Combined application of wounding stress and extrusion as an innovative tool to obtain carrot powders with modified functional properties

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ABSTRACT

Wounding stress induces the accumulation of phenolics in carrots. However, its effect on cell-wall constituents has not been studied. Extrusion generates modifications in high-fiber food matrices. In this study, the combined effect of wounding stress and extrusion on cell-wall constituents and functional properties of carrots was evaluated. Wounding stress was applied by shredding carrots and storing the tissue (48 h/15°C). The stressed tissue (wounding stress carrot, WSC) was dehydrated and then extruded at temperature, 60 or 100°C, and screw configuration, continuous or expansion. Extrudates were milled and analyzed for cell-wall constituents and other physicochemical parameters. Cellulose content increased (112%) as a response to wounding. Furthermore, extrudates obtained from WSC showed higher content of cell-wall components. For instance, insoluble and total lignin content increased (54–84%) with extrusion conditions. Furthermore, WSC showed higher oil absorption index and lower water solubility index (WSI); whereas extrudates showed the highest WSI.

INTRODUCTION

The application of postharvest abiotic stresses in horticultural crops has been proposed as an innovative approach to increase the concentration of secondary metabolites with health-promoting properties (Cisneros-Zevallos, 2003; Jacobo-Velázquez & Cisneros-Zevallos, 2012). In this context, wounding stress induces the accumulation of antioxidant phenolic compounds in carrots (Jacobo-Velázquez & Cisneros-Zevallos, 2012). Following this approach, Santana-Gálvez, Pérez-Carrillo, Velázquez-Reyes, Cisneros-Zevallos, and Jacobo-Velázquez (2016) developed a carrot powder with improved functionality and enhanced levels of phenolic compounds by applying wounding stress (shredding and storing the tissue at 15°C for 48 h) prior to dehydration and milling of carrots. The ingredient was applied to...
modification plant’s cell-wall components. Gao, Yan, Xu, Ye, and Chen (2015) isolated and characterized the increase of soluble dietary fiber from extruded carrot pomace residues, reporting further beneficial effects of extrusion processing such as improved functional properties (i.e. water-holding capacity, oil-binding capacity) and lead (Pb) cation absorption capacity.

Therefore, the objective of the present study was to evaluate the effect of wounding stress and extrusion treatments applied alone and combined on cell-wall constituents and functional properties of powders obtained from treated carrots.

2. Materials and methods

2.1. Reagents and raw materials

Methanol, ethanol, acetone, methyl tert-butyl ether (MTBE, HPLC grade), and Folin & Ciocalteu’s (F-C) phenol reagent (2N) were purchased from Fisher Chemical (Fisher Scientific, Waltham, MA). Sodium hydroxide (NaOH), sodium carbonate (Na₂CO₃), sodium lauryl sulfate (C₁₂H₂₅C₅Sn), sodium tetraborate decahydrate (Na₂B₄O₇·10H₂O), orthophosphoric acid (H₃PO₄), ethyl acetate, hexane and methanol (solvents reagent grade) were obtained from Desarrollo de Especialidades Químicas (San Nicolás de los Garza, NL, Mexico). Hydrochloric acid ([HCl] 98% v/v) and sulfuric acid ([H₂SO₄] 96% v/v) were obtained from J. T. Baker (Fisher Scientific). Lutein, β-carotene, butylated hydroxytoluene (BHT), sodium carbonate (Na₂CO₃), disodium phosphate (Na₂HPO₄), triethylene glycol and hexadecyltrimethylammonium bromide (CTAB) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Thermoresistant α-amylase was manufactured by Novozymes (Bagsvaerd, Denmark). Finally, corn oil (Aveca®, chlorine (Cloralex®, 6% sodium hypochlorite) and fresh carrots were purchased from a local supermarket (HEB, Monterrey, Mexico).

2.2. Application of wounding stress

Vegetable material was conditioned as described by Santana-Gálvez et al. (2016). Whole carrots without visual damage or fungi development were selected. Carrots were washed with tap water to remove dirt; thereafter, they were sanitized with a chlorine solution (200 ppm, pH 6.5–7.0) for 5 min. Carrots were dried using a paper towel and their ends were cut before shredding them with a food processor (Waring Commercial, WFP11, Torrington, CT, USA). To stimulate the production of transport elements, and the expansion configuration according to Cortés-Ceballos, Nava-Valdez, Pérez-Carrillo, and Serna-Saldívar (2015) with reverse, mixing and kneading elements. The screw speed was set at 200 rpm. A die with a single hole (4 mm diameter) was used to obtain round extrudates. Expansion index from extrudates was measured using a Vernier Caliper Micrometer (Mitutoyo America Corporation, Aurora, IL, USA), Recorded data from extrusion parameters are shown in Table 1.

Extrudates were further dried using a convection oven (Electrolux EOG Gas single oven X 601, Stockholm, Sweden) overnight at 50°C. Afterward, extrudates were ground in a knife mill (Wiley Mill, Wednesboro, NJ, USA), through a 2 mm sieve, followed by a 1 mm sieve. Finally, samples were separated into different particle sizes using No. 20 (mesh whole = 846 μm) and No. 40 (mesh whole = 425 μm) sieves (Randor, PA, USA). Particles were separated in three sizes depending on the intended trials: > No. 20 sieve (not analyzed), between No. 40 and No. 20, suitable for dietary fiber assays, and < No. 40 used for functional

2.3. Extrusion processing

A twin-screw corotating extruder (BCTM-30, Bühler AG, Uzwil, Switzerland) was used. Before extrusion, all carrot dehydrated shreds were passed through the solids feeder of the extruder until a constant flow rate was obtained. Two screws of 800 mm total length and a ratio L/D = 20 were used. The extruder barrel was composed of five sections or zones, maintaining the last barrel zone (or 5th zone) at 63°C or 109°C using a heat exchanger device (Tool Temp, Bühler AG, Uzwil, Switzerland). Samples moisture (25%) was enough for the extrusion process. Screws were configured with a continuous or expansion configurations, depending on the shear force intended. The feed rate was 49.4 kg/h. The continuous configuration propose was assembled using only transport elements, and the expansion configuration according to Cortés-Ceballos, Nava-Valdez, Pérez-Carrillo, and Serna-Saldívar (2015) with reverse, mixing and kneading elements. The screw speed was set at 200 rpm. A die with a single hole (4 mm diameter) was used to obtain round extrudates. Expansion index from extrudates was measured using a Vernier Caliper Micrometer (Mitutoyo America Corporation, Aurora, IL, USA). Recorded data from extrusion parameters are shown in Table 1.

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**Table 1.** Extrusion conditions applied to extrude carrot samples.a

| Screw configuration | Product temperature (°C) | Feed rate (kg/h) | Screws speed (rpm) | Torque (%) | Motor power (kW) | SME (Wh/kg) | Expansion Index |
|---------------------|--------------------------|------------------|--------------------|------------|-----------------|-------------|-----------------|
| Continuous          | 61.3                     | 49.4             | 200                | 16.5       | 0.834           | 12.148      | 1.10 ± 0.11     |
| Expansion           | 65.6                     | 49.4             | 200                | 53.4       | 2.722           | 55.112      | 2.01 ± 0.28     |
| Continuous          | 99.8                     | 49.4             | 200                | 32.8       | 1.586           | 33.809      | 1.20 ± 0.15     |
| Expansion           | 119.5                    | 49.4             | 200                | 43.8       | 2.234           | 45.213      | 2.07 ± 0.35     |

aValues represent the mean of two data points registered during the main stable zone. bExtrusion profiles were previously described by Cortés-Ceballos et al. (2015). cAny additional moisture was fed during the trials. Actual feed rate was calculated using the constant weight of material that passed through the hopper screw before extrusion. dSpecific Mechanical Energy (SME) and torque were measured by the Bühler Extrusion System (BCTM-30, Bühler AG, Uzwil, Switzerland).

*Los valores representan la media de dos puntos de datos registrados durante la zona estable principal. **Los perfiles de extrusión fueron descritos previamente por Cortés-Ceballos et al. (2015).*
properties essays, smaller particles presented more exposed surface to interact with oil and water.

2.4. Evaluation of physicochemical properties

A colorimeter (Konica Minolta, CM-600d, Japan) was used to measure the L*, a* and b* color parameters, at 10°/D65 light mode. Chroma (C*) and Hue angle (H°) were calculated using Equations (1) and (2), respectively.

\[ C^* = \sqrt{(a^*)^2 + (b^*)^2} \]  

\[ H^° = \tan^{-1}(b^*/a^*) \]  

Extrudate powders were tested for water absorption index (WAI) based on the protocol reported by Serna-Saldivar (2012), water retention capacity (WRC) was determined as described by Bello-Pérez et al. (2003) and oil absorption index (OAI) measurements were done according to Rodríguez-Miranda et al. (2011). Samples of 1 g were placed in 50 mL pre-weighed centrifuge tubes. Then, 10 mL of water was added, and mixtures were homogenized for 30 s using a vortex. Samples were incubated 30 min at room temperature (21°C) with manual agitation every 5 min. Tubes were centrifuged (5,000 × g, 21°C, 20 min). Tubes were drained on a benchtop drying rack for 10 min while supernatants were recovered in pre-weighed aluminum plates. The sediments were weighed, and WAI was determined by Equation (3). The WRC and OAI were determined following the same procedure, but testing 4 different temperatures (60, 70, 80 and 90°C) in the WRC measurements and with oil instead of water for the OAI.

Water solubility index (WSI) was obtained as described on the protocol of Serna-Saldivar (2012). The supernatants that were drained from WAI centrifuge tubes were recovered in pre-weighed aluminum plates and were dried inside a convection oven overnight at 100°C. WSI was determined using Equation (4).

\[ WAI = \text{wet sediment weight/dry sample weight} \]  

\[ WSI = \text{(soluble solids weight/dry sample weight)} \times 100 \]  

2.5. Dietary fiber analysis

2.5.1. Lignin content determination

Lignin content was determined following the Klason lignin method reported by Templeton and Ehrman (1995). Samples of 1 g were placed in 20 × 150 mm Pyrex glass vials. A solution of 72% (v/v) H₂SO₄ (15 mL, 4°C) was added and mixed with a glass rod. Samples were hydrolyzed for 2 h at room temperature (21°C) with manual agitation every 15 min. Hydrolyzed samples were transferred to a 1 L Erlenmeyer flask and diluted in 560 mL of distilled water. Mixtures were heated at 300°C for 4 h with reflux cooling at 5°C. Samples were filtered under vacuum through a Gooch filter previously weighed.

Absorbance of filtrates was measured at 240 nm with a spectrophotometer (Genesys 10S UV-Vis, Thermo Fisher Scientific, Waltham, MA) and 3% (v/v) H₂SO₄ solution was used as blank. Soluble lignin was calculated using Equation (5). Solids retained in the Gooch filter were used to quantify insoluble lignin. Any remaining solids in the Erlenmeyer flask walls were washed out with hot water (80°C) and transferred to the Gooch filter. Gooch filters were placed in a convection oven to dry the samples overnight at 105°C. Filters were weighed and placed in a muffie (Thermo Fisher Scientific, Waltham, MA) at 575°C for 3 h. Gooch filters were then weighed. Insoluble lignin was calculated using Equation (6). Finally, total lignin was determined by adding the values of soluble and insoluble lignin and lignin concentrations were reported as % DW.

\[ \text{Soluble lignin} = \frac{[A_{240\text{nm}} - 0.576]/\text{absorbivity} \times W_2 + 100}{W_4} \]  

\[ \text{Insoluble lignin} = \frac{(W_3 - W_1) - (W_4 - W_1))}{W_2 \times 100} \]  

where \( A_{240\text{nm}} \) = absorbance at 240 nm, absorbivity = 25, \( W_1 = \) Gooch filter DW, \( W_2 = \) sample DW, \( W_3 = \) gooch filter + hydrolizate DW, and \( W_4 = \) gooch filter + acid ashes DW

2.5.2. Cellulose and hemicellulose profile determination

Cellulose and hemicellulose contents were measured according to Sluiter et al. (2008). Neutral Detergent (ND) solution composition was sodium lauryl sulfate (30 g), EDTA (18.61 g), sodium tetraborate decahydrate (6.81 g), sodium phosphate dibasic (4.56 g) and triethylene glycol (10 mL). For ND solution preparation all reagents except triethylene glycol were weighed and mixed with 450 mL of distilled water in a 1.0 L volumetric flask using a magnetic stirring plate. Then, 300 mL of water was added slowly by the walls of the beaker to avoid foam formation. Finally, triethylene glycol was added to the solution and aforated to 1.0 L, leaving the foam dissolving overnight.

Neutral detergent fiber (NDF), which includes cellulose, hemicellulose and lignin, was determined by placing samples (0.5 g) in 600 mL Berzelius beakers with 0.5 g Na₂SO₄ and 50 mL of neutral detergent (ND) solution. Samples were heated at 300°C using a hot plate and reflux with porcelain capsules filled with ice. After 5 min, 2 mL of 5.0% (v/v) thermoresistant α-amylase solution was added. Mixtures were hydrolyzed for 60 min at 250°C. After 7 min, beakers walls were rinsed with ND solution. Hydrolysates were vacuum filtered with Gooch filters previously weighed and warmed with 10 mL of distilled water (80°C). To achieve enzymatic hydrolysis of starch residues in the filtrate, 30 mL of hot distilled water (80°C) and 2 mL of α-amylase solution were added to Gooch filters and let the reaction for 60 s before vacuum filtration. Finally, samples retained in the filter were rinsed 2 times with 40 mL of hot distilled water (80°C) and 2 times with 40 mL acetone, 2 min intervals between each rinse. Filters were placed in a convection oven to dry samples overnight at 105°C. Then, Gooch filters were weighed. NDF was calculated with Equation (7).

\[ \text{Acid Detergent (AD) solution was made by dissolving CTAB (20 g) in 1.0 L of sulfuric acid (1 N) using a volumetric flask (1.0 L) for aforation and a magnetic stirring plate. Acid detergent fiber (ADF), which includes cellulose and lignin content, was obtained by placing samples (1 g) in 600 mL Berzelius beakers with 100 mL of acid detergent (AD) solution. Samples were heated at 300°C using a hot plate and reflux with porcelain capsules filled with ice. Mixtures were hydrolyzed for 60 min. After 30 min, beakers walls were rinsed with AD solution. Hydrolysates were vacuum filtered with Gooch filters previously weighed and warmed with 10 mL of distilled water (80°C). Retained solids in the filter were rinsed 2 times with 40 mL of hot distilled water (80°C)
2.6. Statistical analysis

Results were expressed as mean values ± standard error of the mean. Each mean was obtained from three replicates (n = 3). To evaluate significant statistical differences between means a one-way ANOVA was done, followed by a Tukey test (p < 0.05), using Minitab software (Minitab 18, Minitab Inc. State College, PA, USA). Pearson’s lineal correlation (r) was obtained using the Data Analysis ToolPak from Microsoft® Excel software (v16.19, Microsoft®, Redmond, Washington, USA).

3. Results and discussion

3.1. Effect of wounding stress and extrusion processing on the content of structural components (lignin, cellulose, and hemicellulose) of powders obtained from treated carrots

3.1.1. Lignin

After acid digestion and filtration, cellulose, hemicellulose, soluble and insoluble lignin were quantified in both control and stressed carrot extrudates (Table 2). In general, insoluble lignin content was higher than soluble lignin and accounted for 68–85% of total lignin. No significant difference was detected in the soluble, insoluble and total lignin content between non-extruded control carrots (CC) and non-extruded wounding stress treated carrots (WSC).

Regarding the effect of extrusion processing on lignin content, an increase (35.32%) was detected only in CC extruded at 100°C with the expansion screw configuration (Table 2). The cross-linked structure of lignin polymers makes it a highly insoluble material, where small fractions are solubilized by chemical treatments (Jaramillo-Carmona et al. 2008). In the case of WSC samples, extrusion did not affect the content of soluble lignin. Furthermore, extrusion did not affect the content of insoluble and total lignin content in CC, whereas all extrusion conditions increased insoluble and total lignin content in WSC. In these samples, a significant increase of insoluble lignin was observed in the continuous and expansion screw configuration treatments both at 60°C (65.13 and 84.48%, continuous and expansion, respectively) and 100°C (54.53 and 73.11%, continuous and expansion, respectively) as compared with non-extruded WSC. This increase in insoluble lignin content may be explained by the temperature and shear force increase during extrusion processing. Rising temperature eases moisture evaporation which promotes thermal softening, while augmenting reaction time and pressure to finally lead to sample pulverization.

Since lignin constitutes the outermost section of lignocellulose materials, bringing strength to cell walls; it can be hypothesized that extrusion opened up the tightly bound complex of cellulose-hemicellulose-lignin (Doherty, Mousavioun, & Fellows, 2011). These events contributed to increasing the availability of insoluble lignin in extrudates. In this context, extrusion processing also was reported as part of saccharification-fermentation, and biofuel production strategies, because it modifies lignocellulose matrices (Choi, Kim, & Oh, 2013; Karunanithy, Muthukumarappan, & Gibbons, 2012).

3.1.2. Cellulose

Concerning cellulose content, wounding stress alone increased cellulose by 112.83% as compared with the control. When plants are subjected to wounding stress, the biosynthesis of cell-wall structural components is induced as a mechanism to prevent water loss and to increase resistance to mechanical, chemical and enzymatic degradation of the tissue (Becerra-Moreno et al., 2015).

Furthermore, results showed higher levels of cellulose when applying extrusion on the control samples under the expansion screw configuration (138.06 and 124.92% of increase for

Table 2. Effect of wounding stress and extrusion conditions (temperature, screw configuration) on structural components of carrot samples.

| Sample                  | Treatment          | Lignin (% DW)  | Cellulose (% DW) | Hemicellulose (% DW) |
|------------------------|--------------------|----------------|------------------|----------------------|
|                        | T(°C)/screw configuration | Soluble | Insoluble | Total | Soluble | Insoluble | Total | Soluble | Insoluble | Total |
| Control carrots (CC)   | Non extruded       | 1.84 ± 0.04    | 5.54 ± 0.36     | 7.38 ± 0.32   | 6.62 ± 0.15   | 5.44 ± 0.17   |
|                        | 60/continuous      | 2.11 ± 0.17    | 6.72 ± 2.32     | 8.94 ± 2.12   | 7.27 ± 0.15   | 6.69 ± 0.52   |
|                        | 100/continuous     | 2.30 ± 0.13    | 4.91 ± 0.55     | 7.30 ± 0.41   | 15.68 ± 0.36  | 11.46 ± 0.65  |
|                        | 60/expansion       | 2.24 ± 0.02    | 7.79 ± 0.32     | 10.03 ± 0.21  | 15.76 ± 0.69  | n.d.          |
|                        | 100/expansion      | 2.49 ± 0.16    | 7.39 ± 0.10     | 14.89 ± 0.23  | n.d.          |
| Wounding stress (WSC)  | Non extruded       | 1.89 ± 0.05    | 6.51 ± 0.55     | 8.39 ± 0.49   | 14.09 ± 1.04  | n.d.          |
|                        | 60/continuous      | 1.92 ± 0.03    | 10.06 ± 0.13    | 12.12 ± 0.12  | 21.81 ± 1.22  | n.d.          |
|                        | 100/continuous     | 2.06 ± 0.03    | 12.01 ± 0.46    | 14.10 ± 0.40  | 20.57 ± 0.02  | n.d.          |
|                        | 60/expansion       | 2.09 ± 0.07    | 11.27 ± 0.51    | 13.52 ± 0.47  | 18.75 ± 0.07  | n.d.          |
|                        | 100/expansion      | 2.25 ± 0.07    | 11.27 ± 0.51    | 13.52 ± 0.47  | 18.75 ± 0.07  | n.d.          |

\(^{1}\)Values represent the mean of three replicates ± standard error of the mean. \(^{a,b,c,d,e,f}\)Differing superscripts indicate statistical difference within each variable using the Tukey test at a significance level of (p < 0.05).

\(^{a}\)Los valores representan la media de tres repeticiones ± desviación estándar de la media. \(^{a, b, c, d, e, f}\)Los superíndices diferentes indican la existencia de una diferencia estadística dentro de cada variable utilizando la prueba de Tukey a un nivel de significación de p < 0.05.
60 and 100°C, respectively) and the continuous screw configuration at 100°C (136.85% of increase). Interestingly, for the wounding stress samples only the continuous screw configuration and 60°C showed a significant increase (54.79%). Other studies have reported changes in cellulose content after extrusion. For instance, cellulose concentration increased under mild extrusion conditions in blue agave bagasse (50°C, 85 rpm screw speed). In contrast, refined wheat straw decreased its cellulose content (50°C, 150 rpm screw speed) under aqueous extraction (Jacquemin et al., 2015; Montiel, Hernández-Meléndez, Vivaldo-Lima, Hernández-Luna, & Bárzana, 2016). Thus, these reports suggest that cellulose extraction output will depend on the adequate combination of extrusion conditions and the pretreatments suffered by the vegetable fiber. To explain this higher content of cellulose in extruded samples it is relevant to recall that walls surrounding plant cells are mainly composed by thick, rigid cellulose microfibrils embedded in a matrix of hemicellulose (Asp, 1987). Then, hydrogen bonding within microfibrils confers its mechanical strength, chemical and biological resistance to degradation (Dhingra, Michael, Rajput, & Patil, 2012). As Montiel et al. (2016) observed in the SEM analyses, extrusion processing may provide the shear force and temperature necessary to disrupt the cellulose structure of cell-walls, promoting its release and finally increasing availability for further enzymatic hydrolysis.

### 3.1.3. Hemicellulose

Hemicellulose was not detected in most treatments, and none of the samples treated with wounding stress showed hemicellulose. Guo et al. (2017) reported an overexpression of BpIMYB46, a key transcription factor in growing and development of plant tissue and observed that cell-wall thickness in birch plants significantly augmented after it was subjected to abiotic stress (water scarcity), when compared to wild-type plants. Likewise, authors also observed an increase in lignin (24.56 to 25.80% DW) and cellulose (42.17 to 44.34% DW), though hemicellulose decreased (25.09 to 21.46% DW), which is consistent with our results. Hemicellulose is a heteropolymer composed by shorter ramifications of sugars (xylose, galactose, mannose, and arabinose) more prone to degradation via chemical or enzymatic hydrolysis (Dhingra et al., 2012; Montiel et al., 2016). Thus, the absence of hemicellulose in wounding stress treated carrots may be explained as the up-regulation of transcription factors through incubation to diminish moisture loss, increasing cellulose deposition on the cell-wall but decreasing hemicellulose levels.

On the other hand, hemicellulose significantly increased as a result of extrusion processing in the continuous screw configuration and 100°C treatment as compared with the control (110.66%). This effect can be explained as an increase in the extractability of hemicellulose constituents. Extrusion processing was previously applied with this purpose. Jacquemin et al. (2015) used extrusion as a pretreatment to increase the extraction yield of arabinoylans (major hemicellulose components in wheat bran), whereas Montiel et al. (2016) increased the cellulose and hemicellulose availability of blue agave bagasse for enzymatic saccharification.

Extrusion processing in combination with alkali and acid treatments has been studied as pretreatment to deconstruct hemicellulose; however, it has been reported that pentoses and hexoses may turn into furfural or suffer decomposition under harsh conditions such as high temperature and acid concentration (Choi et al., 2013; Singkhornart, Lee, & Ryu, 2013), which may explain the absence of hemicellulose in carrot extrudates obtained under expansion screw configuration on both temperatures (60 and 100°C).

Tan et al. (2009) discussed the challenge that represents the fractionation of lignocellulosic materials, specifically lignin. During lignin cleavage, its fragments present reactive ether groups that can bind with each other or with the chemicals of extraction. This process is called condensation and the structures created de novo form stronger carbon-carbon linkages. Extrusion may have induced condensation reactions, releasing high amounts of inner cellulose molecules while breaking down hemicellulose. This result is also consistent with previous reports of hemicellulose conversion into sugar monomers after extrusion of lignocellulosic biomass aiming biofuel production (Heredia-Olea, Pérez-Carrillo, & Serna-Saldívar, 2015).

### 3.2. Effect of wounding stress and extrusion processing on the physicochemical properties (color, WAI, WSI, AOI, and WRC) of powders obtained from treated carrots

#### 3.2.1. Color

The effects of wounding stress and extrusion on color parameters \(L^*, a^*, b^* \) and hue angle (H) is presented in Table 3. Non-significant difference was detected when comparing the \(L^* \) value of non-extruded WSC and CC samples. Regarding \(a^* \) value (redness), non-extruded CC showed the highest value (20.60) and was significantly higher when compared with non-extruded WSC (13.76). Reduction of redness intensity in carrots due to wounding stress can be related to a white color formation during storage as a result of cell-wall constituents biosynthesis in its surface (Cisneros-Zevallos, Saltveit, & Krochta, 1995) and has been used as a quality index (whitening index-WI) in wounded carrots (Formica-Oliveira et al., 2016). This is consistent with the positive linear correlation between lightness values and the increment of insoluble lignin in WSC (Table 2) \((r = 0.658; \alpha = 0.1)\). In addition, no significant difference was detected in \(b^* \) value (yellowness) between non-extruded CC and WSC. Wounding stress also had a significant effect on the hue angle (H). This parameter represents the angular value of color ("red", "blue", etc.). WSC orange color (65.84) was lighter as a result of the response to wounding after 48 h of incubation as compared to CC orange color (57.67). This increment may be explained by the lignification of carrot’s shreds surface (Jacob-Velázquez & Cisneros-Zevallos, 2012).

Extrusion over carrot samples caused a general drop of color parameters \(L^*, a^*, b^* \). The decrease of \(L^* \) was significant and progressive while the intensity of extrusion parameters increased among treatments as compared with CC and WSC. Extrusion effect over lightness (\(L^* \)) had a negative linear correlation with the rise of insoluble lignin \((r = −0.653; \alpha = 0.1)\). Loss of lightness in extrudates may be due to the rearrangement of lignin fragments after condensation reactions (Tan et al., 2009). Lignin fragments bound each other disorderly creating an amorphous less-bright structure than the unextruded CC and WSC source materials.

Regarding \(a^* \), the higher the temperature and the shear force applied, the lower its values became. Likewise, relating
Figure 1. Effect of wounding stress and extrusion conditions (temperature, screw configuration) in color parameters of carrot samples.

| Sample                        | Treatment                     | L*         | a*         | b*         | C*         | H*         |
|-------------------------------|-------------------------------|------------|------------|------------|------------|------------|
| Control carrots (CC)          | Non extruded                 | 63.89 ± 1.04 | 20.60 ± 0.95 | 32.54 ± 1.34 | 38.51 ± 1.63 | 57.67 ± 0.31 |
|                               | 60/continuous                 | 60.08 ± 0.26 | 13.13 ± 0.11 | 34.67 ± 0.26 | 37.08 ± 0.26 | 69.25 ± 0.18 |
|                               | 100/continuous                | 55.42 ± 0.23 | 11.98 ± 0.19 | 28.87 ± 0.08 | 31.26 ± 0.03 | 67.47 ± 0.38 |
|                               | 60/expansion                  | 51.32 ± 0.10 | 11.63 ± 0.10 | 24.85 ± 0.25 | 27.15 ± 0.23 | 64.63 ± 0.31 |
|                               | 100/expansion                 | 47.76 ± 0.51 | 11.18 ± 0.13 | 18.56 ± 0.59 | 21.67 ± 0.57 | 58.92 ± 0.56 |
|                               | Non extruded                 | 65.87 ± 0.30 | 13.76 ± 0.17 | 30.66 ± 0.70 | 33.61 ± 0.13 | 63.54 ± 0.22 |
|                               | 60/continuous                 | 51.82 ± 0.29 | 10.37 ± 0.03 | 24.29 ± 0.35 | 26.41 ± 0.31 | 66.87 ± 0.34 |
|                               | 100/continuous                | 50.75 ± 0.21 | 10.13 ± 0.04 | 23.08 ± 0.20 | 25.21 ± 0.20 | 66.30 ± 0.11 |
|                               | 60/expansion                  | 48.99 ± 0.19 | 9.62 ± 0.08  | 20.55 ± 0.12 | 22.69 ± 0.09 | 64.92 ± 0.31 |
|                               | 100/expansion                 | 49.08 ± 0.05 | 9.97 ± 0.08  | 20.32 ± 0.13 | 22.64 ± 0.10 | 63.86 ± 0.27 |

Wounding stress carrots (WSC)

| Sample                        | Treatment                     | L*         | a*         | b*         | C*         | H*         |
|-------------------------------|-------------------------------|------------|------------|------------|------------|------------|
| Non extruded                 | 60/continuous                 | 66.87 ± 0.34 | 32.54 ± 1.34 | 57.67 ± 0.31 | 69.25 ± 0.18 |
|                               | 100/continuous                | 69.25 ± 0.18 | 32.54 ± 1.34 | 57.67 ± 0.31 | 69.25 ± 0.18 |

Values represent the mean of three replicates ± standard error of the mean. a, b, c, d, e, f, g. Differing superscripts indicate statistical difference within each variable using the Tukey test (p < 0.05).

Los valores representan la media de tres repeticiones ± desviación estándar de la media. a, b, c, d, e, f, g. Los superíndices diferentes indican la existencia de una diferencia estadística dentro de cada variable utilizando la prueba de Tukey a un nivel de significación de p < 0.05.

3.2.2. Water absorption index (WAI)

Results of WAI, WSI, and OAI from wounding stress and extrusion treatments are presented in Figure 1. Regarding WAI (Figure 1(a)), values were not significantly different between CC and WSC. Moreover, extrusion processing did not have in general a significant effect on WAI; however, a significant reduction of WAI was measured only for CC treated with expansion screw configuration at 100°C (5.43 g wet sediment/g DW) (Figure 1(a)). Upadhyay, Sharma, and Sarkar (2010), reported a significant decrease of WAI in extruded mixtures of rice, gram pulse and carrot pomace powder caused by variations in the feed rate and die temperature. Therefore, when the final extrusion temperature is increased a higher extent of cooking is expected in extrudates. As a consequence, the carbohydrate inner structure suffers changes, such as the increment of insoluble dietary fiber (IDF) (Table 2). The reduction of WAI in extruded cereal blends with high fiber content [up to 20% (w/w)] is related with high expansion of the extrudates and starch gelatinization (Jin, Hsieh, & Huff, 1995; Upadhyay et al., 2010). However, the information regarding the effect of increasing IDF on the functional properties (WAI, WSI) of extrudates is limited. In this study can be inferred that extrusion processing modified the compact structure of dietary fiber polymers, inducing its fragmentation and reducing the water binding sites; thus, the WAI capacity of the extrudates was diminished.

3.2.3. Water solubility index (WSI)

The effects of wounding stress and extrusion processing on WSI parameter are presented in Figure 1(b). WSI values from WSC were significantly lower than those from the control group (10.39% of decrease). The WSI indirectly measures the quantity of soluble solids that migrates from the polysaccharide matrix to an aqueous medium. The WSI is also an index of the soluble solids within the food matrix, including simple sugars. In this context, it has been reported that stressed carrot tissue metabolizes these sugars as a source of energy to biosynthesize polyphenols for further cell-wall components biosynthesis (Jacobo-Velázquez, González-Aguero, & Cisneros-Zevallos, 2015), thus reducing the amount of soluble solids. WSI also presented a negative linear correlation when comparing with the effect of wounding stress over cellulose content (r = −0.755; α = 0.05). Cellulose structures are ordered polymer chains that tend to absorb solvent molecules rather than migrate, which is consistent with the results.
Interestingly, extrusion conditions increased the WSI of all samples (Figure 1(b)). For instance, extrudate samples obtained from CC showed a significant increase in WSI when extruded at 100°C (25.46% and 31.86% for continuous and expansion screw configuration, respectively). Furthermore, extrusion also increased WSI of WSC samples, where the treatment of expansion screw configuration at 100°C showed 39.71% of increase. Samard et al. (2017) reported contrasting WSI data at different die temperatures; declining (WSI = 13.94 to 9.15%) from 80 to 100°C and augmenting (WSI = 9.15 to 11.98%) from 100 to 120°C. Thus, WSI is sensitive to temperature changes over extrusion processing. In addition, Kumar, Sarkar, and Sharma (2010) reported increasing WSI values when screw speed augmented from 270 to 310 rpm, as a strategy to add mechanical shear on the extrudates during processing. This tendency is consistent with the results of this study. As mentioned, higher WSI values are linked to the quantity of soluble solids and has been well characterized its relation for high content of starch extrudates, with few studies for high-fiber matrices. The effect of extrusion on high-fiber matrix like carrot exhibited an increase of WSI as shear force and temperature augmented. Thus, it can be hypothesized that under these conditions soluble dietary fiber molecules are released from the tightly packed structure mainly composed by cellulose and lignin.

3.2.4. Oil absorption index (OAI)

The effect of wounding stress on the OAI exhibited a significant increase as compared with the control (26.69%, Figure 1(c)). The OAI is an indirect parameter of the hydrophobicity of the food matrix. Interestingly, the accumulation of cellulose in WSC samples showed a positive correlation with the increase of OAI \((r = 0.833; \alpha = 0.02)\). Greater cellulose levels favor the availability of hydrophobic binding sites for oil retention. This has relevant advantages in the development of enriched food products such as sausages (Alvarado-Ramírez et al. 2018).

On the other hand, the extrusion effect was detrimental on the OAI values of CC and WSC by 11.21% and 28.02%, respectively (Figure 1(c)). Interestingly, this reduction was not significant when comparing between extruded conditions. Reduction of OAI in the CC group may be associated with an increase in the content of DF with poor hydrophobic properties; for example, CC extrudates treated under 100°C and expansion screw configuration presented higher levels of cellulose and hemicellulose compared with control (Table 2). On the other hand, WSC extrudates exhibited higher levels of insoluble lignin, which was characterized for having a strong interconnected structure of phenylpropanoid subunits (Dhingra et al., 2012). This increase of a component of DF that interacts poorly with other molecules could have caused the decline of OAI value. Ai, Cichy, Harte, Kelly, and Ng (2016) also reported a decrease of the oil-binding capacity (OBC) of bean flour after extrusion. To obtain this
Parameter, it should be followed basically the same procedure as the one followed to calculate the OAI. In this case, the significant reduction of OBC values was associated with starch gelatinization that caused an increase in the exposure of hydrophilic functional groups (e.g., hydroxyl groups) and consequently diminished the capacity to bind hydrophobic molecules, such as those from vegetable oils.

3.2.5. Water retention capacity (WRC)

The effect of wounding stress and extrusion conditions on WRC of carrot samples is shown in Table 4. Wounding stress treatment did not significantly modify the WRC values as compared with the control. On the other hand, extrusion significantly decreased the WRC of carrot samples as compared with non-extruded CC (26.32, 16.71, 23.71 and 21.61% average decrease for 60, 70, 80 and 90°C, respectively) and WSC (30.43, 23.71 and 21.61% average decrease for 60, 70, 80 and 90°C, respectively) within the tested incubation temperatures. Karkle, Alavi, and Dogan (2012) also reported a decrease in WRC of corn composites enriched with apple pomace and extruded at 120°C, where authors correlated lower WRC from extrudates with enhanced total fiber content.

The hydrophilicity of extrudates will depend on the specificity of the interactions between water molecules and fibrous substrates. In the present study, increasing extrusion shear force and barrel temperature modified the fiber profile of the carrot samples, augmenting the content of (IDF) (total lignin and cellulose) but interfered with its capacity to bind water at high temperatures.

Specifically, when comparing the WSC only with extruded samples, the increment of lignin was higher than cellulose (Table 2). Lignin highly cross-linked structure of phenylpropane units presents poor holding water properties. On the contrary, cellulose swelling capacity is well recognized. Interestingly, WRC of extrudates did not diminish at 90°C when compared between extrusion treatments, which reflects the stability of water interactions and the fiber hydrophilic-binding sites.

4. Conclusions

In the present study, it was reported the effect of wounding stress on the modification of cell-wall constituents of carrots. Increases in cellulose were detected after wounding stress, which modified the functional properties of carrot when transformed into powders. Likewise, the application of extrusion further modified the functional properties obtained in WSCP, resulting in a food ingredient with unique functional characteristics. Results demonstrated that wounding stress combined with extrusion can be used as an effective strategy to generate novel food ingredients with increased functionality.

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