

Final Report - Ecoberries Project

The report is related to the activity of WP3 coordinated by Marco Dalla Rosa (Dipartimento di Scienze e Tecnologie Agroalimentari) within the *CORE Organic Plus "Innovative and eco-sustainable processing and packaging for safe and high quality organic berry products with enhanced nutritional value" (ECOBERRIES)*

Report on organic pre-treatment technologies to avoid sulphites and improve functionality of semi-dried products according to organic requirements

Introduction

Osmotic dehydration (OD) is a non-thermal technology and it was used to obtain semidried products. It consists of the immersion of cellular tissue (e.g. whole fruits or pieces) in hypertonic solution causing mass transfer phenomenon due to the difference in the water chemical potential between the food and the osmotic medium. Therefore, during OD a partial dewatering of plant tissue takes place, reducing both freezable water content and the water activity of the system (Tylewicz et al., 2011).

However, OD treatment, especially when applied at room temperature, is a time-requiring process, therefore it was coupled with other pretreatments such as Pulsed electric filed and ultrasound (in order to increase the velocity of mass transfer kinetics). Pulsed electric field (PEF) is a process which promotes the modification of the membrane permeability by application of high voltage short time pulses to the product placed between two electrodes, thus causing the creation of pores in the biological membrane which affect the mass transfer in tissues (Barba et al., 2015). The high intensity ultrasound (Power US) is a mechanical wave operating at the frequency range of kHz, promoting the compression and expansion of the material, usually referred as sponge effect. This is believed to have a double effect on moisture removal and solid gain. On one side, microscopic channels are created in fruits tissue. On the other side, expansion and escape of the gas trapped in the pores are eased, so that the empty pores are filled by the osmotic solution. This mechanism may explain the increase in mass diffusion when ultrasonic treatment is used (Nowacka et al., 2014).

The OD treatment was conducted on three selected organic berry fruits: strawberry, kiwifruit and cranberries. Moreover, edible coating application was studied to increase the shelf-life of fresh blueberries. The application of edible coating seems to be a promising technique to increase the shelf-life of fruits and their nutritional values. It represents an alternative way of conservation, because of their ability to reduce moisture, solute migration, respiration and transpiration rate; to maintain firmness and overall product quality during storage (Tezotto-Uliana et al., 2014).

Case Study 1: PEF assisted osmotic dehydration of organic strawberries.

Experimental plan

Organic strawberries *var. Alba* (10 ± 1 °Brix) were used for the experiment. The strawberries were washed, hand stemmed and cut into rectangular shape pieces of the dimension $5 \times 10 \times 20$ mm (height x width x length). Two pieces (approximately 1.3 g) were placed into a treatment chamber equipped with two stainless steel electrodes with a gap between them of 30 mm. For the treatment, the sodium chloride solution was used with the same

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conductivity as the strawberries (1.6 mS/cm). PEF pre-treatments were carried out by applying a train of 100 near-rectangular shape pulses at three different pulsed electric field strength - E (100, 200 and 400 V/cm), a fixed pulse width of 100 ms and a repetition time of 10 ms (100 Hz). Immediately after PEF pre-treatment the samples were immersed in 40% (w/w) hypertonic sucrose or trehalose solutions, with 1% (w/w) of Calcium lactate (CaLac) as a structuring agent. The osmotic treatment was performed at 25 °C for 120 min. The samples were analyses for mass transfer parameters (water loss, solid gain), water distribution, physical parameters (colour, texture) and cell viability at different treatment times: 0, 15, 30, 60 and 120 min.

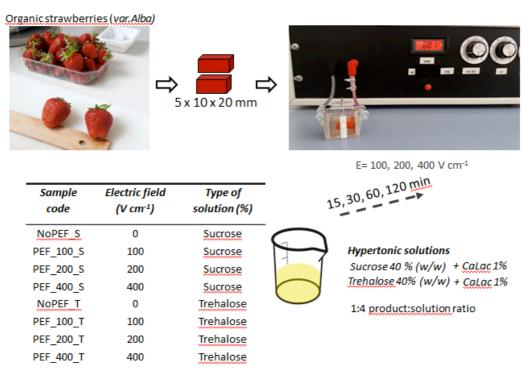


Fig.1. Experimental procedure for osmotic dehydration of organic strawberries.

Results

The results showed that the application of PEF treatment prior to osmotic dehydration positively affected the mass transfer, in term of water loss from the strawberry tissue. Fig.2 and Fig.3 show the water loss and solid gain of untreated and PEF pre-treated strawberry samples, as a function of OD treatment time in sucrose- and trehalose-based solutions, respectively. It could be observed that the application of the lowest electric field intensity (100 V/cm) resulted already sufficient to increase the water loss by 12% and 6% after one hour of osmotic dehydration, respectively for samples dehydrated in sucrose and trehalose solutions. The application of higher electric field strength promoted a further increase in the water loss, however the effect resulted similar whether 200 or 400 V/cm was applied.

Solid gain increased by the application of all electric field strength in samples dehydrated in sucrose solution, while the treatment at 200 and 400 V/cm reduced the trehalose uptake due to a lower initial mass transfer rate.



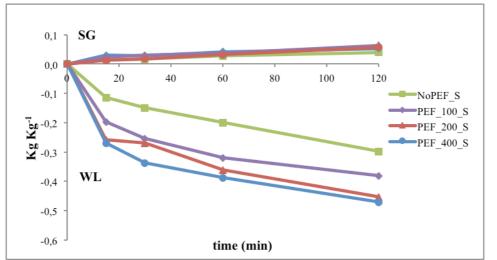


Fig.2. Water loss and solid gain of untreated and PEF pre-treated strawberry samples, as a function of OD treatment time in sucrose-based solution.

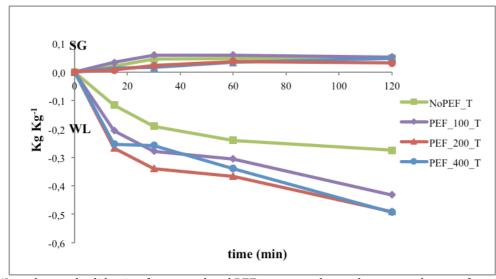


Fig.4. Water loss and solid gain of untreated and PEF pre-treated strawberry samples, as a function of OD treatment time in trehalose-based solution.

Water mobility and distribution was studied by Time Demand-Nuclear Magnetic Resonance (TD-NMR) in terms of T2 relaxation time and its related signal intensities. It permitted to separately observe two main water populations located in vacuoles and cytoplasm plus extracellular spaces of strawberry tissue (Fig.4). OD treatment promoted the decrease of the signal intensity related to the water protons located in the vacuole throughout 120 min, as a consequence of the shrinkage phenomenon. This led to an increase of the intensity of the water protons belonging to the cytoplasm and extracellular space. Moreover, TD-NMR results showed that the diffusion of inner water was eased by PEF application because of a marked effect on membranes permeability, however applying the lowest E, still both water populations were observed suggesting that the PEF treatment has a small impact on cell disintegration. This was also confirmed by partial preservation of cell viability in these samples by using fluorescence diacetate (FDA) dye.

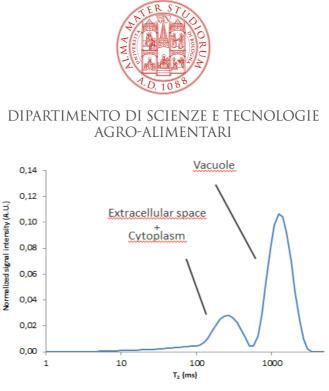


Fig.4 T2-transverse relaxation time of two main water proton populations in fresh strawberry samples.

Concerning the physical parameters, strawberry fruits pre-treated with PEF showed a decrease of red colour in comparison with untreated samples, which was proportional to the electric field strength applied. This probably could be due to both solubilisation of pigments in the osmotic solution and degradation of anthocyanin induced by PEF-treatment. Moreover, softening of the tissue was observed following the application of PEF, which could be due to the alteration of the membrane permeability due to the pores creation and the rupture of internal structure. The slight increase of the texture after longer OD times was observed, which could be probably due to the penetration of CaLac into the strawberry tissue. This increase has not been observed in the samples treated at 400 V/cm, probably because the tissue was already completely disintegrated after the PEF treatment, and did not permit the incorporation of calcium ions in the cell walls.

Conclusions

- Similar effect on the investigated parameters was observed by using sucrose or trehalose solutions, however, the combination of PEF with trehalose allowed to obtain a higher dewatering effect without increasing solute uptake or even reducing it.
- The lowest E (100 V/cm) applied to strawberry samples was able to positively affect the mass transfer. Therefore, the application of 100V/cm and subsequent dewatering in trehalose solution seem to be the optimal combination for obtaining a semi-dried strawberry product maintaining at the same time the fresh-like characteristics of strawberries, that is a fundamental requirement for an organic food production.

Case Study 2: PEF assisted osmotic dehydration of organic kiwifruit.

Experimental plan

The aim of this work was to analyze the effect of Pulsed Electric Fields (PEF) pre-treatment coupled with osmotic dehydration (OD) on mass transfer, water distribution, internal transport and microstructure of organic kiwifruit (*Actinidia deliciosa cv Hayward*). For this purpose, cylinders of 8 mm diameter and 10 mm length were obtained with a cork borer and the PEF pre-treatments were performed by applying three different electric field strengths (100, 250 and 400 V/cm) and a train of 60 pulses. The OD of the kiwifruit was carried out by immersing the samples in 61.5% sucrose solution at 25°C, maintaining a relationship 1:4 (w/w) between the fruit and the OD solution for a contact period of 0, 10, 20, 30, 60 and 120 min. Mass transport phenomena and water distribution into the cellular tissue were studied by time domain nuclear magnetic resonance (TD-NMR)



and by applying the thermodynamic models. Moreover, water activity, water content and soluble solid content were measured.

Results

The application of PEF as a pre-treatment to the OD in kiwifruit increased the water mass transfer and reduced the final sugar concentration compared with samples that have not been pretreated. The application of 100 V/cm increased the water removal from the kiwifruit samples from about 44 % (no PEF) to 50 % after 120 min of OD. The application of 250 and 400 V/cm promoted a further increase of water loss till 55%, however no significant differences were obtained, suggesting that it is not necessary to apply very high electric field strength to achieve a high level of dewatering. Fig. 5 shows the water activity values immediately and 24h after OD treatment. From this figure, it is possible to observe that equilibrated samples show the higher water activity values in comparison to these immediately after treatment. This is due to the concentration profiles observed in the non-equilibrated samples. Moreover, the equilibrated samples seem to be ordered as a function of the intensity of the pretreatment, observing that the higher the pre-treatment the less quantity of water in the liquid phase in the samples was present.

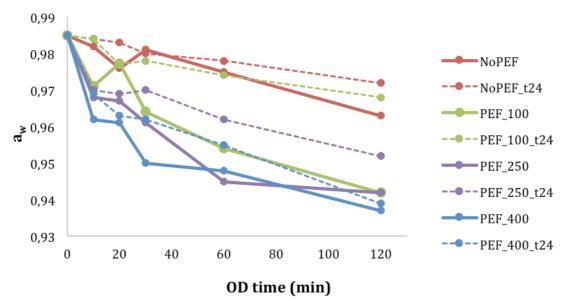


Fig.5 Water activity of kiwifruit samples immediately after OD (solid line) and 24h after OD (dashed line).

The results of water distribution in kiwifruit tissue (vacuoles, cytoplasm and extracellular spaces, cell wall), measured by TD-NMR, showed that PEF treatment caused, for each of the electric field applied, a decrease of average T2 of vacuole and cytoplasm protons populations. Chemical exchange between water (with a T2 around 2 s) and exchangeable sites of the biopolymers of the structures (with a T2 of milliseconds) dominates T2 of these protons populations. The simultaneous shortening of the T2 of both compartments suggests therefore that in both cases exchangeable protons of carbohydrates induced increase their contribution to the overall protons populations upon PEF treatment, but in minimum sense considering negligible. It is worth noticing that the T2 decrease was not proportional to the applied E, with 100 V/cm giving rise to marginal modifications, and the stronger treatments giving rise to similar and much higher T2 decreases. The T2 of each group does not converge to the same value, this suggests that the liquid phase of samples are not mixed, remaining in each compartment.

At higher electric field strength application (250 and 400 V/cm), it was not possible to segregate the signals from some of the compartments of the tissue, suggesting cellular membranes damage. The intensity of 100 V/cm was therefore identified as an optimal compromise between efficacy for mass transfers during OD and the need to preserve the fruit micro-structure.

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Moreover, with NMR analysis it was possible to monitor the internal transport. It was observed that in a normal OD process, the main transport is the symplastic, whereas if previously treated with PEF, the apoplastic transport is as important as the symplastic, considerably increasing the rate of dehydration.

Conclusions

- > PEF pre-treatment could be used to design of new products of candying or osmodehydrated fruits with high dehydration level and lower sugar content.
- > The intensity of 100 V/cm was therefore identified as an optimal compromise between efficacy for mass transfers during OD and the need to preserve the fruit micro-structure.
- > PEF pre-treated involves both apoplastic and symplastic internal transport, considerably increasing the dehydration rate of kiwifruit samples.

Case Study 3: Ultrasound assisted osmotic dehydration of organic cranberries.

Experimental plan

The aim of this work was to investigate the effect of ultrasound assisted osmotic dehydration on dehydration kinetics and quality parameters during storage of cranberries (*Oxycoccus macrocarpon*). Figure 6 presents the experimental procedures used in this study. Ultrasound treatment was performed at the frequency of 21 kHz for 30 min in three osmotic solutions - 61.5% sucrose and 30%

sucrose with an addition of 0.1% of steviol glycosides and 40 % trehalose. The cranberries were cut into a half before the treatment. Afterwards, the cranberry samples were subjected to OD treatment at 40°C for 0, 3, 6, 24, 48, 72 h. The samples OD treated for 72 h were collected and stored at 10 ° C in climatic chamber in microperforated plastic bags (PLA) for 8 weeks. The kinetics of dehydration were studied during OD treatment and modeled by applying Peleg equation.

Moreover, the analysis of colour, dry matter, water distribution, thermal analysis, water activity and microbial analysis were performed after 1, 2, 4 and 8 weeks of storage.



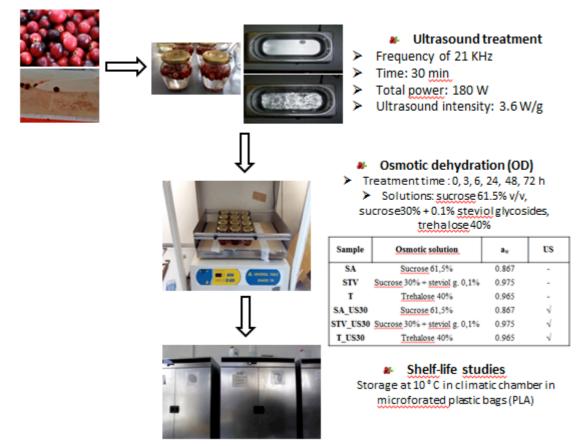


Fig.6. Experimental procedure for ultrasound assisted osmotic dehydration of organic cranberries sults

Results

The kinetics of water loss during OD, modelled according to the Peleg's equation, are shown in Fig. 6. Constants of the Peleg's equation (k1 and k2) and the R2 are reported in Table 1. Comparing the observed and calculated values of mass fraction in the figures and considering the R2 values (between 0.977 and 0.998), the model confirmed to be able to efficiently describe mass transfer phenomena during OD. Samples subjected to the OD treatment in 30 % sucrose solution + 0.1% steviol glycosides presented the lowest water loss compared to the samples treated in T or SA solutions. The significantly higher water loss was observed in SA treated samples (about 60%) in comparison to the untreated samples, due to the lowest water activity of this solution. The application of US before OD treatment of cranberries seems not to have any effect on water loss parameter. This was not in agreement with these presented in the literature, where it has been found that ultrasonic treatment increases the osmotic dehydration rate. However, it has been also reported that not all products respond in the same way to the combination of ultrasound with osmotic dehydration treatment, mainly due to the difference in plant tissue structure (Simal et al., 1998).



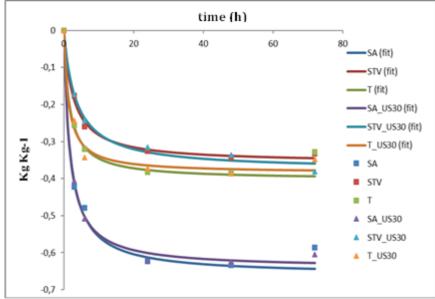


Fig.7. Water loss, modeled by Peleg equation, of untreated and US pre-treated cranberry samples, as a function of OD treatment time.

	Water loss								
Soluzione	k1	SE	k2	SE	(RZ)	1/k1	1/k2		
SA	1,514	0,028	2,837	0,245	0,991	0,660	0,353		
STV	2,800	0,485	7,523	0,039	0,992	0,357	0,133		
Т	2,480	0,255	4,118	0,030	0,996	0,403	0,243		
SA_US30	1,556	0,129	2,586	0,015	0,998	0,643	0,387		
STV_US30	2,656	0,634	9,431	0,043	0,990	0,377	0,106		
T_US30	2,599	0,058	3,543	0,534	0,977	0,385	0,282		

Table 1. Kinetic model of water loss in all investigated osmotic solutions according to Peleg's model.

The samples treated with three investigated solutions for 72h were used for shelf life studies. It was possible to observe the general increase of L*, probably due to the fact that the dehydrated material has less water and therefore the light was reflected differently from the wet surface with different porous structure. General decrease of a* values instead was due to solubilization of pigments in osmotic solution and degradation of anthocyanins. From NMR analysis seems that there are not substantial differences in water mobility, although small differences in dry matter were observed during storage, suggesting that the product was stable over time of storage in terms of water migration and redistribution in the various cell compartments.

Osmotic dehydration improves the shelf-life from the microbial point of view as it results in the reduction of water activity. The sucrose absorption has probably promoted an increase in the viscosity of the liquid phase, affecting the microbial growth kinetics. From table 2 it is possible to observe that samples treated with sucrose at 61.5 % with or without US showed the lowest count of mesophylic aerobic bacteria, yeast and molds.

 Table 2. Mesophylic aerobic bacteria, yeast and molds count on organic cranberries treated with different OD treatment.



Mesophylic aerobic bacteria log ufc/g)

	T0	T1	T2	T4	T8					
SA	2.20	3.26	2.85	2.88	4.92					
STV	2.43	3.75	5.40	7.91	7.99					
T	2.04	4.76	6.00	7.16	8.15					
SA US30	2.53	1.48	2.78	2.85	5.47					
STV US30	2.04	5.08	6.68	7.92	8.55					
T_US30	2.48	5.15	7.01	7.39	8.39					
Yeast (log ufc/g)										
	TO	Tl	T2	T 4	T 8					
SA	2.54	1.48	2.69	\triangleleft	4.90					
STV	2.67	3.12	5.27	7.92	8.26					
T	1.92	4.56	6.07	7.09	8.11					
SA US30	2.09	< <p><1</p>	2.10	2.78	5.15					
STV US30	1.90	4.64	6.59	7.75	8.14					
T_ŪS30	2.53	5.15	6.75	7.25	8.32					
Molds(log ufc/g)										
	T0	TI	T2	T4	T8					
SA	2.00	<1	<	2	3					
STV	<	<1	<	4	<6					
T	1.00	3.00	2	<	<6					
SA_US30	<	<1	< <	< 2	5.11					
STV_US30	<	<2	\triangleleft	<	<6					
T_US30	<	<2	2	4	<6					

Conclusions

- The obtained results indicated that ultrasound application did not significantly affect the mass transfer kinetics during osmotic treatment, as well as the water activity and the content of soluble solids.
- During the storage, ultrasound pretreatment led to the changes in the qualitative characteristics, in particular of colour leading to a general increase in brightness (L *) and to decrease of the red index (a *) in comparison to the untreated fruit.
- NMR analysis showed that the product was stable over time of storage in terms of water migration and redistribution in the various cell compartments.
 The sample that better preserved the physico-
 - The sample that better preserved the physicochemical and microbiological characteristics during storage was the one treated with 61.5% sucrose solution, due to the lowest water activity.

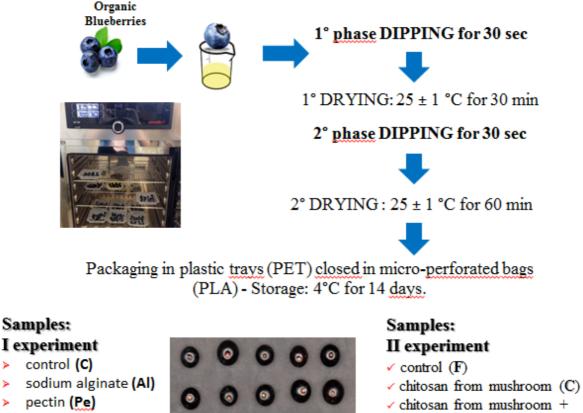
Case Study 4: Edible film application on blueberries

Experimental plan



The aim of this study was to investigate the effectiveness of different edible coatings in improving the shelf-life of blueberry fruits. Whole fruits were dipped in coating solutions, in two-steps process, each one of duration of 30 sec as reported in Fig. 8.

In the first experiment three different types of coatings were used: sodium alginate (Al), pectin (Pe) and sodium alginate + pectin (Al + Pe), while in second experiment the innovative edible coating, based on chitosan from mushrooms (C) and procyanidins extract from grape seeds (C+P) were used. The control and coated blueberry samples were investigated for some quality parameters, cell vitality and microbial growth during 14 days of storage at 4°C. Moreover, in the second experiment antioxidant activity measurements were performed.



sodium alginate
 + pectin (Al + Pe)

 chitosan from mushroom (C)
 chitosan from mushroom + procvanidins from grape seeds (C+P)

Fig.8. Experimental procedure for the application of edible coating on blueberries.

Results

In general, the obtained results showed that the application of coating maintained (C, C+P) or in some cases improved (Al, Pe and Al + Pe) the firmness of blueberries. All type-coated samples did not show significant difference in weight loss, pH, soluble solid content and dry matter. Changes in the surface reflection properties in the coated blueberries induced a general lower lightness and more intense blue hue colour than the control sample. This is probably due to the glossy effect of coating and also the intensity of blue colour might be caused by a possible anthocyanins synthesis during ripening. All kind of edible coatings were able to reduce the growth kinetics of yeasts and mesophilic aerobic bacteria.



Conclusions

- > All applied coatings showed a positive effect mainly on maintaining the firmness and decreasing the microbial growth of treated blueberries samples.
- Results from this study demonstrated the efficacy of new type of coating ingredients (chitosan and natural procyanidins) to maintain the overall quality and high antioxidant activity of fresh blueberries during storage.

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Scientific production

Oral presentations:

- Tylewicz Urszula Oral presentation of ECOBERRIES Project "**Innovative and ecosustainable processing and packaging for safe and high quality organic berry products with enhanced nutritional value**" at WORKSHOP on "Strategies to improve quality of organic products: an European perspective". University of Teramo, Teramo, 3 March 2016
- Marco Dalla Rosa Oral presentation on "**Overview on mild technologies applied to organic berries to increase stability and functionality**" at the Ecoberries workshop during the 2nd Euro-Mediterranean Symposium on Fruit and Vegetable Processing, Avignon, 4-6 April 2016
- Tylewicz U., Mannozzi C., Romani S., Dellarosa N., Laghi L., Ragni L., Tappi S., Rocculi P., Dalla Rosa M. - Oral presentation on "Effect of PEF pre-treatment coupled with osmotic dehydration on physico-chemical characteristics of organic strawberries". At 4th International ISEKI_Food Conference, 6-8 July, 2016, Vienna, Austria, p.81. Book of Abstract ISBN 978-3-900932-34-3



- Tylewicz Urszula Oral presentation on "**Mild drying technologies for organic berries**" at Workshop "Strategies to improve quality of organic products in an European perspective" within the ongoing EcoBerries project, as a side event of FoodInnova 2017 Conference, 31 January 2017, Cesena, Italy
- Traffano-Schiffo M. V., <u>Tylewicz U.,</u> Laghi L., Castro-Giraldez M., Romani S., Rocculi P., Ragni L., Dalla Rosa M., Fito Pedro J. Orat presentation on "**Effect of pulsed electric field pretreatment on microstructure and internal transport throughout osmotic treatment of organic kiwifruit**" at 2nd World Congress on Electroporation, Norfolk 24-28 September, 2017.
- Tylewicz Urszula Oral presentation on **«New technologies for transformation and conservation of berries-Ecoberries project**» at Workshop on Innovative Strategies to improve quality and safety of organic products. Teramo (Italy), 10th November 2017.

Poster presentations:

- Mannozzi C., Cecchini J.P., Tylewicz U., Romani S., Rocculi P., Dalla Rosa M. Poster Presentation PI-35 on **"Study on the efficacy of edible coatings on quality of blueberry fruits during shelf-life**" at 2nd Euro-Mediterranean Symposium on Fruit and Vegetable Processing, Avignon, 4-6 April 2016, p 88.
- Traffano-Schiffo, M.V., Laghi L., Tylewicz U., Castro-Giraldez M., Fito P.J., Ragni L., Dalla Rosa M.
 Poster presentation –P2 on "Study of the water distribution during osmotic dehydration of kiwifruit and the effect of pulsed electric fields as pre-treatment" at 9th International Conference on Water in Food Leuven, May 22-24, 2016
- Nowacka M., Tylewicz U, Romani S, Dalla Rosa M., Witrowa-Rajchert D. Poster presentation on "Selected chemical and physico-chemical properties of ultrasound-assisted osmodehydrated kiwifruit" at 4th International ISEKI_Food Conference, 6-8 July, 2016, Vienna, Austria, p.344. Book of Abstract ISBN 978-3-900932-34-3
- Tylewicz U., Mannozzi C., Tappi S., Dellarosa N., Rocculi P., Dalla Rosa M., Romani S. "Effect of different freezing methods on the physico-chemical characteristics of organic strawberries" –poster presentation, at 30th EFFoST International Conference Targeted Technologies for Sustainable Food Systems on 28-30 November 2016 in Vienna, Austria
- Mannozzi C., Tylewicz U., Chinnici F., Siroli L., Rocculi P., Dalla Rosa M., Romani S (2017). Effect
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- Nowacka M., Tylewicz U., Tappi S., Dellarosa N., Laghi L., Siroli L., Lanciotti R., Dalla Rosa M., Witrowa-Rajchert D. (2017) "Ultrasound assisted osmotic dehydration of organic cranberries (Vaccinium oxycoccus). Study on osmotic dehydration kinetics and quality parameters during storage" In 31st EFFoST International Conference, 13-16 November 2017 Melia Sitges (Spain) [P1.147].

Publications

- Traffano-Schiffo, M. V., Laghi L., Castro-Giraldez, M., Tylewicz, U., Rocculi P., Ragni, L., Dalla Rosa, M., Fito, P. J. (2017). "Osmotic dehydration of organic kiwifruit pre-treated by pulsed electric fields and monitored by NMR". Food Chemistry, 236, 87-93.

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MSc Thesis

- Master Thesis in Formulazione ed Innovazione di Prodotto (Qualità e Formulazione degli Ingredient c.i.).Title: Disidratazione osmotica assistita con ultrasuoni di cranberries (Oxycoccus macrocarpon). Cinetiche di disidratazione e studio delle proprietà chimico-fisiche e microbiologiche in conservazione. Presented by Enrico Casadei. Tutor: prof. Marco Dalla Rosa, Co-tutor. Dr. Urszula Tylewicz. AA 2015-2016.
- Master Thesis in: "Analisi fisiche e reologiche degli alimenti" (Qualità e formulazione degli alimenti c.i.). Title: Modulazione delle caratteristiche chimico-fisiche e della mobilita dell'acqua di fragole sottoposte a differenti processi di trasformazione. Presented by Alessio Lops. Tutor: prof. Pietro Rocculi, Co-tutor. Dr. Urszula Tylewicz, Cinzia Mannozzi, AA 2015-2016.
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Cesena, 25th of May 2018

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