Flavonoid-Enrichment of Fresh-Cut Apple through Osmotic Dehydration-Assisted Impregnation

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Abstract

Inclusion of bioactive compounds in foods is promising for developing novel functional foods. The feasibility of incorporating flavonoids and low-calorie sugar substitutes in fresh-cut apple through osmotic dehydration (OD) was investigated. The impregnation of quercetin and fisetin in apple cubes was tested. The effects of different osmotic agents, sucrose and sorbitol:mannose, on the water loss and sugar gain of the samples were studied at 25 °C and 40 °C for 8 hours. Temperature was a significant factor in the mass transfer kinetics, higher temperatures resulting in higher rates. The molecular weights of the solutes in the osmotic solution also affected the OD kinetics and flavonols uptake, as well as the physico-chemical quality. Overall, the results suggest that OD using alternative low-calorie and health- promoting solutes can be an effective treatment to simultaneously enrich fresh-cut apple with senolytic flavonoids, presenting, therefore, a great potential for a novel functional food.

Keywords: fisetin, quercetin, mannose, sorbitol, osmotic dehydration, functional food

1. Introduction

Apples are a rich source of polyphenols, particularly flavonoids. Additionally, apples contain lower fructose and sucrose contents than most fruits. Hence, they are associated with a reduction of the risk of diabetes, which is especially related with the flavonol quercetin (Ferretti et al., 2014). Quercetin is one of the most widely studied flavonol found in apple (Schieber et al.,

2001). While quercetin possesses biological functions common to most flavonoids, Gulati et al. (2006) found that it specifically inhibited cancer cell growth. Quercetin also showed immunomodulating properties by inhibiting mediator enzymes, which could effectively terminate allergic response (Mlcek et al., 2016). Snyder et al. (2016) also found that quercetin significantly reduced blood glucose concentrations and hepatic lipid accumulation. Ali et al. (2011) found another flavonol, fisetin, in apple, which exceeded the amount of quercetin. Currais et al. (2014) found that fisetin has potential to prevent cognitive and neuropsychiatric problems that develop as a consequence of Alzheimer's disease by reducing oxidative stress in the nerve cell. Yousefzadeh, et al. (2018) added that fisetin significantly reduced the progression of aging-related senescent cells compared to other flavonoids, including quercetin. Moreover, Sung et al. (2007) reported that fisetin was more effective in suppressing cancer activating enzymes compared to quercetin.

As a biological product, the chemical composition of apple may change over time depending on its postharvest handling. Carbone et al. (2011) reported a 50 % phenolic loss in apples stored at 0-1 °C for 3 months. More drastic reductions were observed during further processing of apples. A 10-fold reduction of quercetin was perceived in apple juice compared to fresh whole apples (Van der Sluis et al., 2002). Osmotic dehydration (OD) could be a solution for this phenolic loss in apples. Rózek et al. (2010) mentioned that osmotically-treated fruits presented less reduction in total phenolics during subsequent air-drying processes compared to the untreated samples.

The osmotic agent is an important consideration during the OD process since it may affect the sensory and physical properties of the end product. Sucrose is the most common osmotic solution used in fruits. However, Cichowska et al. (2018) concluded that polyol (erythritol,

xylitol, maltitol, inulin and oligofructose) solutions showed higher or similar efficiency as suitable alternative to OD sucrose solution. Furthermore, Brochier et al. (2015) found that polyols, such as glycerol and sorbitol, allowed a higher level of dehydration in yacon in relation to sucrose and maltodextrin did not promote dehydration. Additionally, the use of OD with oligofructose and a high DE maltodextrin solutions as a pre-freezing treatment significantly improved quality, including vitamin C retention, and sensory properties of strawberry (Dermesonlouoglou et al., 2016). Assis et al. (2017) showed that the use of sorbitol, as osmotic agent resulted in a higher mass transfer rate in fresh apple cubes compared to sucrose, which presented an advantage in terms of process time. Sorbitol is a sugar alcohol with low caloric value and low glycemic index and it is non-cariogenic (Deis and Kearsley, 2012), which makes it a "healthier" option than sucrose and makes it suitable for diabetic people. Mannose is another potential sucrose-alternative as osmotic agent in OD processes. Besides having a slightly lower caloric value, mannose is known to effectively prevent urinary tract infection by inhibiting bacterial adherence to uroepithelial cells (Kranjčec et al., 2014). Other studies also presented potential prebiotic advantages for both sorbitol and mannose: Chou and Hou (2000) found that sorbitol could support the growth of *Bifidobacteria* spp. in soymilk; Umemura et al. (2004) showed that the inclusion of mannose-derived mannooligosaccharides in diets could improve defecation due to the observed growth of *Bifidobacterium* spp. in the large intestine. The inclusion of bioactive compounds in conventional foods is one of the innovation areas for functional foods (Morais et al., 2018). The OD process may be carried out without light and, as the samples are immersed in the OD solution, they are exposed to low oxygen concentrations (Landim et al., 2016). In addition, the solubility of oxygen in a solution decreases with °Brix (Sadler, Roberts and Cornell, 1988). Also, the optimal temperature of polyphenol oxidase in

apples is around 30 °C (Eidhin, Murphy and O'Beirne, 2006). Therefore, the contribution of these factors to the oxydation of flavonols may be considerably reduced during the OD process. In fact, Landim et al. (2016) reported that temperature and solute concentration are the main factors related to the oxidation of phenolic compounds and Maillard reactions in osmodehydrated products. High temperatures (60 °C) used in OD of apple reduces phenolic and vitamin C contents (Devic et al., 2010).

The main objective of this study was to determine the feasibility of incorporating flavonoids, quercetin and fisetin, and low-calorie sugar substitutes, sorbitol and mannose, in fresh-cut apple through OD. The effect of the osmotic agent and temperature (25 and 40 °C) on the OD process of apple cubes was assessed, focusing specifically on the water loss and sugar gain. The impregnation of the bioactive compounds, quercetin, fisetin, mannose and sorbitol, were also evaluated and quantified. The effect of the osmotic process on the quality of the apple cubes, specifically on total soluble solids, water activity and colour, was evaluated..

2. Materials and Methods

2.1. Sample Preparation

The apples (*Malus* spp. cv. Royal Gala) were graciously provided by Campotec, Portugal, and were stored at 4 °C. After washing with running water, the fruits were then sanitized in 150 ppm chlorine solution for 5 minutes. Apples were cut into 12 mm cubes using a vegetable cutter (Actuel, France) and immediately immersed in a 0.9 % sodium chloride solution, acidified until pH≈3 using lemon juice extract (Solevita, Portugal). The apple cube samples were kept in this solution for 3 minutes to prevent enzymatic browning. The samples were then blotted gently with tissue paper to remove the excess of sodium chloride solution.

2.2. Osmotic Solutions Preparation

Commercial sucrose, 68 % sorbitol aqueous solution (Fagron Iberica SAU, Spain), and D-mannose (KEB Biotechnology, China) were used as solutes for the preparation of the osmotic solutions. 60 °Brix sucrose (Assis et al., 2017) and 60 °Brix sorbitol:mannose (56:4) were prepared using deionized water. Separate 3 % solutions of quercetin (Sigma Aldrich, Germany) and fisetin (TCI, Japan) were prepared by mixing in glycerol:polysorbate (50:50) to create a water-soluble blend (Sowbhagya et al., 2005), making sure they was protected from light (dark vessels and aluminium foil). The flavonoid solution (0.13 g/100 mL) was added to each osmotic solution (0,0039 % quercetin/ fisetin), again protected from light with aluminium foil.

2.3. Osmotic Dehydration

The apple samples were immersed into the different osmotic solutions mentioned above, the control sucrose and sorbitol:mannose solutions and the correspondent solutions with flavonol, quercetin or fisetin, at 1:4 sample to solution mass ratio based on similar studies by Assis et al. (2017). These authors had found an increased rate of OD mass transfer at 60 °Brix solute concentration, while a different sample to solution mass ratio, 1:10, did not affect mass transfer kinetics at atmospheric pressure; hence a recommended lower quantity of osmotic solution was preferred. The process time had already been previously studied by Assis et al. (2017), who found that 8 h was enough to have a reasonable water loss and solute gain (close to or at the equilibrium values). Therefore, this was the maximum process time used in the present work.

The OD process was performed in opaque glass jars covered with aluminium foil placed on orbital shakers (Wiggenhauser, Germany) at 50 rpm and 25 °C and 40 °C. The apple samples were immersed in the solution (plastic net just below the surface solution). The apple samples were removed from the osmotic solution every 2 hours and quickly rinsed with deionized water to remove the excess of osmotic solution adhered to the surface and blotted gently with tissue paper to remove the excess of water.

2.4. Moisture Content Determination

The moisture content was determined by placing the fresh and osmotic dehydrated apples in an oven (FP115, Binder, Tuttlingen, Germany) at 105 ºC until constant weight (A.O.A.C. 2002). The determinations were performed in triplicate.

2.5. Osmotic Dehydration Parameters

Water loss (WL) and solute gain (SG) during the OD process were determined as follows:

$$WL = \frac{w_{w0} - w_w}{w_0} \quad (3)$$

$$SG = \frac{w_s - w_{s0}}{w_0} \quad (4)$$

where w_{w0} is the initial moisture content before OD, w_w is the moisture content after OD, w_0 is the initial sample weight before OD, w_s is the dry matter weight after OD, w_{s0} is the initial dry mater weight, all in gram.*2.6. Impregnation Effectivity*

Impregnation effectivity (IE) was calculated to determine the effectiveness of the applied OD treatment on the infusion of solutes. IE of the treatments were calculated as follows:

$$IE = {SG_t}/{WL_t} \quad (5)$$

where WL_t and SG_t are the water loss and solute gain, respectively, after 8 hour-OD.

2.7. Colour Evaluation

Colour parameters of the L*a*b* colour space (CIE, 1978) were determined using a colorimeter (Minolta CR-300, Japan). The lightness (L^*) and colour parameters a^* and b^* for red-green and yellow-blue, respectively, were measured on the control samples and osmotically-dehydrated apple samples. The total colour difference (TCD) was computed as follows:

$$TCD = \sqrt{(L_0^* - L_t^*)^2 + (a_0^* - a_t^*)^2 + (b_0^* - b_t^*)^2} \quad (6)$$

L_0^* and L_t^* is the lightness value, a_0^* and a_t^* is the a^* colour parameter and b_0^* and b_t^* is the b^* colour parameter, before OD and after 8 hour-OD, respectively.

2.8. Quantification of Flavonoids and Sugars

2.8.1. Flavonoid Analysis

Flavonoid extraction was performed by adding 5 mL of 50 % ethanol to 20 mg ground freeze-dried (liquid nitrogen) apple sample, protected from light. The samples are placed on a orbital shaker at 100 rpm and 25 °C for 60 minutes. Samples were then filtered through a 0.45 µm membrane and the filtrate was analysed by HPLC (Waters e2695, USA) with photodiode array detector (Waters 2998, USA). A C18 column (XDB-C18 Zorbax Eclipse, 250 x 4.6 mm x 5 µm Agilent) was used at 25 °C with water/acetonitrile/trifluoroacetic acid (94.8:5:0.2) as mobile phase. The flow rate was set at 1.0 mL/min and the injection volume was 20 µL. Detection was performed for 62 minutes at 360 nm. Quercetin and fisetin concentrations were determined using Empower 3 software (Waters, USA) and quantified using previously established standard curves ranging from 10 to 40 mg/L. The concentrations of each flavonoid in the apple sample were calculated in mg/kg based on the peak area at the retention time (30.926 and 38.336 min for fisetin and quercetin, respectively), the correspondent concentration in the calibration curve, the volume of the extract (5 mL) and the exact mass of the apple sample.

2.8.2. Sugar Analysis

Samples of 16 mg ground freeze-dried apple were mixed with 6 mL of deionized water to extract the sugars. The samples were then centrifugated at 4 °C (Boeco U-320R, Germany) at 10,000 rpm for 10 minutes. The filtrate was passed through a 0.45 µm membrane for HPLC analysis (Beckman Coulter System Gold, USA) equipped with a refractive index detector (Knauer). Samples were passed through an Aminex HPX-87P column (Bio-Rad, USA) using ultrapure water at 85 °C as the mobile phase, a flow rate of 0.5 mL/min and a running time of 30 minutes. After the analysis, sorbitol and mannose concentrations were determined using 32 Karat software (Beckman Coulter, USA). Standard curves were established using concentrations

ranging from 0.125 to 2 mg/mL. The concentrations of each sugar in the apple sample were calculated in mg/kg based on the peak area at the retention time (14.644 and 16.680 min for mannose and sorbitol, respectively), the correspondent concentration in the calibration curve, the volume of the extract (6 mL) and the exact mass of the apple sample.

2.9. Determination of Total Soluble Solids and Water Activity

Total soluble solids (TSS) and water activity (a_w) of the control samples (point 2.1) and the 8-hour osmotically-dehydrated apple were determined. Samples were ground and filtered to obtain a 0.5 mL extract where TSS was measured using a hand refractometer (Atago HSR-500, USA). The a_w was measured by placing approximately 2-3 g sample in the hygrometer (AquaLab 3TE, USA), which was maintained at 23 ± 1 °C. Moreover, 0.5 mL OD solutions were also analysed for TSS.

2.10. Statistical Analysis

The statistical analysis was performed using Microsoft Excel 2013 (Microsoft Corporation, USA). Data was subjected to two-way analysis of variance to detect significant differences between treatments after which post-hoc analysis was carried out for multiple comparisons using Tukey's Range Test. Significance level was assumed 5 % for all statistical data analysis.

3. Results and Discussion

3.1. Mass Transfer Kinetics

Both WL and SG kinetics (Figures 1 and 2) showed a rapid increase in the first two hours and slight increases in the succeeding hours of the OD process. Assis et al. (2017) found similar

results to those of the present work, using 60 °Brix sucrose and sorbitol solutions. The WL of the apple cubes that were immersed in OD solutions with quercetin continuously increased with time. In relation to SG, for all conditions, except for apple cubes treated with the sucrose solution with quercetin, there was no significant change in SG between 6 to 8 hours of immersion time and it might be assumed constant at this time. Moreover, no significant difference between 4 to 8 hours immersion times was found for the SG of sorbitol:mannose-treated apple cubes.

Temperature affected the kinetics of mass transfer, as processing at 40 °C increased both WL and SG of apple cubes. Assis et al. (2017) found similar results in relation to WL; regarding the SG, these authors found that only the processing temperature of 60 °C produced a significant difference in comparison to 25 °C. The type of solute also affected the WL and SG. Sorbitol:mannose promoted a higher WL than sucrose, whereas sucrose favoured a higher SG. Works by Rodrigues and Fernandes (2007) and Pattanapa et al. (2010) showed higher rates of WL and SG using a mixture of solutes, the lower molecular weight ~~(MW)~~ component enhancing the mass transfer. Furthermore, Azoubel and Murr (2004) concluded that the addition of a higher molecular weight~~MW~~ solute, sucrose, in a salt solution decreased the driving force during the OD of cherry tomatoes. In the present work, there was a higher WL in sorbitol:mannose solutions, which could be explained by their lower molecular weights than sucrose. It could be expected to be likewise for SG. However, sucrose resulted in a significantly higher SG rate than sorbitol:mannose. Atarés et al. (2008) found similar results: apple cylinders OD-treated in glucose solution presented lower SG kinetics compared to sucrose and trehalose solutions, both these solutes having higher molecular weights than glucose. Likewise, Chauhan et al. (2011) found higher WL and lower SG of OD-treated apple slices in sorbitol solutions in relation to sucrose. The addition of fisetin seems to have had an influence on these findings as well, because

the solute uptake was significantly higher in OD with sucrose solutions with this flavonol (Figure 2). This could be due to chemical properties of flavonoids, which might support their affinity to sucrose. Studies by Plaza et al. (2014) and Slámová et al. (2018) pointed out that glycosylation was effective in increasing the solubility and stability of flavonoids in water, the substrates involved in this process being usually saccharides. In this case, chemical glycosylation might have happened when water of the OD solution might have split the sucrose into glucose and fructose, at 40 and 25 °C. These monosaccharides have an available hemiacetal group likely to form a glycosidic bond with the available hydroxyl group in flavonols, quercetin and fisetin. The addition of a slightly lower molecular weight~~MW~~ solute, fisetin, in comparison with quercetin, might have made the SG higher with sucrose OD solution (Figures 1 and 2). Comparing with Assis et al. (2017)'s results, quercetin did not seem to affect the SG in relation to the OD with solutions without flavonol.

Furthermore, the results of WL and SG could also be related to the overall effect of solute type on IE (Table 1). IE values were higher when sucrose solution was used during OD treatment. This could be related to the higher SG values using sucrose rather than sorbitol:mannose. Moreover, the significant influence of the type of solute can be observed on the OD-solutions using fisetin. Overall, based on the WL and SG kinetics of the OD processes with different solutions, OD-treated apple with sorbitol:mannose solution at 25 °C showed to be the most successful in terms of dehydration (lower IE). On the other hand, an effective solute impregnation was achieved using sucrose solution at 40 °C, with the highest IE among all treatments.

Please insert Table 1

3.2. Impregnation of Bioactive Compounds

3.2.1. Flavonoids

Initial analysis of the flavonoid contents showed the presence of both fisetin and quercetin in the fresh apple, a higher fisetin content, 0.29 mg/kg, being detected compared with quercetin level, 0.22 mg/kg (Figure 3). Kimira et al. (1998) reported relevant quantities of fisetin in whole apple, following strawberry. Ali et al. (2011) found higher levels of fisetin than quercetin in extracted apple juice. During the OD process, both flavonoids, quercetin and fisetin, penetrated the apple fruit matrix, presenting maximum quantities after 4 and 8 hours of OD time (Figure 3). Nevertheless, fluctuations in flavonoid content could be noticed over time (Figure 3). These were most likely due to the high solute concentration at the surface layer, creating a barrier, as evidenced by a low and approximately constant SG. Bellary et al. (2011) studied the infusion of curcuminoids in coconuts and observed that an osmotic solution concentration higher than 25 °Brix resulted in a mass transfer of these compounds from the fruit to the OD solution instead of solute infusion from the OD solution to the fruit, the highest incorporation of solutes being achieved at the lowest solution concentration.

Temperature affected the flavonoid uptake after the OD process (Figure 3): OD-treated apple cubes at 25 °C tended to a have higher flavonoid content than samples treated at 40 °C. This might show the overall heat lability of flavonoids, specifically these flavonols, fisetin and quercetin. Wang & Zhao (2016) studied the degradation kinetics of fisetin and quercetin. They reported a noticeable decrease in concentration of both flavonols during 3 hours at 37 °C, with higher reductions at higher temperatures and for longer times. In this study, neither the immersion time nor the temperature affected the flavonoid uptake during the OD process.

The type of solute also affected the flavonoid content during the OD process. There was an increased gain of quercetin and fisetin using sucrose as osmotic agent (Figure 3), which could be related with the SG kinetics, higher for sucrose solutions. While solutes with lower ~~MW~~molecular weights, like sorbitol and mannose, would easily penetrate the fruit matrix, there is no theory about an increased flavonoid impregnation being supported by the affinity of the flavonoids towards these sugars. The tendency to an increased flavonoid content seems to be more associated with sucrose than sorbitol:mannose. Moreover, here was a higher gain of fisetin than quercetin regardless the solute type used in the OD solution. In agreement, Wang & Zhao (2016) found a lower thermal stability of quercetin than fisetin.

Apple cubes were impregnated with fisetin presenting a maximum quantity of 32 mg/kg after OD with sucrose solution at 25 °C. This sample exceeded the fisetin level of fresh strawberry by a two-fold increase. There was a maximum of 8.9 mg/kg quercetin, achieved in OD-treated apple cubes also in sucrose solution at 25 °C.

3.2.2. Sorbitol and Mannose

As one of the major sugar-alcohol in apples, Aprea et al. (2017) reported sorbitol in some apple varieties (except 'Royal Gala') with a maximum of 12.9 g/kg. In this study, initial quantification of sorbitol in the fresh 'Royal Gala' apple showed an amount three-fold higher than this value. Gheyas et al. (1997) reported apples to contain an average of 0.6 g/kg mannose. However, mannose was not detected in fresh 'Royal Gala' apple in this study.

Although sorbitol and mannose contents showed fluctuations during the process (Figure 4), similar to flavonoids, tentatively explained by a reverse mass transfer of solutes at certain moments (Bellary et al., 2011), statistical analysis implied that the immersion time and

temperature did not have an influence on the uptake of these compounds. Mannose intake was higher than sorbitol, but there was an overall increase of both osmotic agents, sorbitol and mannose in the OD-treated apples at the end of the process. Sorbitol suffered a maximum increase of 59.7 mg/kg (153 %), whereas there was a maximum 836 mg/kg uptake of mannose, after eight hours of OD at 25 °C.

3.3. Physico-chemical Quality

The mass transfer kinetics of OD after eight hours could further be related to the results on some physico-chemical parameters (Tables 2 and 3). The increase of TSS in the OD-treated apple cubes was at least more than two-fold in relation to the fresh apple. Apple colour was critical during the OD process due to several factors. A proper pre-treatment was performed to avoid the browning process. Another crucial factor was the effect of the addition of quercetin and fisetin to the OD solution. Like most flavonoids, quercetin and fisetin are yellow-pigmented compounds from natural plant source. While the flavonoid uptake might affect the colour, the yellow colour might also be an indicator of a noticeable flavonoid uptake.

Temperature had an effect on the soluble solute uptake. Likewise, for the mass transfer kinetics of WL and SG, higher temperatures related to increased TSS values. This solute uptake resulted in a decrease of water activity (Table 2). Solutes move at a higher rate at an increased temperature and with a high cell permeability in the fruit tissue, which allows solutes to penetrate the apple fruit matrix efficiently. Generally, treatments at lower temperature presented lower reductions in lightness (L*) and redness (a*). While higher temperatures promoted an increase in yellowness (b*), which was especially significant using sucrose as osmotic agent (Table 3). Colour changes could be observed at the end of the process for both temperatures 25 and 40 °C.

Please insert Table 2

The type of solute used during the process in this work also affected both TSS and water activity. Apple cubes treated with sorbitol:mannose solution presented a greater TSS and a more reduced water activity than the ones treated with sucrose solution (Table 2~~1~~). In this case, mixed solutes with lower molecular weights~~MW~~ showed to favour the soluble solids uptake. Similarly, a study by Chauhan~~HAUHAN~~ et al. (2011) showed significantly lower a_w of apple slices at the end of the OD in sorbitol compared to sucrose solutions. On the other hand, the use of different osmotic agents with addition of quercetin did not significantly affect TSS at the end of the OD process. Moreover, the addition of flavonoids did not have a significant effect neither on the TSS nor on a_w in OD with sucrose.

Please insert Table 3

OD-treated apple in the sorbitol:mannose solution had higher a* in comparison to the sucrose solution (Table 3). According to Mandala et al. (2005), a decrease in L* and an increase in a* parameters were well correlated to colour changes in fruit tissues due to enzymatic browning. In this case, it seemed that the solute type highly affected the a* values and more than the L* parameter. On the other hand, b* tended to increase along with time, which indicates an increase in yellowness. The presence of increased TSS in apple cubes at the end of the OD-process could explain this observation, especially with the addition of fisetin and quercetin to the osmotic solution. Mandala et al. (2005) also attributed the increase of yellowness to the solute uptake. A closer look on the L* parameter showed a reduction of 10 % maximum at 40 °C for OD with the sucrose solution. On the other hand, the b* parameter presented a maximum of 36 % increase when this OD solution was used, which might possibly be related to a higher uptake of yellow-pigmented flavonoids, likely forming glycosidic bonds in sucrose solutions. However,

the type of solute seemed to have only a slight effect on TCD. This is in agreement with Kowalska et al. (2019)' study, the smallest TCD being found in apples osmotically dehydrated in sucrose and xylitol solutions in comparison with erythritol and maltitol solutions. The use of OD solution with sorbitol:mannose presented a higher a* increase compared to sucrose solutions. Likewise, Assis et al. (2018) found a similar higher increase in a* of apple cubes using sorbitol than sucrose. In the end, the TCD was the lowest using sucrose at 25 °C.

Conclusions

The efficiency of applying OD-assisted impregnation of flavonoids in apple cubes was evaluated with respect to two different types of OD 60 °Brix solutions, with sucrose and sorbitol:mannose, at two temperatures, 25 and 40 °C. SG reached an equilibrium after 6 hours of OD, while WL increased continuously during the 8 hours duration of the process. The higher temperature promoted higher WL and SG kinetics. The type of solute also affected the WL and SG kinetics, which were promoted by sorbitol:mannose and sucrose, respectively. Overall, an effective osmotic dehydration was achieved by using sorbitol:mannose solution, whereas effective solute and flavonoid impregnation was most successful when the sucrose solution was used.

There was a significant increase of quercetin, fisetin, sorbitol, and mannose, after 8 hours of OD, indicating successful impregnation of bioactive compounds, despite fluctuations during the process.

Both temperature and solute type had great influence on the quality of the OD-treated apples, specifically on TSS, water activity, and colour. The type of solute affected the water

activity of the apple cubes, which was lowered by lower molecular weight ~~MW~~ solutes. There was a lower reduction in the TCD when the sucrose OD solution was used at lower temperature.

The results of the present work suggest that OD using alternative low-calorie and health promoting solutes is also an effective treatment to simultaneously enrich fresh-cut apple with senolytic flavonoids, resulting, therefore, in a great potential for a novel functional food.

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Figure captions

Figure 1. Water loss (WL) and solute gain (SG) of apple cubes during osmotic dehydration (OD) at 25 and 40 °C and 1:4 sample to solution mass ratio, using 60 °Brix sucrose and sorbitol:mannose (56:4) OD solutions with added quercetin (0,0039 %).

Figure 2. Water loss (WL) and solute gain (SG) of apple cubes during osmotic dehydration (OD) at 25 and 40 °C and 1:4 sample to solution mass ratio, using 60 °Brix sucrose and sorbitol:mannose (56:4) OD solutions with added fisetin (0,0039 %).

Figure 3. Fisetin and quercetin concentrations in apple cubes during osmotic dehydration (OD) at 25 and 40 °C and 1:4 sample to solution mass ratio, using 60 °Brix sucrose and sorbitol:mannose (56:4) OD solutions.

Figure 4. Sorbitol and mannose concentrations in apple cubes during osmotic dehydration (OD) at 25 and 40 °C and 1:4 sample to solution mass ratio, using 60 °Brix sucrose and sorbitol:mannose (56:4) OD solutions (no available data for samples treated in sorbitol:mannose solution with fisetin for 4 hours at 40 °C and for 8 hours at 25 °C).

Figure 1

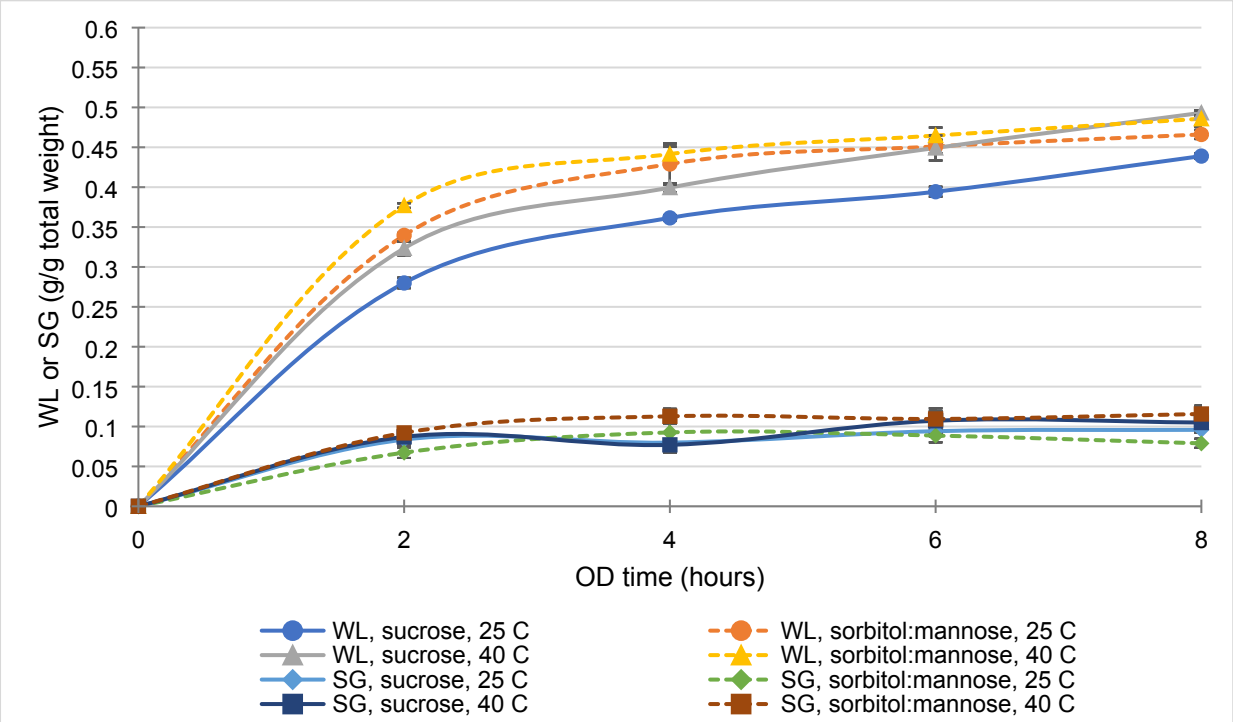

Figure 2

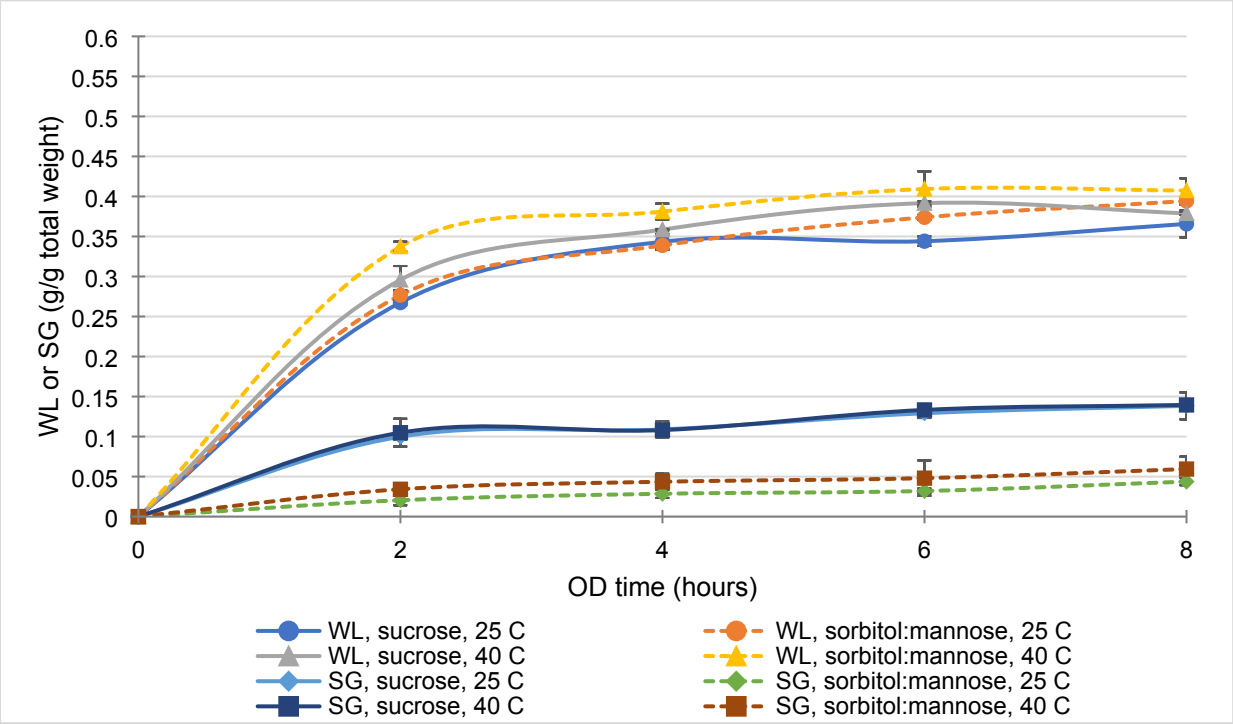

Figure 3

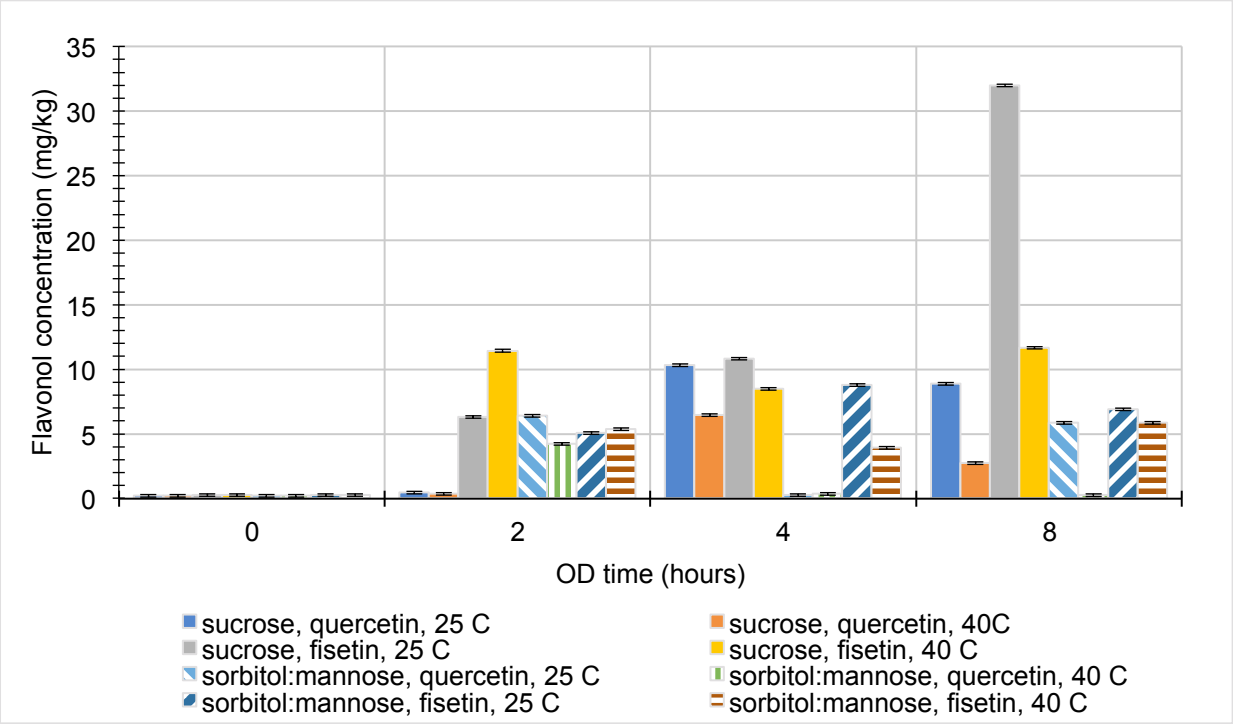

Figure 4

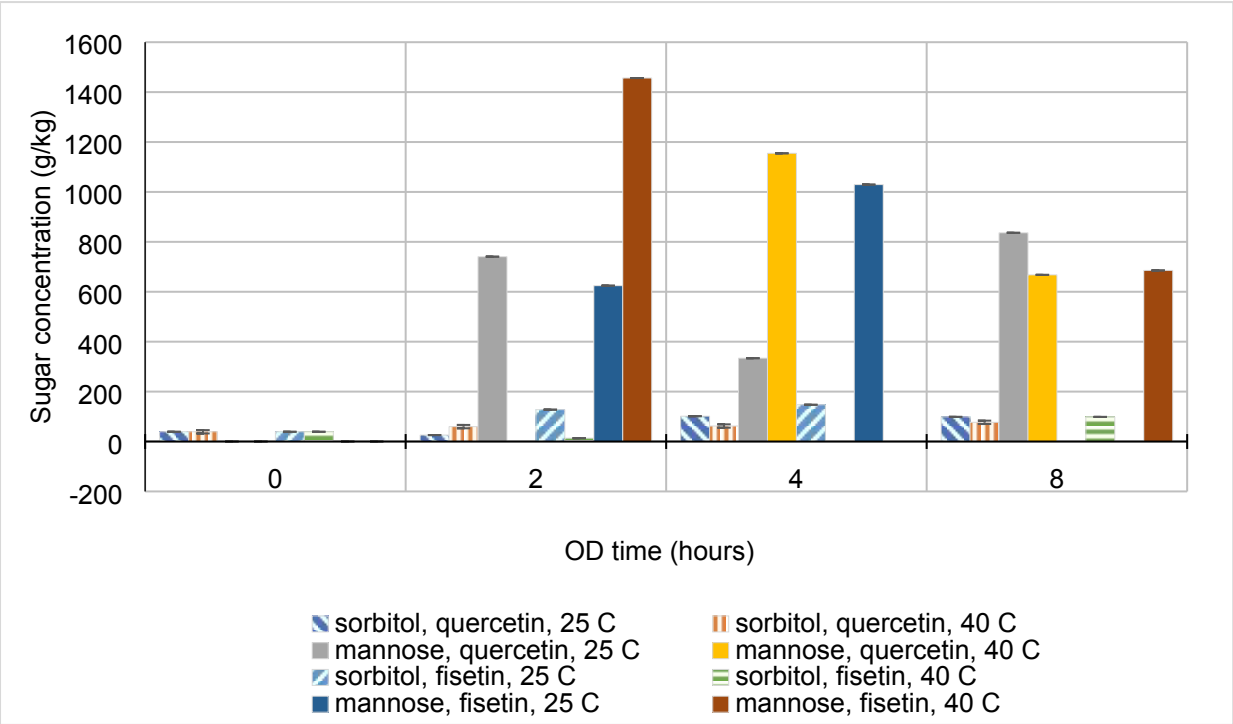

Table 1. Impregnation ~~values~~ efficiency of different treatments after 8h-OD

Treatment	Impregnation effectivity	
	25 °C	**40 °C**
sucrose with quercetin	0.422^{acd}	0.470^{acd}
sorbitol:mannose with quercetin	0.169^{bd}	0.238^{bd}
sucrose with fisetin	0.378^{c}	0.368^{c}
sorbitol:mannose with fisetin	0.111^{d}	0.146^{d}

The same statistical letter for values in the same column means there is no significant difference

Table 2. Total soluble solids (TSS) and water activity (a_w) of apple cubes after 8h-OD at 60 °Brix and 1:4 sample to solution mass ratio

Treatment	TSS (° Brix)	a_w
Control	11.4 ± 1.1	0.995 ± 0.001
After OD, 25 °C		
sucrose with quercetin	30.4 ± 2.6^{ac}	0.964 ± 0.002^{aef}
sorbitol:mannose with quercetin	30.7 ± 0.8^{ad}	0.964 ± 0.004^{b}
sucrose with fisetin	26.2 ± 2.0^{c}	0.976 ± 0.002^{e}
sorbitol:mannose with fisetin	34.1 ± 1.5^{acd}	0.971 ± 0.001^{f}
After OD, 40 °C		
sucrose with quercetin	37.8 ± 3.2^{be}	0.961 ± 0.004^{cgh}
sorbitol:mannose with quercetin	35.2 ± 0.2^{bf}	0.936 ± 0.006^{d}
sucrose with fisetin	30.9 ± 0.8^{e}	0.971 ± 0.002^{g}
sorbitol:mannose with fisetin	34.7 ± 0.6^{bef}	0.964 ± 0.001^{h}

The same statistical letter for values in the same column means there is no significant difference

Table 3. Colour parameters of apple cubes during OD at 60 °Brix and 1:4 sample to solution mass ratio

Treatment	L*	a*	b*	TCD
Control	73.6 ± 1.7	-4.2 ± 0.4	21.9 ± 2.9	
After OD, 25 °C				
sucrose with quercetin	67.6 ± 0.7^{ae}	-2.5 ± 0.5^{a}	28.7 ± 1.9^{a}	6.3 ± 1.6^{a}
sorbitol:mannose with quercetin	71.6 ± 2.1^{b}	0.2 ± 0.5^{b}	27.5 ± 1.1^{ac}	9.7 ± 1.3^{bg}
sucrose with fisetin	70.3 ± 1.3^{e}	-2.7 ± 0.5^{c}	24.4 ± 0.8^{e}	8.8 ± 1.2^{eg}
sorbitol:mannose with fisetin	72.3 ± 1.2^{abe}	0.7 ± 0.3^{d}	25.8 ± 0.9^{ce}	10.0 ± 0.9^{e}
After OD, 40 °C				
sucrose with quercetin	67.7 ± 1.7^{c}	-2.6 ± 0.3^{e}	29.7 ± 1.9^{b}	8.3 ± 1.6^{c}
sorbitol:mannose with quercetin	69.4 ± 2.2^{d}	1.3 ± 1.3^{f}	28.3 ± 1.1^{bd}	11.6 ± 1.6^{dh}
sucrose with fisetin	65.9 ± 2.2^{ce}	-0.8 ± 0.5^{g}	27.1 ± 1.2^{f}	14.0 ± 1.8^{fh}
sorbitol:mannose with fisetin	66.2 ± 0.6^{dc}	2.8 ± 0.7^{h}	28.3 ± 0.8d^{f}	14.4 ± 1.1^{f}

The same statistical letter for values in the same column means there is no significant difference