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SPECTROPHOTOMETRY

Shelf Life Extension and Quality Improvement of Cucumber Slices Impregnated in Infusions of Edible Herbs

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ABSTRACT

Development of functional food through their enrichment with herbal extracts is gaining much attention from the food industry. The present study evaluates the benefits, such as the oxidative stability and quality retention, of the enrichment of cucumber (*Cucumis sativus*) with natural phenolic compounds through immersion into a variety of herbal infusions. After a preliminary experiment with gallic acid solutions, the beneficial effect of hypotonic solutions was observed and the optimal process conditions were defined. The second step of the study involved the immersion of cucumber in the herbal infusions for 2.5 h at 70 °C. The total phenolic content (TPC), antiradical and antioxidant activity of osmo-treated slices were determined to evaluate the extent of bioactive compounds' impregnation. During the immersion, TPC, antioxidant and antiradical activity significantly increased irrespective of the type of the herbal infusion. The TPC varied greatly, ranging from 1.41 to 781.14 mg GAE/kg of cucumber. The highest total phenolics were found in cucumber treated with *Origanum vulgare* infusion, followed by *Jasminum officinale* and *Mentha spicata* infusions. The aforementioned osmo-treated slices were also highly appreciated by the sensory panel; therefore, for those best performing herbal infusions, the third phase of this work involved the study of quality degradation under subsequent storage of the osmo-treated slices vs. the untreated ones. The results regarding color, texture, and visual assessment demonstrated the superior quality retention of the osmo-treated samples that exhibited a shelf life extension ranging from two- to almost four-fold compared to the untreated tissue.

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Antiradical-antioxidant activity; cucumber; herbal infusions; osmotic treatment; sensory analysis; total phenolic content (TPC)

Introduction

Nowadays, there is an increasing commercial and nutritional interest associated with the production of functional and healthy foods. The positive benefits of phytochemicals in human health such as anti-inflammatory, antioxidant, antibacterial, antifungal,

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chemopreventive, hypolipidemic, neuroprotective, and antiaging agents (Fotakis et al. 2016) have prompted food industry to use them for the production of functional foods and dietary supplements.

Herbs and aromatic plants are recognized as a potential source of phytochemicals of nutraceutical importance, such as terpenoids, phytosterols, and polyphenols. Specifically, aromatic plants of the Lamiaceae family, such as *Salvia officinalis*, *Mentha spicata*, *Origanum vulgare*, *Mentha pulegium* L., are well-known raw materials that contain a plethora of bioactive substances that include protocatechuic acid, ferulic acid, rosmarinic acid, caffeic acid, apigenin, luteolin, kaempferol, myricetin, quercetin, eriocitrin, and naringenin with antimicrobial, anti-inflammatory, antiaging, and antioxidant properties (Pereira and Cardoso 2013). The flowers of *Jasminum officinale* (Family, Oleaceae) contain in high concentration flavonoids and saponins, and their extracts are reported to show anti-lipid peroxidative effects, and antibacterial, antioxidant, and antiradical activity (Akhtar and Mirza 2018).

Moreover, aromatic plants of the Lauraceae family, such as *Laurus nobilis*, *Cinnamomum burmannii*, *Cinnamomum verum*, are rich sources of terpenoids, phenols, flavones, and flavonols, and have shown many biological properties including anti-inflammatory, antioxidant, antibacterial, antiseptic, digestive, diuretic, and antiviral, improving insulin response and lowering serum lipids (Patrakar, Mansuriya, and Patil 2012; Pandey and Chandra 2015; Ervina, Nawu, and Esar 2016). Furthermore, aromatic plants of the Myrtaceae family, such as *Eucalyptus globulus* and *Syzygium aromaticum*, are good sources of bioactive terpenoids, tannins, flavonoids, flavonoid glycosides, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid derivatives, galloyl-glucose derivatives, and ellagitannins (Nassar et al. 2007; Pombal et al. 2014).

On the other hand, fruits and vegetables are foods of superior nutritional value, supplying vitamins, nutrients, natural antioxidants, and essential minerals as well as of increased significance for consumers, food industries, and food market. However, the majority of these cellular matrices of vegetative origin are susceptible to physicochemical deterioration and, thus, are highly perishable, having short shelf life with their rapid degradation leading to significant economic losses. The deterioration occurring in fruits and vegetables is based on their post-harvest spoilage, oxidation of phenolic substances in plant tissues by phenolase, lipid oxidation, chlorophyll pigments degradation, off-flavor production, non-enzymatic browning and bacteria, yeasts, and mold growth (do Nascimento Nunes 2008).

Cucumber (*Cucumis sativus*) is one of the most cultivated and consumed vegetables in the world due to its sensory and physicochemical properties. Cucumbers are low in calories, carbohydrates, sodium, and fat, and provide many health benefits, including hydration properties due their high water content and valuable nutrients such as vitamin K (Joshi et al. 2018).

The extension of the shelf life of fruit and vegetables has been previously achieved through the application of several technologies such as osmo dehydration (OD), cryo-concentration with ohmic heating (OH), pulsed vacuum (PV), and modified atmosphere packaging (MAP) (Manjunatha and Anurag 2014; Guerra-Valle et al. 2018).

Food matrix enrichment with bioactive compounds including vitamins, phenolic compounds, curcuminoids, and probiotics has been extensively studied through the

application of a non-thermal treatment, namely the OD (Barrera, Betoret, and Fito 2004, Rózek et al. 2009; Rózek et al. 2010). OD is a mild method frequently used for a variety of foods with the aim to partially remove the inherent moisture and thus decrease water activity, a_w, while simultaneously enhancing the selective uptake of solutes, resulting in the desired modification of the characteristics of the original product (Dermesonlouoglou and Giannakourou 2019). This technique involves the immersion of a solid food in a water solution (either hypotonic or hypertonic) to induce water loss from the food into the solution and solute transfer from the solution into the food; the direction of this mass transfer depends on the osmotic pressure of the surrounding solution as well as the osmotic pressure in the food matrix and takes place through the selective cellular membrane. Focusing on the actual mechanism describing this mass transfer, several mechanisms such as osmosis, diffusion, and hydrodynamic mechanisms have been proposed in the literature (Rastogi, Angersbach, and Knorr 2000; Rastogi et al. 2002).

Osmotic treatment has many advantages which include selective food formulation and enriching the food matrix with compounds that improve its functional, structural, and nutritional properties. The extent and rate of infusion of bioactive compounds can be changed by varying the type of surrounding solution (concentration and content) and the process conditions (temperature and time). This impregnation technique, accomplished mainly through diffusion phenomena, can be helpful in designing novel foods with improved functional attributes, increased bioactive compounds, besides producing modified final products in terms of sensory and nutritional aspects (Bellary and Rastogi 2012).

In this study, the immersion of a model-solid food into a surrounding hypotonic solution, based on herbal infusions, was explored as a method to introduce antioxidants in cucumber slices without altering their matrix. The basic idea was to select a food tissue of low initial natural antioxidant content, so as to be able to systematically study the potential of impregnating bioactive compounds into fruits or vegetables in order to enhance their antioxidant profile, quality attributes, and shelf life.

Materials and methods

Chemicals, standards, and solvents

The chemicals 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ), sodium persulfate, ferrous sulfate heptahydrate, iron(III) chloride hexahydrate, ABTS [2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid)], Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), gallic acid (3,4,5-trihydroxybenzoic acid), and Folin–Ciocalteu reagent, as well as solvents of analytical grade were purchased from Mallinckrodt Chemical Works (St. Louis, MO, USA), Alfa Aesar GmbH (Karlsruhe, Germany), Tokyo Chemical Industry (Japan), and Sigma-Aldrich Chemie GmbH (Germany).

Sampling and osmotic solutions preparation

Fresh cucumbers (*C. sativus*) of the same maturity level and the same lot were purchased from Agricultural Vegetable Cooperative of Megara (AGROMEG), in order to

Table 1. Herb samples.

Herbs	Scientific name	Family
Chamomile	*Matricaria chamomilla* L.	Asteraceae
Sea buckthorn seeds	*Hippophae rhamnoides*	Elaeagnaceae
Alexandria leaves	*Senna alexandrina*	Fabaceae
Alexandria pods	*S. alexandrina*	Fabaceae
Sage	*Salvia officinalis*	Lamiaceae
Spearmint	*Mentha spicata*	Lamiaceae
Oregano	*Origanum vulgare*	Lamiaceae
Mint	*Mentha pulegium* L.	Lamiaceae
Bay laurel leaves	*Laurus nobilis*	Lauraceae
Cinnamon sticks	*Cinnamomum burmannii*	Lauraceae
Cinnamon sticks	*Cinnamomum verum*	Lauraceae
Liquorice root	*Glycyrrhiza glabra*	Leguminosae
Roselle calyces	*Hibiscus sabdariffa*	Malvaceae
Eucalyptus leaves	*Eucalyptus globulus*	Myrtaceae
Clove flower buds	*Syzygium aromaticum*	Myrtaceae
Jasmine flowers	*Jasminum officinale*	Oleaceae
Goji Berry fruits	*Lycium barbarum*	Solanaceae
Annual nettle leaves	*Urtica urens*	Urticaceae
Lemon verbena leaves	*Lippia citriodora*	Verbenaceae
Turmeric	*Curcuma longa*	Zingiberaceae

minimize compositional variability. After manual skin removal, cucumbers were cut in slices of 20-mm length and 2-mm thickness.

Aqueous solutions of gallic acid standard (1.0 and 2.0%, w/v) and aqueous solutions of the combination of gallic acid standard (2.0% w/v) and glycerol (40% w/v) were used as the hypotonic and hypertonic osmotic solutions, respectively, for preliminary experiments. Gallic acid solutions, in order to be characterized as hypotonic, should have lower osmotic pressure when compared to that of cucumber. Twenty herbal species, in a dry form, were purchased from the Anatolia Spices Company and selected as the source of natural antioxidants for the preparation of the herbal infusions for the osmotic procedure (Table 1). To prepare the herbal infusions, 4 g of each herb sample were added to 60 mL of boiling distilled water in a stainless steel pot, left to stand for 15 min (Fotakis et al. 2016), and filtered under reduced pressure and immediately used.

Osmotic treatment in model solutions of gallic acid and herbal infusions

The first set of preliminary experiments was carried out to select between hypotonic and hypertonic osmotic solutions for cucumber enrichment. Therefore, cucumber slices were immersed into hypotonic (gallic acid 2.0% w/v) and hypertonic (gallic acid 2.0% and glycerol 40% w/v) solutions at 70 °C for 1, 2, and 4 h in order to determine their efficiency to infuse gallic acid in the cucumber. The second set of preliminary experiments was performed using different temperatures, immersion times, and gallic acid concentrations in order to determine the efficiency of osmotic enrichment. For this purpose, fresh-cut cucumber slices were immersed into osmotic solutions of gallic acid (1.0 and 2.0%, w/v), at 60, 70, and 80 °C for 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h. The osmotic solution/fresh-cut cucumber slices ratio (w/w) was higher than 10:1, in order to avoid any dilution of the osmotic solution. The experiment was performed in duplicate for two concentrations, three temperatures, and eight immersion times.

Based on the results of the aforementioned experiments, the optimal process conditions were selected in order to immerse fresh-cut cucumber slices into the infusions of the 20 plant species. Therefore, the temperature was maintained at 70 °C; the herbal infusion/fresh-cut cucumber slices ratio (w/w) was higher than 10:1; the immersion time was 2.5 h. All experiments were performed under atmospheric pressure in triplicate.

At the end of immersion time, the samples were removed, blotted softly with tissue paper to remove adhering herbal infusion and then weighed. Approximately 3 g of each sample (in triplicate repetition) were pulped and water was added to a final volume of 20 mL. The extraction took place for 24 h at 25 °C. The extracts were passed through a Buchner filter and were diluted to a final volume of 20 mL. All extracts were stored at 4 °C until analysis.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of enriched cucumber extracts was determined according to a modified micromethod of Folin–Ciocalteu's colorimetric assay (Andreou et al. 2018). The absorbance was measured at 750 nm with a visible spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed Inc., USA). Standard solutions of gallic acid ranging in concentrations from 50 to 2500 mg/L were used. The TPC was expressed as mg or g gallic acid equivalents (GAE) per kg of cucumber, using the standard calibration equation $y = 0.0005x + 0.0786$, R^2 of 0.9989.

Scavenging activity on 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$)

The antiradical activity of the enriched cucumber extracts was determined according to the method described by Lantzouraki et al. (2015). Standard solutions of Trolox ranging in concentrations from 0.20 to 1.50 mM were used. Absorbance was measured at 734 nm with a visible spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed Inc., USA). The antiradical activity was expressed as mg Trolox equivalents (TE) per kg of cucumber, using the standard calibration equation $y = 0.2876x - 0.002$, R^2 of 0.9995.

Ferric reducing/antioxidant power assay (FRAP)

The ferric reducing antioxidant power (FRAP) assay of enriched cucumber extracts was carried out according to a minor modification of the method of Benzie and Strain (1996), as described by Lantzouraki et al. (2016). Standard solutions of $FeSO_4.7H_2O$ were used ranging in concentration from 50 to 1800 μM. The absorbance was measured at 595 nm with a visible spectrophotometer (Spectro 23, Digital Spectrophotometer, Labomed Inc., USA). The antioxidant activity was expressed as mg $FeSO_4 \times 7H_2O$ per kg of cucumber, using the standard calibration equation $y = 0.0003x + 0.0081$, R^2 of 0.9969.

Sensory analysis

The sensory analysis of the cucumber slices enriched with herbal infusions took place in a special room which met the requirements of ISO 8589:2007 to enable the sensory

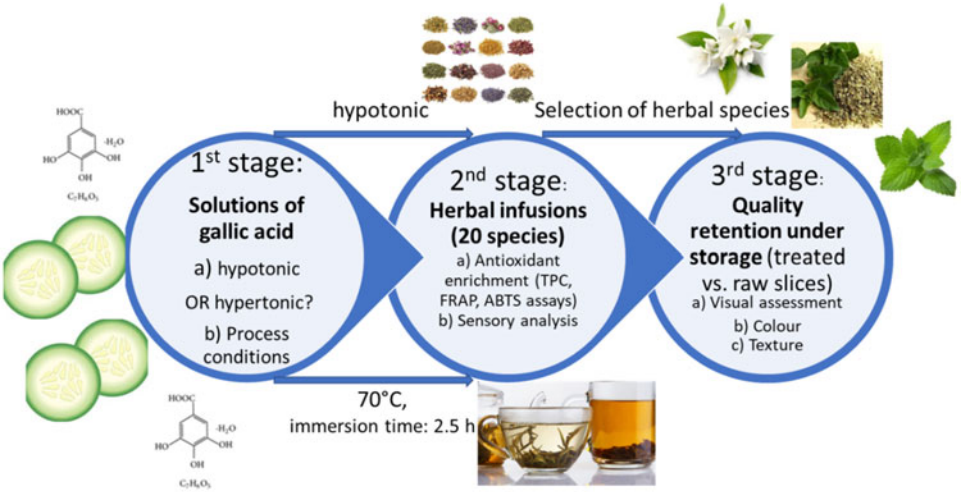

Figure 1. Flow chart of the experimental design implemented.

panel to identify and score the main descriptors. Sensory properties were evaluated applying quantitative descriptive analysis (QDA) with a 20-member trained panel and using an appropriate questionnaire with a scoring scale from 1 (lowest) to 9 (highest) for main quality parameters. Since organoleptic assessment is of major importance, special training was performed, and specific guidelines were given to assessors, including a detailed explanation of the terminology used. Not only sensory attributes related to the visual assessment, such as the color, the overall appearance, but also attributes related to the taste (sweetness/bitterness/the observation of an after taste) were evaluated for all cucumber slices immersed into the different herbal infusions.

In order to better illustrate the structure of the experimental design implemented and the purpose of each stage, the following flow chart (Figure 1) is provided.

Infusion selection and quality evaluation under constant storage conditions

Cucumber slices, immersed into the different herbal infusions under the optimal impregnation conditions, were evaluated in terms of total antioxidant power and sensory acceptability in order to select the infusions for further quality evaluation study.

To assess the effect of storage, cucumber slices immersed into the selected herbal infusions were compared to untreated, fresh samples, in terms of instrumental color and texture as well as visual quality. Samples were examined at 4 and 37 °C up to 21 days depending of storage temperature. Six replicates per osmotic treatment, storage temperature, and sampling day were prepared.

Color measurement

The chromatic characteristics of the cucumber slices enriched with herbal infusions were defined by the colorimetric coordinates L* (lightness), a* (redness/greenness), b* (yellowness/blueness), C (chroma), and h (hue angle in degrees). The above values were measured with a tristimulus chromatometer (model CR-400, Minolta, Tokyo, Japan) calibrated with a white

standard plate (L*: 97.83, a*: −0.45, b*: +1.88). Color parameters were evaluated at days 0, 3, 8, 11, 15, 18, and 21, at 4 °C. Three random readings per sample were taken and averaged.

Texture measurement

Cucumber slices were left to equilibrate at room temperature, and texture measurements were conducted by means of a TA-XT2i texture analyzer (Stable Micro Systems, Godalming, UK) with a load cell (Heavy Duty Platform/90) of 25 kg. The measurement was conducted through texture profile analysis (TPA), which was performed on a non-lubricated flat platform using an aluminum cylinder of 25-mm diameter with which samples were pressurized at a fixed rate and depth. Important mechanical properties of cucumber slice such as hardness calculated as the maximum peak force (N), springiness, and elasticity were considered. Texture parameters were evaluated at days 0, 3, 8, 11, 15, 18, and 21, at 4 °C, and measurements were carried out in duplicate at 20 ± 1 °C.

Visual assessment of microbial growth and spoilage

Visual quality was examined every day for a week period at 37 °C. In this study, a slight discoloration was noticed on the slices' surface which was characterized by a darker brown color. However, the main abnormality observed was fungal infection, characterized by mycelial growth of fungi, which was visible to the naked eye. Other deterioration identified was a decay shown as an extended injury, as well as a surface shrinkage, which was characterized by shriveling or wilting of the center of slices. All samples were documented on photo at each evaluation. This parameter is deemed quite important, considering that it may represent the overall acceptability/marketability of cucumber samples (Chandra et al. 2018).

Statistical analysis

All values were averaged and reported along with their standard deviation. The data regarding TPC, antiradical-antioxidant activity, and color-texture parameters were analyzed using one-way ANOVA and relevant *post hoc* tests. Probabilities lower than 0.05 were considered as statistically significant ($p < 0.05$). Regarding sensory scores, for all attributes assessed, *F*-value and *p*-values of each effect of the ANOVA and of the *post hoc* Duncan discrimination test were calculated. The correlation among the results was performed by the Pearson correlation test. All statistical calculations including partial correlations were performed with the SPSS package (IBM SPSS Statistics, version 19.0, Chicago, IL, USA) statistical software for Windows.

Results and discussion

Preliminary experiments

The main goal of this study is the investigation of the migration of bioactive compounds into fruits or vegetables to enhance their antioxidant profile and quality retention. Therefore, the preliminary experiments of the present study aimed to compare the efficiency of hypotonic (gallic acid 2.0% w/v) and hypertonic (gallic acid 2.0% and

Table 2. Total phenolic content (g gallic acid/kg of cucumber).

	60°C		70°C		80°C	
	hypotonic osmotic solution concentrations					
Time (h)	1.0 % w/v	2.0 % w/v	1.0 % w/v	2.0 % w/v	1.0 % w/v	2.0 % w/v
Total phenolic content						
0.5	0.47 ± 0.01	1.07 ± 0.03	1.06 ± 0.02	2.21 ± 0.04	1.22 ± 0.03	2.44 ± 0.05
1.0	0.50 ± 0.01	1.25 ± 0.04a	1.34 ± 0.06a	2.38 ± 0.08b	1.41 ± 0.04b	2.79 ± 0.06a
1.5	0.74 ± 0.04	1.59 ± 0.07	1.46 ± 0.02	2.51 ± 0.07a	1.78 ± 0.06a	3.03 ± 0.10
2.0	0.87 ± 0.05	1.84 ± 0.05	1.52 ± 0.03	2.82 ± 0.05	1.94 ± 0.11a	3.56 ± 0.16
2.5	1.17 ± 0.06	2.33 ± 0.11a	1.84 ± 0.10	3.28 ± 0.15	1.79 ± 0.07a	2.73 ± 0.07a
3.0	1.30 ± 0.05a	2.53 ± 0.07	1.37 ± 0.03a	2.64 ± 0.10a	1.36 ± 0.04b	2.26 ± 0.12b
3.5	1.77 ± 0.13	2.86 ± 0.08	1.25 ± 0.08a	2.38 ± 0.09b	1.37 ± 0.03b	2.23 ± 0.09b
4.0	1.27 ± 0.08a	2.20 ± 0.06a	1.25 ± 0.06a	2.35 ± 0.03b	1.35 ± 0.08b	2.32 ± 0.06b

The results represent mean ± standard deviation (N = 6). The same letters after each value, in the same column, indicate non-statistically significant differences ($P > 0.05$).

glycerol 40% w/v) solutions for cucumber enrichment with gallic acid. For the most precise calculation of the gallic acid migration extent, it was necessary to evaluate the initial TPC of the raw material (cucumber slices) that was found to be lower than 0.02 g gallic acid E/kg. Therefore, after 1, 2, and 4 h of slices immersion into the hypotonic and hypertonic solutions, the migration of gallic acid in cucumber was almost five-fold higher when the slices were subjected to hypotonic solution (> 2.40 g gallic acid E/kg) as compared to that of hypertonic solution (< 0.40 g gallic acid E/kg), irrespective of the immersion time. In accordance with our results, other researchers (Rózek et al. 2010; Bellary, Sowbhagya, and Rastogi 2011; Bellary and Rastogi 2012) also demonstrated that the migration of phenolic compounds is strengthened in hypotonic solutions than in hypertonic ones.

Therefore, the use of hypotonic solutions was selected as the most appropriate for cucumber enrichment with bioactive compounds. In a further step, preliminary experiments were performed to select the optimum conditions of hypotonic osmotic treatment for cucumber enrichment. Temperature, immersion time, and phenolic concentration are crucial factors to determine the efficiency of hypotonic osmotic enrichment. Based on the findings of Réblová (2012), gallic acid (amongst other phenolic acids, namely gentisic, protocatechuic, and caffeic acids) showed a significant antioxidant activity even at 150 °C in a study that tested the effect of temperature on the antioxidant activity of phenolic acids.

In this study, the main target of slices' immersion was their enrichment with antioxidants, i.e., process parameters should be selected so as to favor solid uptake. Higher temperatures result in a significant increase in the rate of solute uptake, followed by a marginal increase in the rates of water loss (Lazarides and Mavroudis 1996; Lazarides 2001; Lazou et al. 2016). Therefore, the use of higher process temperatures is preferably suggested in treatments aiming at product impregnation, bearing however in mind the heat tolerance of the specific tissue to be processed and especially the heat stability of its cell membrane. Therefore, in our case, the choice of 60, 70, and 80 °C was based on maximizing solid uptake to obtain the desired antioxidant impregnation, in a short osmosis step, without altering tissue integrity.

Table 2 presents the TPC of enriched cucumber extracts derived from the immersion of cucumber slices into hypotonic osmotic solutions of gallic acid (1.0 and 2.0% w/v) at 60, 70, and 80 °C after processing for 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h. Regarding the

Table 3. Total phenolic content, antiradical and antioxidant activity of the post-treatment cucumber extracts at 70 °C.

Herbs	TPC	ABTS	FRAP
Chamomile	138.74 ± 2.83c	651.08 ± 1.77	465.23 ± 12.76
Sea buckthorn seeds	23.25 ± 0.36a	204.34 ± 0.94	115.89 ± 1.17
Alexandria leaves	72.17 ± 3.86b	438.16 ± 9.24	349.45 ± 4.67
Alexandria pods	117.58 ± 3.77	536.54 ± 6.34	302.14 ± 4.90
Sage	45.78 ± 1.59	468.03 ± 3.16	255.15 ± 3.81
Spearmint	513.82 ± 12.26	4075.71 ± 28.54	1480.80 ± 15.33
Oregano	781.14 ± 16.89	5246.60 ± 39.06	2867.93 ± 43.68
Mint	245.36 ± 3.41	1741.30 ± 21.49	685.23 ± 4.67
Bay laurel leaves	21.65 ± 0.46	189.86 ± 0.71	122.19 ± 7.69
Cinnamon burmannii sticks	35.05 ± 1.10	242.48 ± 1.68	105.86 ± 1.64
Cinnamon verum sticks	23.87 ± 0.28a	133.37 ± 3.37	97.62 ± 1.62
Liquorice root	92.05 ± 3.92	452.38 ± 8.14	382.44 ± 2.57
Roselle calyces	77.00 ± 3.46	482.25 ± 22.43	385.92 ± 5.85
Eucalyptus leaves	134.47 ± 1.66c	771.36 ± 1.42	437.79 ± 15.08
Clove flower buds	170.43 ± 2.98	947.81 ± 7.78	411.68 ± 9.03
Jasmine flowers	537.68 ± 3.71	3898.03 ± 8.40	1674.43 ± 13.99
Goji Berry fruits	26.39 ± 0.99	251.69 ± 4.96	242.59 ± 1.87
Annual nettle leaves	137.63 ± 3.97c	812.75 ± 2.99	374.62 ± 3.57
Lemon verbena leaves	299.42 ± 18.55	3284.81 ± 76.04	659.44 ± 16.53
Turmeric	70.59 ± 1.02b	368.92 ± 2.95	268.58 ± 2.45

The results represent the mean ± standard deviation (N=9). The same letters in the same column indicate nonstatistically significant differences at $p > 0.05$.

treatment at 60 °C, higher ($p < 0.05$) impregnation of gallic acid in cucumber was achieved after 3.5 h of slices immersion into the hypotonic solution, irrespective of gallic acid concentration. Accordingly, at 70 and 80 °C, higher ($p < 0.05$) impregnation was achieved after 2.5 and 2.0 h of slices immersion, respectively. Based on the above results, the most interesting findings are discussed below.

First, considering that cucumber without osmotic treatment showed TPC lower than 0.02 g gallic acid E/kg, the hypotonic osmotic treatment succeeded cucumber tissue enrichment with gallic acid. Moreover, the increase of temperature and gallic acid concentration of the hypotonic solutions resulted in a significant positive effect ($p < 0.05$) in gallic acid migration to the cucumber tissue. Interestingly, since the enriched cucumber extracts obtain the maximum gallic acid concentration, further osmotic process causes its significant ($p < 0.05$) reduction (Table 2). A possible explanation may be that by immersing the cucumber slices in the hypotonic solution, water is transferred from the dilute solution to the slices, apart from gallic acid diffusion into the tissue through the selectively permeable membrane, a mechanism which may gradually affects the mass transfer of gallic acid.

Furthermore, the increase of osmotic treatment duration in conjunction with heating may eventually have an adverse effect on gallic acid impregnation by affecting its stability and antioxidant activity. According to Volf et al. (2014), exposure of catechin, gallic acid, and vanillic acid standard solutions to 60–100 °C revealed degradation of phenolic compounds ranged from 15 to 30% after 4 h of heating. Moreover, Horvathova, Suhaj, and Šimko (2007) reported that thermal treatment at 130 °C for 5 minutes caused significant decrease of all antioxidant activity parameters of some spices. As the main purpose of the study was the impregnation of the bioactive compounds of herbal infusions into the cucumber tissue and considering all the above results, the optimal hypotonic osmotic conditions should both limit the risk of phenolic degradation and ensure maximum cucumber enrichment.

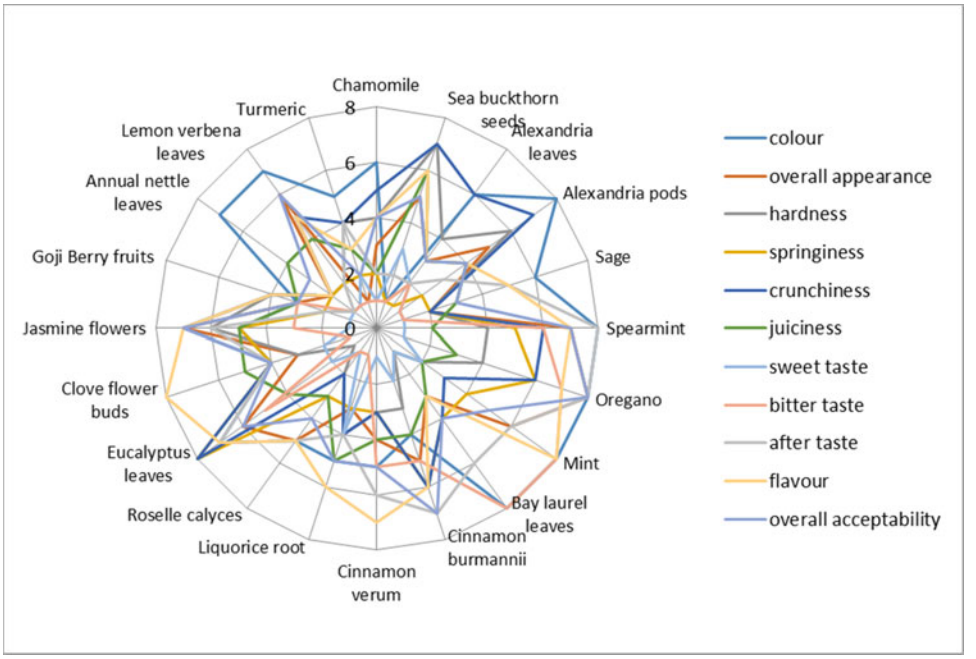

Figure 2. Sensory scoring of osmo-treated cucumber samples at 70 °C.

Impregnation of phenolics in cucumber slices in hypotonic solution of herbal infusions

Based on the results of the preliminary experiments, freshly cut cucumber slices were immersed into the infusions of the 20 plant species listed in detail in Table 1 at 70 °C and processed for 2.5 h. The herbal infusion/fresh-cut cucumber slices ratio (w/w) was higher than 10:1.

Freshly cut cucumber samples without any treatment were assessed for their TPC, antiradical and antioxidant activity presenting nonsignificant values. TPC, antiradical and antioxidant activity of the osmo-treated cucumber extracts varied considerably (Table 3). The results showed that cucumber treated with *Origanum vulgare* infusion had the highest TPC (781.14 mg GAE/kg) while those with *Laurus nobilis* infusion had the lowest value (21.65 mg GAE/kg). Moreover, extracts, derived from cucumber treated with *Origanum vulgare*, *Jasminum officinale,* and *Mentha spicata* infusions, demonstrated remarkably higher TPC, antiradical and antioxidant activity than all of the other samples.

Strong positive correlations ($p < 0.01$) between TPC and antiradical activity (0.978), between TPC and antioxidant activity (0.979), as well as among antiradical and antioxidant activity (0.932) were found. Therefore, it seems that the phenolic compounds infused into cucumber tissue contribute significantly to the in vitro antiradical and antioxidant activity of cucumber extracts. Moreover, TPC could be used as an indicator of the antioxidant properties of osmo-treated cucumber samples.

The osmo-treated cucumber samples were evaluated for their sensory properties as explained in the Materials and methods section. A list of 12 attributes and the panel scores obtained are depicted in the characteristic radar plot (Figure 2). Osmotic treatment significantly affected the appearance, texture, taste, flavor, and overall acceptability

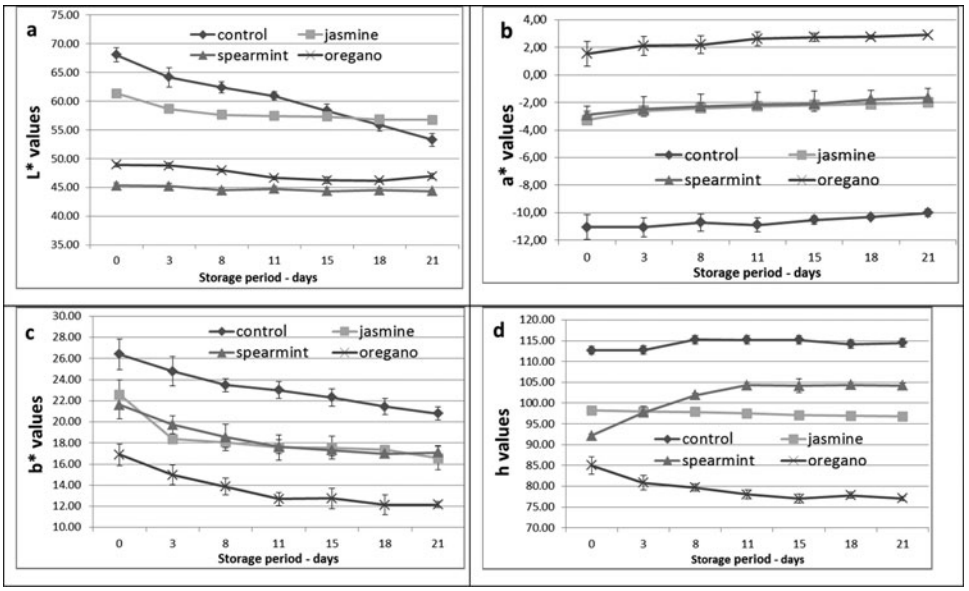

Figure 3. Effect of storage at 4 °C for 21 days for the (a) L* values, (b) a* values, (c) b* values, and (d) h values of fresh and osmo-treated cucumber slices. Vertical bars represent the standard deviation at the 5% level. The mean values were reported for N equals 2 replicates × 10 samples to provide 20 measurements.

of the cucumber samples. In the overall appearance test results, the samples immersed into spearmint, oregano, and jasmine flower infusions received the highest evaluation (7, 8, and 7, respectively). The after taste of the samples immersed into spearmint and oregano infusions were highly appreciated at 8, followed by samples immersed into *Cinnamon burmannii* and eucalyptus leaf infusions, scored with 7.

Flavor gave better scores for the samples immersed into mint and clove flower buds' infusions, given a score of 8, followed by samples immersed into spearmint, oregano, jasmine flowers, *C. burmannii*, and eucalyptus leaf infusions, given a score of 7. Moreover, the ranking of overall acceptability demonstrated as best the sample immersed into oregano infusion, followed by the samples immersed into spearmint and jasmine flower infusions. High positive correlations ($p < 0.01$) were found between overall appearance and overall acceptability (0.890), among overall appearance and TPC-antiradical-antioxidant activity (0.702, 0.715, 0.667), as well as among overall acceptability and TPC-antiradical-antioxidant activity (0.734, 0.751, 0.695). Therefore, combining the TPC and antiradical and antioxidant activity results with the overall appearance and acceptability scores, oregano, spearmint, and jasmine-treated samples can be selected as the optimum, in order to proceed to the next experimental set up, concerning the quality evaluation of the impregnated cucumber slices compared to the untreated samples.

Impact of storage on osmo-treated cucumber slices

The cardinal aim of this study was the enrichment of fruits and vegetables with herbal bioactive constituents, using cucumber slices as the vegetative matrix. The goal was to improve its biological activity, and thus, extend its shelf life enhancing, at the same

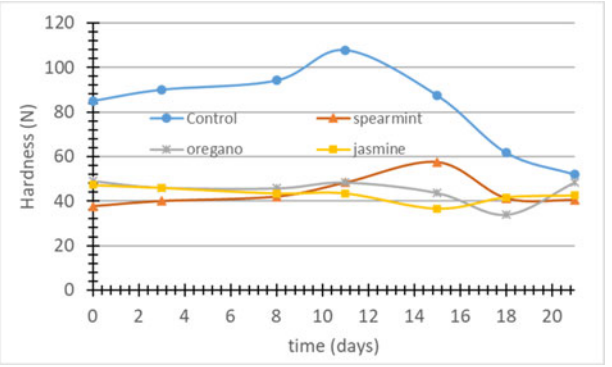

Figure 4. Effects of storage at 4 °C for 21 days on the hardness of fresh and osmo-treated cucumber slices.

time, its organoleptic characteristics. Therefore, a quality study during storage was performed for the cucumber slices, previously immersed in spearmint, oregano, and jasmine flower infusions, including also raw, untreated cucumber samples. Color and texture of fresh and osmo-treated cucumber samples were evaluated during a three-week storage time at 4 °C. Moreover, visual quality of the above samples was evaluated during a week storage time at 37 °C, based on the principles of accelerated microbial degradation (mold evolution), visually observed on samples' surface.

Regarding color changes, osmotic treatment significantly affected the color parameters such as L* (lightness), a* (redness/greenness), b* (yellowness/blueness), and h (hue angle) of cucumber samples (Figure 3). Specifically, osmo-treated cucumber samples immersed into spearmint, oregano, and jasmine flower infusions showed significantly ($p < 0.05$) lower L*, b*, and h values and higher a* values than the fresh samples, probably due to the flavonoid pigments transfer from the infusions to cucumber tissue. According to the results, osmotic treatment caused a significant ($p < 0.05$) decrease of hue-angle values, from a dull green color in the fresh cucumber samples to a green-yellow color in the osmo-treated cucumber samples.

Storage at 4 °C for 21 days significantly affected color parameters of fresh and osmo-treated cucumber samples. Interestingly, L* parameter was significantly ($p < 0.05$) decreased throughout the preservation period only for the fresh samples, whereas osmo-treated cucumber samples immersed into jasmine flower infusion showed a significant decrease up to the first three days. Redness (a*) values showed slight or significant increase throughout the storage period. Regarding yellowness (b*), the osmo-treated samples showed a rapid b* values decrease at the initial storage period and stabilization from day 11 to day 21, whereas b* values of fresh samples significantly decreased throughout the storage period.

The hue (h) angle varied with an opposite trend during the initial storage period, increasing in fresh and post-treated into spearmint infusion cucumber samples and decreasing in the samples post-treated into oregano and jasmine flower infusions. Moreover, hue angle values showed stabilization from day 11 to day 21. Color changes of cucumber during storage could be attributed to the dehydration of cucumber surface, chilling injury, oxygen penetration into the tissues, and decay (Kasim and Kasim 2008). An important conclusion resulting from the color measurement was that the treatment of cucumber samples tended to better maintain its color values during refrigeration

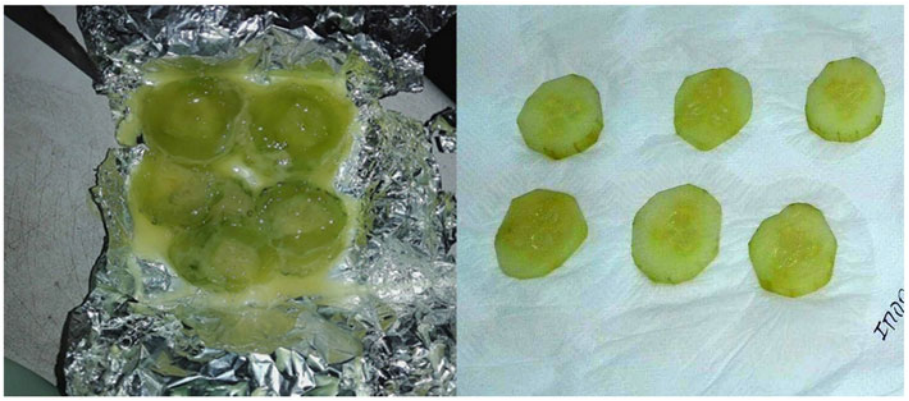

Figure 5. Untreated cucumber slices (left) after three days and osmo-treated samples (indicatively with oregano) (right) after one week of storage at 37 °C.

storage. Therefore, the phenolic compounds impregnation into cucumber tissue may play an important inhibitory effect on the cucumber appearance degradation.

Regarding texture alterations, osmotic treatment significantly affected samples hardness, as shown in Figure 4. Regarding the other TPA parameters calculated, namely springiness and elasticity, statistically significant differences were not observed for the whole storage period (data not shown); initial values for hardness were significantly higher for the untreated sample, as expected, due to the softening of the osmosed tissue. However, raw cucumber firmness was gradually degraded throughout chill storage; on the other hand, osmo-treated samples of all categories (immersed in spearmint, oregano, and jasmine infusions) were initially softer due to the mild water uptake, resulting from their immersion in the hypotonic herbal solutions. Nevertheless, during their subsequent storage, they maintained their firmness, as well as the other mechanical properties evaluated, such as the springiness and elasticity.

The visual quality of fresh and osmo-treated cucumber samples was evaluated every day for a week at 37 °C. Cucumber samples, previously immersed into oregano infusions, maintained consistently their good initial appearance macroscopically and microscopically during the whole storage period. It is worthy noticing that the osmo-treated samples had a fresher appearance throughout their short storage while the untreated slices seemed drier at the surface and more brittle.

Furthermore, throughout the storage period, no colony growth was observed, neither macroscopically nor microscopically in all osmo-treated cucumber slices in contrast to untreated samples, on which fungal infection and decay were clearly detected at the third day of abusive storage (37 °C) (Figure 5). Therefore, all treated cucumber samples showed acceptable overall visual quality through seven days of storage. Conclusively, the impregnation of cucumber samples in herbal infusions significantly extended their shelf life and improved the antioxidant activity and organoleptic quality.

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