used to refer to those applications with the potential for being transferred to the production environment. We identified three main classes of applications for cold atmospheric plasmas (CAPs):

1. Disinfection/hygienization
2. Functional modification of food matrices
3. Functional modification of packaging material

The purpose of class 1 applications (disinfection/hygienization) is to decrease the microbial load of foods, food contact surfaces and packaging material in order to reduce health risks for the consumer and to extend the shelf life of food products. The rationale of these applications is that CAPs produce a variety of active agents (chemical species, radical, UV radiation) which are demonstrated to be effective, to a certain extent, on all kind of pathogens (e.g. bacteria, fungi, spores and viruses) and on food mycotoxins and residual pesticides. Due to their negligible thermal load, CAPs are suitable for processing thermosensitive materials and heat sensitive food products, such as fresh products for raw consumption (e.g. meat, eggs, vegetables, fruit, fresh dairy products) and low water-activity foods (e.g. powders, spices, semi-dried and dried foods). Further advantages of plasma technologies with respect to competing ones are not needing chemical products to operate, the absence of long-lasting chemical residual and the possibility of small/local scale application (e.g. on-field, in-packaging, during transportation, on-shelf). CAPs can either be directly applied to the target (located either in proximity or remotely from the plasma), resulting in a dry technology, or the target can be washed with a liquid previously treated with CAPs; in this configuration, the so-called plasma treated liquids act as vectors for plasma produced reactive species and possess peculiar anti-microbial characteristics. These aspects make CAPs an interesting technology for minimally processed food and suggest that CAPs can play an important role in the quest for a green and sustainable agriculture system, which is one of the main goals marked by the European Commission in the "Strategic plan 2020-2024 – Agriculture and Rural Development" (*https://ec.europa.eu/info/files/strategic-plans-2020-2024-agriculture-and-rural-development_fr*).

The advantageuos aspects of CAP technology carry over to class 2 applications (functional modification of food matrices), which are, among others, the inactivation of anti-nutritional factors, allergenes and degradative enzymes on minimally processed fruit and vegetables, the modification of edible coatings and the tailored modification of product properties along value-added chains of plant and animal related products. These applications are still less explored than CAP disinfection/hygienization.

Examples of class 3 applications are the production of active food packaging with the deposition of antibiofilm coatings and the development of barrier coatings to reduce food contact with oxidative species. Both these strategies are aimed at extending the shelf life of food products and reducing health risks for the consumer.

Requirements posed by key food applications on CAP processing

Two main classes of requirements have been identified: technological requirments and product quality requirements.

1. Technological requirements

   This category refers to challenging technological aspects to increase LTP technology maturity with the aim of translating into commercial products. Examples of technological requirements are:

   - guaranteeing the homogeneity and reproducibility of plasma treatment under an ample range of environmental conditions (ex. Temperature and humidity);
   - adapting the processes to the characteristic production rates and processing times of the process line of interest to enable the integration of plasma processes in standard agro-food chain processes;

- guaranteeing an economically viable process and up-scalable process:
  - reducing the required input resources (e.g. energy, water);
  - relying on air as plasma gas to avoid using expensive gas and introducing additional technical gas lines in food production plants;
    - Note: in-package tretments, namely the treatment of packaged food, may otherwise employ plasmas processes of modified atmospheres, among which: O_2, CO_2, N_2, Ar, N_2O, He;
- safeguarding process operators by reducing possible hazards (e.g. emission of ozone, high voltage contact);
- identifying suitable indicators for monitoring operation and enabling process control strategies

2. Product quality requirements

   This second category broadly refers to guaranteeing the maintainment of the quality of the product after the plasma treatment. Here the most challenging applications are the ones where plasma is applied to foods (either direct or mediated by a plasma treated liquid), which pose the most challenging requirements, among which:

   - avoiding the production of toxic components;
   - minimizing (or avoiding) non-toxic residuals;
   - avoiding the modification of physical-chemical and sensorial quality and stability of treated foods.

   A relevant aspect of food quality requirements is the presence of regulations to which new processess must be compliant with; a critical aspect of these regulations is being region, or even country, specific (e.g. FDA food code standards in USA, EMEA food code standars and Novel Food regulation in Europe).

Standardized procedures

The standardization of procedures has been identified as appropriate strategy to systematically approach the requirements posed by key food applications on CAP processing, enabling objective comparisons of results obtained by different research groups and this taking advantage of the efforts of the entire European community of scientists working in this field. The adoption of three classes of standards is suggested:

- standards to evaluate the characteristics of the employed CAP or plasma treated water;
- standards to evaluate the treatment efficacy;
- standards to evaluate the safety and quality of plasma treated products.

The first class of standards should be aimed at producing an ID card of the plasma treatment in order to allow for comparisons between different plasma technologies and the effects induced in a given application. This approach requires 1) the identification of key parameters describing technical aspects of applied CAPs, preferentially focusing on indicators suitable for in-line monitoring to enable process control strategies, and 2) the adoption of standardized procedures for the measurement of such parameters. Similarities to this approach can be found in the DIN SPEC 91315 covering the standardization of procedures for measuring key plasma parameters in the context of therapeutical applications, which could be adopted as a stepping stone for introducing standards in the context of plasma processing for the food industry. Given the fundamental difference between direct plasma application and liquid mediated application, a different set of key parameters should be identified for each approach. As a final note, it should be mentioned that all members of the WG5 are convinced that standardization should not limit the range of plasma technologies under investigation: currently there is no clue that any plasma generation or application method is better than any other, even if it's understood that some applications might pose specific requirements making a certain solution preferrable at first sight (e.g. decontamination/hygienization of pipings with plasma treated liquids).

The second class of standards should be aimed at identifying indicators suitable to compare the effectiveness of performed treatments. Performance indicators are often defined by regulations and are application-specific: each application, such as microbial inactivation, enzymatic inactivation, chemical compounds degradation, functional properties modification, has to be associated with a specific performance indicator. Given the pressing economic requirements posed on new technologies by agro-food industries, hybrid indicators accounting for both process efficacy and energy expense might be an interesting option. For the case of food processing, standard procedures to be introduced to measure the efficacy indicators might also consider different levels of complexity: a) model systems; b) real food products; c) packed real food products during storage in controlled storage conditions. Also, for the case of disinfection/hygienization, suitable standardized procedures might require to consider both strains from international Culture collection and strains naturally contaminating foods.

The third class of standards should be aimed at identifying indicators suitable to assess and compare the safety and quality of the treated products. For the case of food processing, the choice of proper indicators depends on the product category and is guided, at least to a certain extent, by regulations (e.g. FDA food code standards in USA, EMEA food code standars and Novel Food regulation in Europe). Some examples of indicators suitable for different food products are:

- fresh-cut fruit and vegetable: respiration, metabolic activity, colour, visual quality, texture, sensorial properties;
- dried fruit: colour, visual quality, texture, oxidative indexes;
- fresh/minimally processed fish: colour, visual quality, texture, oxidative indexes, biogenic amines;
- dairy products: pH, odour, taste and colour.

Other indicators suggested during the discussion are microbiota, vitamin content, weight loss, polyphenols and flavonoids of the processed food. Standard procedures to be introduced to measure the safety/quality indicators should focus both on the value of the indicators immediately after the treatment and during the shelf life in different storage conditions (e.g. using climate test chambers). The application of standard mathematical models for quality degradation parameters, or the development of new ones specific for plasma technology, might be helpful to develop a modular exposure assessment model integrated on an Industrial Risk Assessment framework.

4) Plasma treatment of berries

This chapter summarizes the results of a round table performed in the context of a virtual mobility grant (grantee: Pia Staric) as a first attempt to identify suitable indicators and standard procedures for a specific class of products (berries) and application (disinfection to reduce health risks for the consumer and to extend the shelf life of the product).

- Standards to evaluate the characteristics of the employed CAP or plasma treated water
  Direct application of CAPs is suggested to be more effective for this product category, as washing with plasma treated water might result in faster water loss, decay, shorter shelf-life and reduction of sensory and nutritional values. The following CAP characteristics are suggested as suitable key parameter:
    - temperature;
    - ultra violet (UV) and vacuum ultraviolet (VUV) radiation;
    - electromagnetic field;
    - reactive species.

  Further discussions are needed to define standard measurement procedures and to better clarify which reactive species should be measured (e.g. O_3, NO_2, …)

- Standards to evaluate the treatment efficacy
  The considered food category is subject to yeast, fungal, bacterial and viral infections. The assessment of disinfection efficacy on berries should be performed according to ISO standards (or adapted ISO standards) such as:
    - Microbiological criteria of the European Comission - Guidance on the acceptability of foodstuffs and their manufacturing processes;
    - SR EN ISO 4833:2003 - Determination of total mesophilic aerobic count;
    - SR ISO 21527-1:2009 - Determination of yeasts and moulds;
    - ISO 15141:2018 - Determination of Ochratoxin A;
    - ISO 16050:2003 - Determination of Aflatoxin B1, B2, G1 and G2;
    - ISO 15216-1:2017 – Determination of viruses.

  It would be wise to use at least two different methods for each type of microorganisms as some methods can give false results or do not cover the entire spectrum of the contaminants on the berry surface.

- Standards to evaluate the safety and quality of plasma treated products
  The following indicators should be adopted to assess and compare the safety and quality of treated products of this category (relevant ISO standard indicated in brackets, when available):
    - Fresh fruit sampling (ISO 874:1980)
    - Determination of dry matter
    - Determination of firmness of a fruit
    - Determination of total soluble solids (ISO 2173:2003)
    - Determination of titrable acidity (ISO 750:1998)
    - Determination of water-insoluble solids (ISO 751:1998) o Sugar/acid ratio
    - Determination of total soluble solids
    - Determination of fruit skin colour
    - Total anthocyanins content

- Determination of total phenolic compounds
- Determination of antioxidant capacity
- Determination of texture of berries
- Bloom presence (blueberries)

As previously mentioned, safety and quality of plasma treated products should be assessed both immediately after the treatment and during the shelf life in different storage conditions. Relevant ISO standards that address the methodology of storage tests for fruits and berries are:

- ISO 3659:1977 - Ripening after cold storage
- ISO 6664:1983 - Cold storage for blueberries
- ISO 6665:1983 - Cold storage for strawberries
- ISO 6949:1988 - Principles and techniques of the controlled atmosphere method of storage
- ISO 2169:1981 - Physical conditions in cold stores

5) Conclusions

The identification of key indicators and the standardization of procedures for their measurement is seen as instrumental for increasing the maturity of plasma technogy with the aim of translating into commercial products and processes in the food industry.

A tentative approach to enable the comparison of results obtained by a broad and multidisciplinary scientific community was recently performed in the neighboring field of plasma treatment of seeds (*Entering the plasma agriculture field: An attempt to standardize protocols for plasma treatment of seeds, Plasma Process and Polymers 19, 2022*). In the context of plasma technologies for the food industry, this will require first to introduce standard indicators and procedures suitable for the description and comparison of the different plasma systems adopted in the field. The definition of these standards will enable to perform the functional characterization of key food applications (T5.2) and the analysis of food safety and quality (T5.3) with comparable plasma treatments, thus facilitating the intermediate scale-up of technologies and processes (T5.4). Given the profound difference between direct CAP application and application mediated by plasma treated water, two different sets of indicators and procedures are expected; two separate events (e.g. round tables) will be organized in the second year of the COST action to discuss suitable technological indicators and associated standard procedures.

The WG5 activitis forseen for the second and third year of the COST action, functional characterization of key food applications (T5.2) and the analysis of food safety and quality (T5.3), respectively, will also strongly benefit from the introduction of efficacy, safety and quality indicators and standardized procedures for their measurement. These indicators are determined by the application (e.g. efficacy), the category of the treated food (e.g. safety and quality) and are often subject of regulations. The definition of detailed protocols and unified methodologies, working groups of experts from different scientific and legal fields for each topic segment should be formed; food producers associations, authorities and partners with toxicology experience are also needed. The round table on plasma treatment of berries, whose results where summarized in the previous chapter, has been the first step in this direction; as next steps to be taken in the second year of the COST action we envision further discussions on the berries food category and the organization of similar round tables for other relevant classes of foods.

6) List of dissemination activites

The following list is limited to activities performed during the first year of the CA19110 COST action.

Publications

- M.C. Pina-Pérez et al., Surface Micro Discharge–Cold Atmospheric Pressure Plasma Processing of Common House Cricket *Acheta domesticus* Powder: Antimicrobial Potential and Lipid-Quality preservation, Frontiers in Bioengineering and Biotechnology, 2021
- M.C. Pina-Pérez et al., *In vivo* assessment of Cold Atmospheric Pressure Plasma technology on the bioactivity of *Spirulina*, Frontiers in Microbiology, 2022.
- I. Muro-Fraguas et al., Durability Assessment of a Plasma-Polymerized Coating with Anti-Biofilm Activity against L. monocytogenes Subjected to Repeated Sanitization, Foods, 2021
- E.F. Ricciardi et al., Effects of plasma treatments applied to fresh ricotta cheese, Innovative Food Science and Emerging Technologies, 2022
- R. Laurita et al., Effect of plasma activated water (PAW) on rocket leaves decontamination and nutritional value, Innovative Food Science & Emerging Technologies, 2021
- F. Capelli et al., Decontamination of food packages from SARS-COV-2 RNA with a cold plasma-assisted system, Applied Sciences, 2021
- D. Abouelenein et al., Effect of Plasma Activated Water on Selected Chemical Compounds of Rocket-Salad (Eruca sativa Mill.) Leaves, Molecules, 2021

Presentations in International Conferences

- E. Pagan et al., The impact of plasma activated water on the cross-protection mechanisms of resistant mutants of *Salmonella enterica* Typhimurium, 35th EFFoST International Conference, 2021
- G. Gozzi et al., Effect of plasma activated water (PAW) on rocket leaves decontamination and nutritional value, 34th EFFoST International Conference, 2020
- S. Tappi, PASS - Plasma assisted sanitation systems for the inactivation of SARS-CoV-2 on food contact materials, International Conference AgroFood, 2020
- Tappi S. PASS "Plasma assisted sanitation systems" for the inactivation of SARS-CoV-2 on food contact materials, IFSTI 49th Annual Food Science and Technology Conference, 2020

Divulgative events and activities

- Green Plasma for Green Future, chair Nina Recek, CA 19110 Plagri online seminars, 2021
- Round Table on Plasma and Berries, chair Pia Staric, CA 19110 Plagri VM grant, 2021
- Presentation of the CA 19110 Plagri to the association AgreenTechValley, 2021
- ational conference of the network HAPPYBIO (https://happybio-sfpb21.sciencesconf.org/) on biomedical (including agro-food) applications of plasma and physical agents (i.e. electric field and photons)
- P. Rocculi et al., Plasma freddo per la decontaminazione di alimenti e imballaggi alimentari da SARS-CoV-2, Foodhub magazine, 2021 (in italian)