Application of non-thermal technologies for minimally processed fruits and vegetables quality increase and product innovation

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1. Stato dell'arte

Vacuum impregnation (VI) is an innovative technology that has been recognized as a promising tool for the introduction of solutes into the internal structure of some porous food products, due to the action of hydrodynamic mechanisms promoted by pressure changes (Tylewicz et al., 2012). Fruits and vegetables have a great amount of intercellular spaces which are occupied by gas and offer the possibility to be impregnated by external solutions. VI has been widely used to incorporate into the internal structure of fruit and vegetable porous matrices with various solutes, such as anti-browning agents, microbial preservatives or cryoprotectants (Panarese et al., 2014). Previous studies have successfully been conducted to insert external solutions into plant intercellular space for quality or nutrition improvements (Betoret et al., 2015), including potatoes (Hironaka et al., 2015).
High Hydrostatic Pressure (HHP) is an innovative, emerging non-thermal technology that causes permeabilization of the cell structure because of the cell disintegration, which leads to significant changes in the tissue structure resulting in increased mass transfer rates during osmotic dehydration (OD) as compared to untreated samples (Rastogi, Raghavarao, Niranjan, & Knorr, 2002). Application of HHP has been successfully used to improve the mass transfer during OD in several fruits such as pineapple, strawberry, banana etc. (Verma, Kaushik & Rao, 2014). Moreover, compared to traditional thermal treatment, HPP allows the retention of nutritional and organoleptic attributes of foods.
This project is aimed to: 1) enrich minimally processed potato sticks with rosemary essential oil through VI in order to obtain an innovative aromatic potato product; 2) evaluate the effects of HHP treatment (50-400 MPa) to improve OD and quality of green plums.

2. Bibliografia

Tylewicz U, Lundin P, Cocola L, Dymek K, Rocculi P, Svanberg S, Galindo FG (2012) Gas in scattering media absorption spectroscopy (GASMAS) detected persistent vacuum in apple tissue after vacuum impregnation, Food Biophys 7(1): 28-34.

Panarese V, Rocculi P, Baldi E, Wadsö L, Rasmusson AG, Galindo FG (2014) Vacuum impregnation modulates the metabolic activity of spinach leaves, Innov Food Sci Emerg 26: 286-293.

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Verma D, Kaushik N, Rao PS (2014) Application of high hydrostatic pressure as a pretreatment for osmotic dehydration of banana slices (*Musa cavendishii*) finish-dried by dehumidified air drying, Food Bioprocess Tech 7(5): 1281-1297.

3. Sviluppo della ricerca

The research was developed according to the following main points:
1) Bibliographic study on innovative non-thermal technologies applied on fruits and vegetables.
2) **Study on the application of VI** for the enrichment of minimally processed potato sticks with rosemary essential oil.
3) **Study of the effect of the application of HHP** (50-400 MPa) as a pre-treatment for the osmotic dehydration of green plum on mass transfer kinetic, water distribution, cell viability and microstructure.
4) **Comparison between HPP and traditional thermal treatments** in the efficiency of the OD process and on the quality and nutritional properties of green plums.

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic study***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A2)	***Study on the application of VI on minimally processed potato***					■	■	■	■	■									
	1) selection of processing parameters					▒	▒	▒											
	2) quality measurement during storage							▒	▒	▒									
A3)	***Study of the effect of the application of HHP***										■	■	■	■	■				
	1) evaluation on OD kinetics of green plum subjected to HHP										▒	▒							
	2) investigation on the effect on water distribution, cell viability and microstructure												▒	▒	▒				
A4)	***Comparison between HPP and traditional thermal treatments***															■	■	■	■
	1) Comparison of the effect on OD efficiency															▒			
	2) evaluation of products quality and nutritional properties																▒	▒	▒
A5)	***Papers and theses writing***								■	■	■	■	■	■	■	■	■	■	■

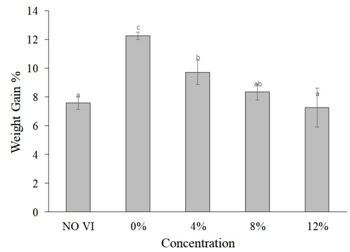
4. Stato di avanzamento della ricerca e principali risultati

4.1. Study on the application of VI for the enrichment of minimally processed potato sticks with rosemary essential oil.

4.1.1. Impregnation efficiency

After immersion in distilled water for 70 min, a weight gain of about 8% was observed (Fig. 1), most likely due to osmotic effects that has caused the entry of water into the cells characterized by a lower water activity.

Figure 1 *Weight gain (%) after dipping in distilled water (NO VI) and vacuum impregnation with aqueous solutions containing 0, 4, 8 and 12% of rosemary essential oil.*



The application of vacuum promotes the inflow of the solution inside the tissue pores. By applying vacuum to the sample immersed in water (0% solution), the weight increase was approximately 12%. Considering that the porosity of potato resulted of only about 1%, this result indicates that vacuum impregnation can foster exchanges of mass due to osmotic phenomena. The results also showed that increasing the concentration of rosemary oil the weight gain proportionally decreased. With the more concentrated solution (12%) an increase in weight equal to that achieved by immersion in distilled water at atmospheric pressure was observed.

4.1.2. Evolution of physico-chemical and microbiological aspects during storage

During storage, a progressive decrease in the value of whitening index (WI) (Fig. 2) was observed for the control sample (NO VI), probably due to enzymatic browning. The reduction of enzymatic browning following VI has already been observed and was attributed to the reduction of the presence of oxygen in the tissue.

However, samples impregnated with solutions containing 8 and 12% of essential oil, were characterized by a higher browning level at the end of storage, compared to the other samples. This effect may have been caused by the oxidation of essential oil components which has led to a change in colour during storage.

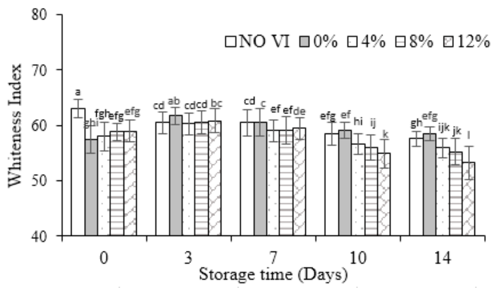


Figure 2 *Changes of whiteness index value of vacuum impregnated potatoes during storage.*

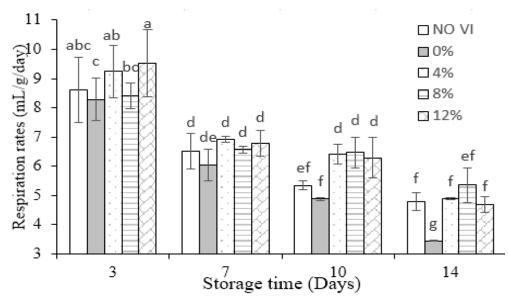


Figure 3 *Respiration rate of vacuum impregnated potatoes during storage.*

In general, the respiration rate (Fig. 3) decreased with storage time in all samples. 0% sample showed lower values compared to NO VI sample. This could be due to the filling of the intercellular spaces as a result of the vacuum application with water. The samples impregnated with solutions containing rosemary extract showed an increase in the respiration rate that could be due to a metabolic change in the tissue caused by essential oils.

Microbial loads relative to total mesophilic counts, psychrophilic bacteria, coliforms, molds and yeasts were increased, but only slightly during storage, without significant differences among the samples.

According to GC analysis, the main detected compound was eucalyptol, followed by camphor, 3-methyl-apopinene, α-pinene, 1,3,8-p-Menthatriene, α-camphene and linalool. The amount of these volatile compounds generally decreases along with storage time and, at the end of storage, camphor, 1,3,8-p-Menthatriene and α-linalool were no longer detectable. At the last day of storage, the presence of ethanol was detected, probably due to the cell metabolism and growth of microorganisms.

4.1.3. Sensorial properties of fried samples

Main results of the sensorial test showed that samples impregnated with higher concentrations of essential oil showed lower values related to colour uniformity at the end of storage; in agreement with the findings of the instrumental assessment, the odour of rosemary was perceived fairly proportionally to the concentration, while the perception of the typical potato flavour tends to diminish with the increase of the concentration of rosemary of the impregnating solution.

4.2. Study of the effect of the application of HHP as a pre-treatment for the OD of green plum

4.2.1. Effect on OD kinetics

HHP treated samples showed higher solids gain and water loss compared to non-treated samples (Fig. 4); the initial rate and equilibrium mass transfer values calculated according to Peleg model, were higher in HHP treated samples. Generally, the cell membranes of fruits exert high resistance to mass transfer. Application of HHP causes permeabilization of the cell structure, which leads to significant changes in the tissue structure resulting in increased mass transfer rates during OD as compared to untreated samples.

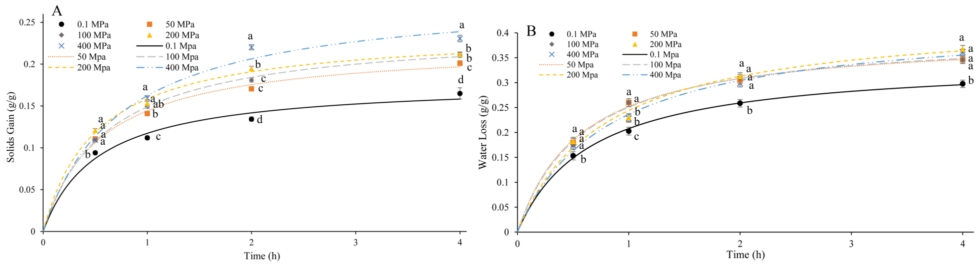


Figure 4 *Fitting curves of Peleg's model on SG and WL of green plum.*

4.2.2. Effect on water distribution by LF-NMR

Water distribution was evaluated through LF-NMR. Because the calculated parameter T_2 depends on chemical exchange of protons among water, solutes and biopolymers, and it represents the mobility of the water molecule, it is possible to separately observe the water in different locations (cell wall, extracellular space/cytoplasm and vacuole of plant cells, characterized by different T_2 and different intensity values as shown on Fig. 5.

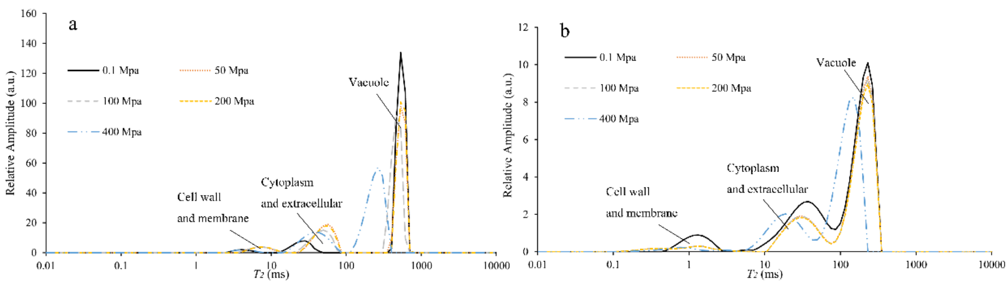


Figure 5. *The distribution of transverse relaxation times of proton pools in different cellular compartments before (Figure 2a) and after 4h OD (Figure 2b) as a function of HHP treatments.*

HHP caused a decrease of the total signal of the water in vacuole and an increase of total signal in cytoplasm space, indicating the water flowed out from vacuole. After treatment at 400 MPa T_2 value showed a significant decreased to 268 ms. After 4h OD, the total signal intensity dropped 5-6 folds in all samples. The values of T_2 also dramatically dropped in the cell compartments, mainly due to the cellular shrinkage caused by OD. After the OD, the HHP treated samples were characterized by lower signal intensity compared to the control. Only after the higher applied pressure (400 MPa) the T_2 of the vacuolar protons were lower compared to the control, while the T_2 relative to cytoplasm/extracellular space and cell wall/membrane was generally reduced by all HHP treatments.

4.2.3. Effect on cell viability

Increasing the applied pressure, a loss of cell viability was observed by FDA staining and fluorescence microscopic observation (Fig. 6).

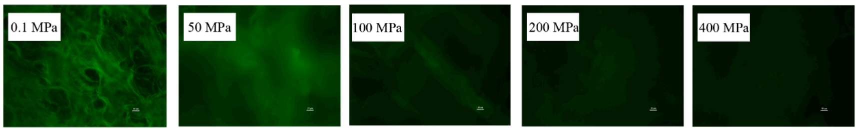


Figure 6. *Micrographs of green plum tissue by fluorescence detection microscope, using Fluorescein Diacetate to identify viable cells. Viable cells are distinguished by a bright fluorescence.*

4.3. Comparison between HPP and traditional thermal treatments

Kinetic of mass transfer (Fig. 7) showed that HHP resulted an effective method to accelerate the OD process of candied green plums, allowing to reduce the processing time from more than 5 days in the control sample (C) to about 2 days. However, traditional heating methods (H and T samples) showed the highest rates of dehydration.

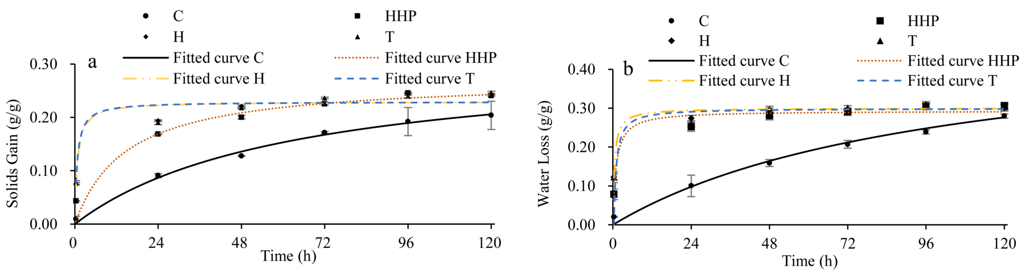


Figure 7. *Experimental data and fitting curves of Peleg's model to SG (a) and WL (b)*

4.2.5. Evolution of physico-chemical of green plum during OD

After the heating treatments, the green plums showed lower titratable acid content compared to HPP and Control samples, possibly due to the leakage of intracellular organic acid promoted by the damages to the cell membranes during the heating process.

Moreover, immediately after the treatment, the color turned from a light green in the control sample to a bright yellow in H and T samples and to a bottle green in HHP sample. During the OD process, HPP sample showed a slight increase of L* and b* and a remarkable increase of a* value, that may be due to enzymatic activity, such as peroxidase and/or chlorophyllase, that promote the degradation of color and the formation of dark pigments. At the end of the OD process, HPP sample was still characterized by a greener hue compared to H and T samples.

Heating showed a strong negative influence on the fruit firmness; although the addition of calcium salt in the dipping solution (sample T) promoted a better retention of texture, firmness was still considerably lower compared to the control and HHP.

4.2.6. Sensory evaluation of candied green plum

Sensory analysis has confirmed the data obtained by instrumental analysis about firmness and color, also indicating that HHP samples retained the typical green plums odor and flavor of the fresh sample, but with a considerably higher overall acceptability (Fig. 8).

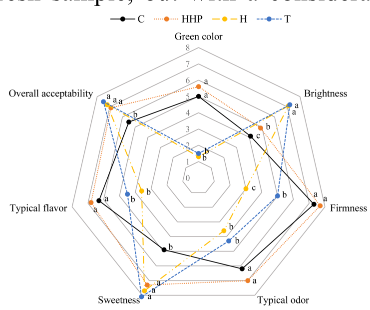


Figure 8. *Sensory scores of candied green plums after OD for 5 days. Each point labelled with different letters indicated the statistical difference from each other.*

5. Elenco delle pubblicazioni prodotte nell'ambito dell'attività di dottorato

Luo W, Wang C, Yong Y, Zhu S, Tappi S, Rocculi P (2018) Study and optimization of High Hydrostatic Pressure (HHP) to improve mass transfer and quality of candied green plums (Prunus mume). LWT-Food Sci Technol Under review.

Luo W, Tappi S, Wang C, Yong Y, Zhu S, Rocculi P (2018) Effect of High Hydrostatic Pressure (HHP) on the osmotic dehydration kinetics of Wumei fruit (Prunus mume) and mechanism study. Under revising by tutor.

Luo W, Tappi S, Rocculi P, Dalla Rosa M (2018) Preparation of Innovative Aromatic Minimally Processed Potatoes by Vacuum Impregnation (VI) with Rosemary Essential Oil. Under revising by tutor.

Luo W, Tappi S, Wang C, Yong Y, Zhu S, Rocculi P (2018) HHP better retained the antioxidant and volatile properties of Wumei during OD progress. Under writing.