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Design and Optimization of Edible Coating and Osmotic Dehydration Processes for the Development of High-value Fruits and Vegetables with Extended Shelf-life

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Berries and mushrooms are some of the most important traditional Mediterranean products that are characterized by high nutritional value and beneficial properties for the human body. Mushrooms are popular for their texture and taste but also for their chemical and medicinal properties. Although they have beneficial properties for human health, the shelf-life of fresh produce is very limited. In addition, sea buckthorn (Hippophae rhamnoides) berry is valued for its high nutrient density, providing high amounts of vitamin C, vitamin E, carotenoids, oils rich in unsaturated fats, etc. However, these berries are fragile and delicate and have a short shelf-life. The aim of the present study was the design and development of new berry and mushroom products, which are characterized by prolonged shelf-life without the addition of chemical preservatives, high nutritional value with low salt and sugar levels, and increased seasonal availability. The design and development of these products was based on the optimization of osmotic dehydration process and the application of edible coatings, using alternative agents. Various osmotic agents, such as glycerol, natural pectins or fruit juices were examined as alternatives to the conventional ones, with the aim to reduce salt and sugar levels in final products. Osmotic dehydration process was further optimized in terms of several parameters (immersion time, temperature, osmotic agent concentration, product to solution ratio). Dehydrated products were then coated using alternative agents, such as chitosan and aloe vera. The dehydrated and coated products were evaluated for their quality and sensory properties, during storage in controlled conditions. The examined alternative osmotic agents had significant effect to the water loss and water activity of the selected products and led to products with advanced characteristics. The use of alternative agents during edible coating application also led to the increase of products' shelf-life and the enhancement of their nutritional value.

1. Introduction

Sea buckthorn (Hippophae rhamnoides) is a fast-growing, deciduous and thorny shrub or small tree, which is cultivated in Asia and Northern Europe and its products are distributed in many countries around the world (Ciesarová et al., 2020; Padmanabhan et al., 2016). The name of the plant is derived from the Greek words "hippo" and "phaos" (Olas, 2016), which mean "horse" and "to shine", respectively. The sea buckthorn berries are valuable because of the carbohydrates, proteins, vitamins (C, K, B, E), organic acids (malic and quinic acid), phytosterols and minerals (Padmanabhan et al., 2016; Teleszko et al., 2015). They are rich in natural antioxidants (² -carotene, lycopene) and bioactive compounds including carotenoids, phenolic compounds, amino acids and chlorophyll derivatives (Olas, 2016; Padmanabhan et al., 2016). Mushrooms are also popular for their texture and taste but also for their chemical and medicinal properties. They comprise a vast source of powerful new pharmaceutical products (Philippoussis et al., 2007).
The demand for traditionally grown plants is increasing and the preference of consumers has altered to healthier, more nutritious and minimally processed food. The valuable bioactive compounds of sea buckthorn berries offer them a unique advantage on the market (Ciesarová et al., 2020), but they are delicate and have a short shelf-

life (Araya-Farias et al., 2011). In addition, although mushrooms have beneficial properties for human health, the shelf-life of fresh produce is very limited. The opportunity of fruits and vegetables with an extended shelf-life as well as quality preservation is feasible if a drying process is applied (Kyriakopoulou et al., 2013). Osmotic dehydration is a pretreatment process of counter-current transfer of mass, which minimizes the thermal degradation of nutrients and leads to high-quality products (Shete et al., 2018). The parameters affecting the process include the temperature, the type and the concentration of the osmotic agent, the osmotic solution to sample mass ratio, the agitation and the process duration (Akbarian et al., 2013). Due to the growing interest in developing low-calorie products, the use of alternative osmotic agents, such as sugar alcohols, natural pectins or fruit juices, is a challenge. The further extension of the shelf-life of the osmo-dehydrated food can be accomplished with the edible coating technology (Lenart & Piotrowski, 2001). Edible coatings are made of edible biopolymers (proteins, polysaccharides, lipids or a combination of them) and applied to food by dipping, spraying or brushing creating a modified atmosphere by controlling respiratory gas exchange (Nussinovitch, 2009). In several studies, various edible coatings have been already applied to blueberries (Mannozzi et al., 2018), grapes (Ali et al., 2016) and strawberries (Gol et al., 2013).
The objective of this work was to optimize the process parameters of osmotic dehydration using alternative osmotic agents to reduce the sugar and salt level of the dehydrated product. Osmotic dehydration in combination with the edible coating technology was applied to berries and mushrooms to achieve a prolonged shelf-life and high nutritional food products.

2. Materials and Methods

2.1 Materials and chemicals

Sea buckthorn berries were stored at -30 ºC and were thawed before experiments. Mushrooms were stored at 4 ºC. Solvents and reagents used in the experiments were of analytical grade. Water, methanol, acetic acid and sodium carbonate were purchased from Fischer Scientific (Leicestershire, UK). Tween 80 was obtained from Acros Organics and glycerol from Moulas Scientific.

2.2 Osmotic dehydration

Initial moisture content of sea buckthorn berries and mushrooms was 76.34±0.72 % and 93.25±0.22 %, respectively. The osmotic agents used were saccharose, glycerol, concentrated apple juice and a mixture of saccharose and apple pectins for sea buckthorn berries. Mushroom samples were dipped in 40 % glycerol for 30 min, followed by immersion in 10 % salt solution. 5 g of fruits were immersed in jars filled with the osmotic solution and then placed on a temperature- and agitation-controlled water bath. Process parameters are described in Table 1. After immersion, the samples were lightly wiped with a tissue paper to remove the excess solution from the surface and then were weighed. All results are based on average values of two replicates.

Table 1: Process parameters of osmotic dehydration

Osmotic agent	Concentration	Temperature	Fruit/solution ratio (w/w)	Agitation	Time (h)
Saccharose	50 % w/w, 60 % w/w	25 ºC, 45 ºC	1:4, 1:9	With and without	1 - 24
Glycerol	40 % w/w, 50 % w/w	25 ºC, 45 ºC	1:4, 1:9	With and without	1 - 24
Saccharose–Pectins	45–5 % w/w, 55–5 % w/w	25 ºC, 45 ºC	1:4, 1:9	With and without	1 - 24
Apple juice	47,2 ºBrix	45 ºC	1:4, 1:9	With	1 - 24
Salt	10 % w/w	25 ºC, 45 ºC	1:4, 1:9	Without	2

2.3 Mass transfer modelling

The osmotic process is characterized by the parameters of water loss (WL) and solid gain (SG) of fruit after time t of osmotic treatment. They are defined as:

$$\mathrm{WL} = \frac{(M_o - m_o) - (M - m)}{M_o} \quad (1) \qquad \mathrm{SG} = \frac{m - m_o}{M_o} \quad (2)$$

where M_o=initial mass of fresh fruit before the osmotic treatment, M=mass of fruit after time t of osmotic treatment, m=dry mass of fruit after time t of osmotic treatment, m_o=dry mass of fresh fruit.

2.4 Preparation of coating solutions

The biopolymers used for the coating formulations were chitosan and aloe vera (gel). Chitosan solution was prepared by dissolving 2 % (w/v) chitosan in 1 % (v/v) aqueous acetic acid solution. Then, 50 % glycerol (based

on the weight of chitosan) was added, as a plasticizer, and 0.2 % (w/v) Tween 80, as a homogenizer. The preparation was carried out under agitation (Han et al., 2004). Aloe vera was diluted to distilled water until the concentration of 25 % (v/v) was attained. The solution was heated at 70 ºC for 45 min and then cooled to room temperature (Ali et al., 2016; Hassanpour, 2015).

2.5 Active coating application

Osmo-dehydrated berries samples were coated with chitosan solution by dipping the sample into the solution for 2 min. The excess of the coating material was allowed to drip off for 1 min and then the coated samples were air-dried for 1 h in a laboratory tray dryer at room temperature with the air-velocity of 2.5 m/s, as proposed by Sakooei-Vayghan et al. (2020) with some modifications. Osmo-dehydrated berries and mushroom samples were coated with aloe vera solution. The same procedure was followed with a dipping time of 5 min. Osmo-dehydrated with saccharose solution berries and with salt solution mushrooms were used as control, which were immersed in distilled water for 2 min. Both coated and control samples were packaged in polyethylene bags and stored at 3 ºC for 7 days.

2.6 Physicochemical properties

Weight loss of samples (berries and mushrooms) during storage was measured by monitoring the weight changes at 0, 2, 3, 4, 5, 6, and 7 days. Weight loss was calculated as percentage loss of initial weight. Color changes of berries were evaluated using MiniScan XE colorimeter (Hunter Associates Laboratory Inc, Reston, Virginia). The values L, a and b were recorded, which indicate the lightness, greenness and yellowness, respectively. Total color difference was calculated using Eq.(3) described by Nair et al. (2018):

$$(\Delta E) = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3)$$

where ” L, ” a and ” b represent the difference in L-, a- and b- values at a particular interval from the fresh product.

Total Soluble Solids (TSS) of berries were determined using a refractometer in diluted juice as described by Amal et al. (2010).

The Titratable Acidity (TA) derived from the method of Ion et al. (2019) by titration with 0.1 N NaOH using 1 ml of diluted juice in 9 ml distilled water. Phenolphthalein was used as indicator. The results were expressed as ºBrix and grams of malic acid equivalent per 100 g^{-1} fresh weight, respectively.

2.7 Microbial growth

The microbiological analysis took place after 7 days of storage. Each sample consists of 3 g of berries diluted in Ringer solution with ratio 1:9 under aseptic conditions and followed by homogenization in a Stomacher. Compact Dry Plates were incubated for the determination of total aerobic bacteria (Total Count), yeasts and molds, *Salmonella*, *Escherichia coli 0157* and *coliforms* and *Listeria monocytogenes*, according to HyServe. The results were expressed as CFU/g of berries.

3. Results and discussion

3.1 Osmotic dehydration

Figure 1 presents, indicatively, the effects of SA and CAJ on the moisture loss and solid gain with respect to time of osmosis at different temperatures and different fruit/solution ratios. Similar plots were obtained using saccharose and glycerol.

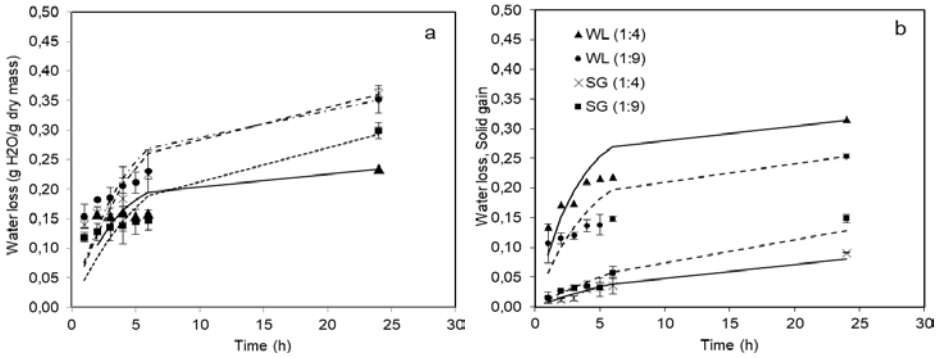

*Figure 1: a) Water loss of berries during osmotic dehydration using as osmotic agent a mixture of 45 % (w/w) saccharose and 5 % (w/w) apple pectins, fruit/solution ratio equal to 1:4 (² : 25 ºC without agitation, Ï : 45 ºC without agitation, :25 ºC with agitation, **x**: 45 ºC with agitation), b) Water loss and Solid gain using CAJ*

During osmotic dehydration the highest water loss was achieved with saccharose-pectins as osmotic agent (0.36 g H_2O/100 g dry biomass (d.b.)), followed by saccharose solution (0.34 g H_2O/100 g d.b.). Less amount of moisture was removed with concentrated apple juice (0.31 g H_2O/100 g d.b.) and glycerol (0.23 g H_2O/100 g d.b.). The solid gain values were 0.02, 0.05, 0.09 and 0.02 g solids/ g d.b. for SA, S, G and CAJ, respectively. It is evident that the moisture loss is faster in the initial period of the process and moderately decreases reaching an equilibrium state. Furthermore, the increase in temperature affected significantly the osmotic dehydration. It is obvious that at the higher temperature the moisture loss was higher due to the improved cell membrane permeability allowing the molecules to be transferred to the solution easier. The solid gain was less affected by the increase in temperature. Moreover, both WL and SG increase when concentration becomes greater, up to a certain level. As the concentration of the osmotic agent increases the presence of agitation leads to higher WL and contributes to a greater extent at lower temperatures. Regarding the fruit/solution ratio, the ratio 1:4 usually appears to be advantageous to WL. Higher SG was obtained with ratio 1:9, with the highest SG achieved using CAJ. Taking all the experimental data into consideration, the optimum process parameters were chosen. The optimum temperature was 45ºC and the time duration 24 h. Regarding the concentration of the osmotic agent, it was proved that the less dense osmotic solutions achieved similar water removal compared to denser ones and lower solid gain. So, the optimum osmotic solutions identified to be 50 % saccharose, 40 % glycerol and a mixture of 45 % and 5 % (w/w) saccharose and pectins, respectively. The preferred fruit/solution ratio of 1:4 was high enough to avoid dilution of the medium. Finally, agitation was not found very efficient.
Figure 2 presents the WL and SG of mushroom samples. The temperature and ratio increase also led to higher WL and SG. Immersion in glycerol solution prior to osmotic dehydration with salt led to lower salt gain.

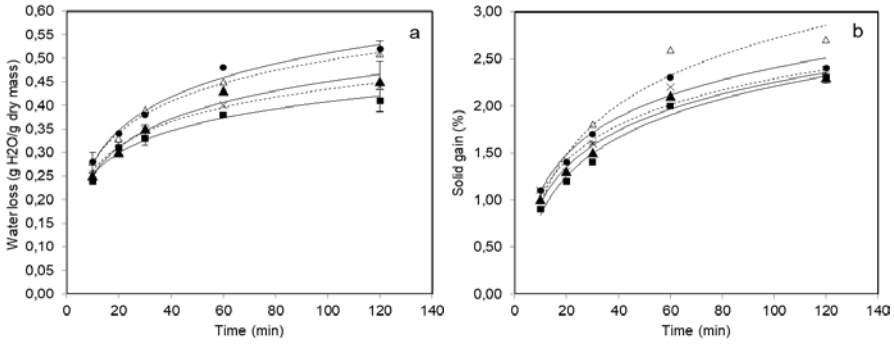

*Figure 2: a) Water loss and b) Solid gain of mushrooms during osmotic dehydration (² : 25 ºC, ratio 1:4, Ï : 45 ºC ratio 1:4, :25 ºC ratio 1:9, **x**: 45 ºC ratio 1:9, • : 45 ºC, ratio 1:4, without immersion in glycerol)*

3.2 Quality evaluation of coated samples during storage

3.2.1 Weight loss and Total color difference

As it can be seen in Figure 3a, the weight loss of berries increased during storage time in all samples because of the surface water evaporation and water vapor permeability (WVP), thus the respiration rate of berries. The lowest weight loss was obtained in control samples and in osmo-dehydrated with SA samples coated with aloe vera. The highest water loss observed in samples coated with chitosan. The polysaccharide films have high WVP and their hydrophilicity causes moisture loss. The relatively high concentration may cause increased anaerobic respiration and higher weight loss, observed also by Ghasemnezhad & Shiri (2010). The addition of lipids to the chitosan coating could retard the moisture loss, but it seems unnecessary in the case of aloe vera coating due to its hygroscopic properties and its hydrophobic nature.

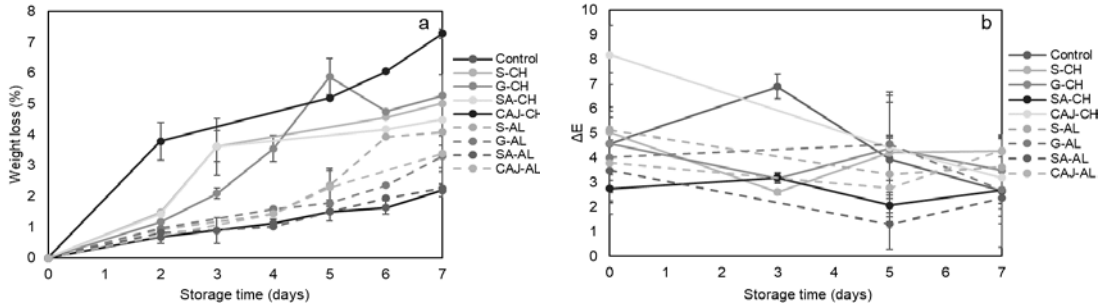

Figure 3: a) Weight loss and b) Total color difference (" •) of coated and control berries samples during storage

Figure 3b shows the color differences of non- and coated-berries during storage. In the first days, the aloe vera-coated samples retained slightly better the color of berries contrary to the control ones that showed significant color changes. From the fifth day of storage, the changes were generally decreased. The osmo-dehydrated with SA and coated berries retained at the most the color of the fruit. The delay in the ripening of the chitosan-coated fruits could be attributed to the positive charges of anthocyanins in their stable form and of chitosan leading to the stabilization of the color. Aloe vera-coating prevents enzymatic and oxidative browning preserving the color. Figure 4 presents the weight loss of mushrooms during storage time in selected samples coated with aloe vera. Aloe vera contributed to the decrease of weight loss of mushroom samples during storage.

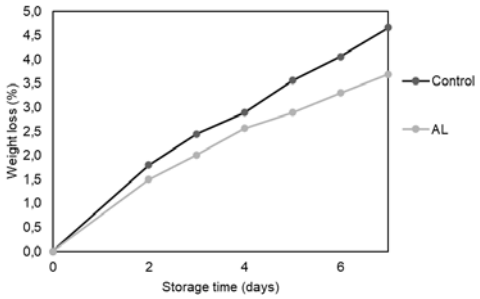

Figure 4: Weight loss of coated and control mushroom samples during storage

3.2.2 Titratable acidity (TA) and Total soluble solids (TSS)

The fresh berries contained 2.08±0.04 g malic acid/100 g fresh fruit, which are similar to Ion et al. (2019), and 7.27±0.12 ºBrix. The osmotic dehydration caused a reduction of TA and an increase of TSS in all samples.

Table 2: Analyses of chitosan (CH) and aloe vera (AL) coating on osmo-dehydrated sea buckthorn berries during storage at 3 ºC

	Days	Control	S-CH	G-CH	SA-CH	CAJ-CH	S-AL	G-AL	SA-AL	CAJ-AL
TSS/TA	0	8.30±0.01	8.30±0.01	8.30±0.01	8.30±0.01	8.30±0.01	8.30±0.01	8.30±0.01	8.30±0.01	8.30±0.01
	7	11.34±0.02	10.35±0.07	8.57±0.07	5.36±0.30	13.12±0.17	9.84±0.01	10.90±0.07	9.26±0.06	8.06±0.42

The TSS/TA ratio increased in all samples except SA-CH during storage, but the control ones had a more pronounced increase, as described in Table 2. That could be explained by the higher respiration rate of the uncoated berries compared to coated samples, whose internal atmosphere was modified by the thin layer of coating delaying the ripening. The changes in TA and TSS may be also due to the water loss during storage, especially of the CAJ-CH samples that showed the highest shrinkage.

3.2.3 Microbial growth

Microbiological analysis showed that chitosan-coated berries had no microbial growth and were considered safe to be consumed, except for SA-CH samples. The addition of pectins in the osmotic solution allowed the growth of microorganisms in the samples coated with chitosan. Chitosan has an excellent antimicrobial effect in all samples except for SA-CH because of its bioactive compounds with antimicrobial activity. The fungistatic properties of chitosan are confirmed through studies in the shelf-life of strawberries by Ribeiro et al. (2007) and of blueberries by Mannozzi et al. (2018). Aloe vera-coated samples did not reach significant microbial spoilage since the counts of *Escherichia coli* 0157 and *Listeria monocytogenes* were below 100 CFU/g. Counts of yeasts and molds as well as total count were not significant (less than 10^3 and 10^5 CFU/g, respectively) (EU, 2005). Samples CAJ-AL were an exception due to *Listeria monocytogenes* growing even though that apple juice provides antimicrobial activity because of its high acidity. *Salmonella* was absent in all samples. The antimicrobial activity of aloe vera is attributed to its compounds with inhibitory effects on microorganism's growth and was verified by Martínez-Romero et al. (2006).

Table 3: Microbiological analysis of coated and control berries samples after 7 days of storage

	Days	Control	S-CH	G-CH	SA-CH	CAJ-CH	S-AL	G-AL	SA-AL	CAJ-AL
Total Count	7	0	0	0	>5000	0	160	120	70	870
Yeasts and Moulds	7	0	0	0	>5000	0	120	10	80	10
E. coli 0157	7	0	0	0	855	0	20	20	20	87
Listeria	7	0	0	0	>5000	0	N/A	N/A	N/A	625
Salmonella	7	0	0	0	0	0	0	0	0	0

4. Conclusions

The osmotic dehydration of sea buckthorn berries and mushrooms at various conditions has been studied. The increase in temperature led to greater WL and SG. Higher values of solution concentration with the presence of agitation exerted little influence on process. The ratio of 1:4 provoked greater water loss. The optimum process parameters for berries were 45 ºC, 1:4 ratio, 24 h at the least solution concentration and without the use of agitation, except for the CAJ, while for mushrooms 45 ºC and 1:4 ratio. The production of high nutritional and prolonged shelf-life products was attempted with CH and AL edible coatings. AL restrained weight loss at a greater extend, but ” • were negligible. Both coatings could extend the shelf-life, even after 7 days of storage.

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