Research Article

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Vacuum impregnation of firming agents in red raspberries

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Abstract

BACKGROUND: Red raspberries are a delicate and highly perishable fruit with a fragile pulp tissue. In this study we used vacuum impregnation (VI) methods to incorporate pectin and calcium chloride into whole red raspberries to improve their firmness. Specifically, we impregnated low methoxyl pectin (LMP) at 10 g of pectin kg^{-1} of solution and calcium chloride ($CaCl_2 \cdot 2H_2O$) at 30 g calcium kg^{-1} of pectin, and on the other side pectin methylesterase (PME) at 10 g of enzyme kg^{-1} of solution, and ($CaCl_2 \cdot 2H_2O$) at 10 g of calcium kg^{-1} of solution, into whole red raspberries. We tested three vacuum levels 33.9, 50.8, and 67.8 kPa, three vacuum impregnation times 2, 7, and 15 min, and two temperatures, 20 and 40 °C, during VI treatment. Maximum force (F_M) and gradient (G_{C3}) were evaluated to assess raspberry firmness.

RESULTS: A vacuum level of 50.8 kPa, processing time of 7 min, and a LMP and calcium infusion at 20 °C resulted in the firmest fruit compared to the other treatments. At these VI treatment conditions, F_M and G_{C3} values of red raspberries obtained were 28 N, and 8.4 N mm^{-1}, respectively.

CONCLUSION: The optimal VI conditions identified in this study can be used to improve firmness and structural integrity of red raspberries by infusion of LMP and calcium. Findings on vacuum-impregnated red raspberries may be used to develop dehydrofrozen berries for incorporation into bakery and dairy products.

Keywords: calcium; low methoxyl pectin; pectin methylesterase; mechanical properties

INTRODUCTION

Raspberries are a high-value fruit crop, and are used as an ingredient in many foods due to their delicious flavor and bright color. They are a good source of both soluble dietary fiber and antioxidants. Raspberries have also been utilized for their functional food properties.[1] Their vitamin C and flavonoids provide high levels of antioxidants, while the fiber helps to maintain a healthy digestive tract.[2] Red raspberries are delicate in nature with a very short shelf life. Texture, sweetness, aroma, and color are important quality attributes of red raspberries, which determine their acceptance by the consumer.[3] Due to their susceptibility to crushing and mechanical collapse during handling and processing, the use of raspberries in commercial baked and dairy products is limited. It is difficult to add whole berries to a batter without them undergoing physical breakage. Although the baking and dairy industry often use frozen fruit in their recipes, freezing causes cell damage and loss of texture. Berries lose turgidity and firmness after thawing, causing quality problems in baking products. For example, juice from berries often turns batter red.

One of the potential solutions to improve the structural integrity of red raspberries before freezing is vacuum impregnation (VI) of firming agents. Vacuum impregnation is a physical treatment to promote compositional changes in fruits and vegetables that improve texture. During the VI process, porous tissues are submerged in a solution containing firming agents and subjected to a partial vacuum. Application of the vacuum results in extraction of air from intercellular spaces, while the restoration of pressure allows the impregnation of the solution into intercellular spaces. Mass transfer during VI is a hydrodynamic mechanism consisting of capillary action and a pressure gradient, coupled with the deformation–relaxation phenomenon.[4] Vacuum infusion of bioactive constituents, including antioxidants, minerals, probiotics, and firming agents in food, has been well documented.[5] Vacuum impregnation conditions, including the level of vacuum, restoration times, type of solution, and solution temperature, can influence the efficacy of the solute infusion. The microstructural properties of fruit and vegetable tissues are also critical factors.

The highest concentration of pectin is found in the middle lamella, where calcium plays an important role in maintaining the cell-wall structure by forming a firm gel-like structure.[6] Low methoxyl pectin (LMP) forms gel in the presence of calcium, which acts as a bridge between pairs of carboxyl groups of pectin molecules on adjacent polymer chains in close proximity. The interactions between Ca^{2+} ions and carboxyl groups of the pectin are described by the egg-box model.[7] Pectin methylesterase

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(PME), a native enzyme in fruits and vegetables, is also postulated to increase firmness of fruits and vegetables by demethylating endogenous pectin, forming LMP that is readily crosslinked by calcium.[8]

Degraeve *et al.*[9] reported that using an infusion of calcium alone, or a calcium and PME solution before thermal processing reduces the loss of firmness in strawberries, raspberries, and apples. Studies show an increase in firmness of pasteurized strawberry halves that underwent vacuum impregnation with PME and $CaCl_2$ compared with strawberry halves that were impregnated in atmospheric conditions. While raspberries infused with PME and $CaCl_2$ under non-vacuum treatment are firmer than those infused under a vacuum, no significant advantage of VI compared to atmospheric infusion was found for apple cubes. Differences in results were attributed to the internal structure of fruit tissues. Banjongsinsiri *et al.*[10] studied fresh-cut mangoes infused with PME and/or calcium chloride. They found that infused mango cubes had increased gumminess and chewiness but that the treatment had no impact on firmness. They also concluded that cellulose and hemicellulose in the mango cell wall might have contributed to inhibition of firming agents. Kirtil *et al.*[11] also reported that vacuum infusion of fresh and frozen mangoes at 10 kPa with PME and $CaCl_2$ is the most suitable treatment to preserve their cell structure. Nuclear magnetic resonance has also been used to examine the effect of PME and calcium on fruit-cell integrity.

Reno *et al.*[12] examined changes in the microstructural characteristics of frozen strawberries after thawing. They infused the fruit with pectin and $CaCl_2$ at different concentrations and at different vacuum levels. Results show that increasing the pectin and calcium chloride concentration reduced the loss of cellular fluid and enhanced the texture of thawed fruit. Sousa *et al.*[13] studied the effect of $CaCl_2$ and LMP at different freezing and thawing rates on the structure and sensory characteristics of raspberries and blackberries. When these fruits were pretreated with $CaCl_2$ and LMP, there was a significant increase in the mechanical strength of the tissue.

Several studies have examined the effectiveness of VI treatment using mechanical measurement of fruits. Firmness is a textural sensory attribute used to describe the resistance to breaking of a solid food product when it is eaten. Firmness depends on such factors as the degree of ripeness, fibrousness, turgidity, and processing, and can be assessed by instrumental or sensory tests such as compression and penetration.[9,12,14] Maximum force (F_M) is defined as the peak force that occurs during the first compression cycle. Gradient (G_{C3}) is the slope of the curve in the linear zone prior to rupture point. F_M and G_{C3} can be used to measure firmness of fruit.[14–17]

Few studies have explored how to improve firmness in raspberries. This study therefore investigated the feasibility of using VI to improve the firmness of red raspberries. Findings may improve the use of raspberries in formulated products such as cakes and muffins with less mechanical damage, breakdown, or bleeding. We examined a range of process conditions, including vacuum level and time, calcium-LMP and calcium-PME infusion solutions, and solution temperatures. Select mechanical properties, maximum force, and gradient were used to assess the fruit firmness.

MATERIALS AND METHODS

Raw materials

Red raspberries (*Rubus idaeus* L. cv. *Meeker*) were purchased from a local grocery store in Pullman, WA. The fruit was stored at 4 °C and processed within three days following purchase. Raspberries were prescreened visually for uniform size, weight, and firmness (by touch) before undergoing vacuum impregnation tests. Deionized water (DI) was used to prepare infusion solutions. All chemicals were of analytical grades: food grade calcium chloride dihydrate (VWR International, LLC, Batavia, IL), LMP (TIC Pretested® Pectin LM 35 powder, a gift from TIC GUMS, White Marsh, MD), commercial *Aspergillus niger* PME (Rapidase® FP Super, a gift from DSM Food Specialties B.V. Delft, The Netherlands, and Centerchem, Norwalk CT), and sucrose Grade II (Sigma-Aldrich, Milwaukee,WI).

Infusion solutions

Vacuum impregnation solutions were prepared with Pectin LM 35 Powder (LMP) at a concentration of 10 g of pectin kg^{-1} of solution and calcium chloride ($CaCl_2{\cdot}2H_2O$) at 30 g calcium kg^{-1} of pectin in DI water. The pH of the solutions was adjusted with granulated citric acid to range from 3.2 to 3.6. PME Rapidase® FP Super preparation solutions were prepared at a concentration of 10 g of enzyme kg^{-1} of solution, and ($CaCl_2{\cdot}2H_2O$) at 10 g of calcium kg^{-1} of solution. The pH was adjusted with granulated citric acid to between 4 and 4.5. The supplier recommended using LMP and PME at a temperature of 40 °C, so we investigated this temperature to assess the fruit's firmness. Vacuum impregnation experiments were conducted at solution temperatures of 20 and 40 °C. Additional experiments were performed to investigate the effect of the infusion of LMP-calcium and PME-calcium in hypertonic and isotonic solutions. Isotonic and hypertonic solutions were prepared by adding predetermined quantity of sucrose in LMP-calcium, and PME-calcium solutions at 20 °C. To determine the quantity of sucrose needed for the preparation of the isotonic solutions, the raspberries were homogenized at 1400 g for 5 min in a 50 ml beaker, in a homogenizer (Model Kinematica Politron pt-2500 E, Bohemia, NY) and °Brix was measured using a hand-held digital pocket refractometer (Model Atago pal – α 0–85%, Itabashi-Ku, Tokyo, Japan). All hypertonic solutions were prepared at 45°Brix. Deionized water alone (without PME, LMP, $CaCl_2{\cdot}2\,H_2O$ or sucrose) was also used as a control treatment. Data from these treatments were used to compare the effect of LMP-calcium and PME-calcium on the mechanical properties of raspberries.

Vacuum impregnation treatment

Three levels of vacuum 33.9, 50.8, and 67.8 kPa, and the three vacuum times 2, 7, and 15 min were investigated. In each experiment, a ratio of 1: 4 (w/w) fruit to impregnation solution was maintained. Raspberries were placed in a beaker containing the impregnation solution and covered with a plastic mesh to keep the fruit immersed in the solution during the treatment. A vacuum chamber (Model No.1410-2 Sheldom Manufacturing, Cornelius, OR) was connected to a vacuum pump (Edwards 12 Two stages, oil sealed rotary vane, Hillsboro, OR). The vacuum time was noted after the vacuum level was reached. Immediately after the vacuum time was accomplished, the chamber was returned to atmospheric pressure and maintained for 5 min. The restoration time of 5 min was selected based on preliminary experiments. The raspberries were then separated from the solution using a stainless-steel strainer. Each berry was individually tap dried with tissue to remove excess solution from the surface and then kept at room temperature (~23 °C) for 1 hour. The berries were individually placed in a plastic holder covered with aluminum foil and kept at 4 °C for 6 days. Each experiment was performed three times.

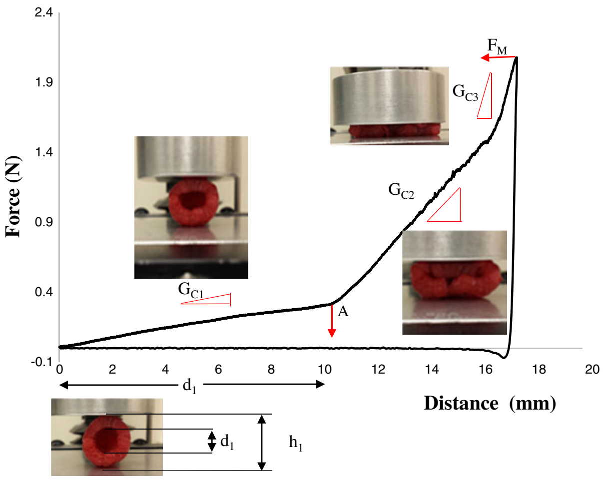

Figure 1. Force-deformation curve. **G_{C1}**: opposite wall compression; **G_{C2}**: berry interstices compression; **G_{C3}**: solids and liquid compression; **F_M**:maximum force. Compression was performed to close the opening of the raspberry by applying a perpendicular force to the berry long axis.

Mechanical properties

The mechanical properties of VI berries were determined by using a texture analyzer (Model TA-XT2, Stable Microsystems, Godalming, England) with a 25 kg load cell and a flat cylinder probe 50 mm in diameter. The measurements were performed at a constant plunger speed of 0.5 mm s^{-1}. Due to variability in the size and shape of the fruit, several preliminary sample preparations and mechanical measurement tests were conducted to determine the most reliable procedure. The raspberries were first tested using a compression-extrusion test using a five-blade Kramer shear cell and then a uniaxial compression test between two plates, with a flat cylinder probe for force measurement. The mean value of these tests was determined, with neither producing consistent results. Cutting the berries for a constant height damaged the integrity of the sample as a whole.

The berries were also centrally placed with their major axis perpendicular to the compression plate. These tests also failed to produce satisfactory results. Finally, a uniaxial compression test with 80% strain produced the most repeatable results. Strain is defined as the change in size of the berry with reference to its original dimension $h_{1,}$ (Fig. 1). Maximum force (F_M) required to achieve 80% strain in a berry and the slope of the curve force deformation prior to maximum force (G_{C3}) were considered to be indicators of fruit firmness. Ten berries were used per experiment and each experiment was performed three times.

Figure 1 shows a typical force-distance curve during compression tests. The different slopes represent the change in force represented by Newton (N) over deformation in millimeters (Δ N /Δ mm) during the compression test. The first region of the curve (gradient G_{C1}) represents the compression of the opposite walls of the raspberries when the probe was lowered perpendicular to the fruit placed on its side. The second region (gradient G_{C2}) shows compression of the fruit interstices filled with solid, liquid, and trapped air.[18] The final/third region (gradient G_{C3}) represents the compression of berry solids and liquid without air. Overall, the berries were compressed to 80% of their initial heights. Due to the concavity of the first two regions of the curve, the G_{C1} and G_{C2} were not easy to characterize. On the other hand, the third region of the curve G_{C3} was mostly linear.

Calcium analysis

Infused fruits were placed in a plastic colander and submerged for 2 minutes in a container with DI water at 23 °C to remove surface traces of $CaCl_2{\cdot}2H_2O$, which would have presumably not reacted. The washed fruit was dried in a natural convection oven (Model ED-53 L Binder GmbH Tuttligen, Germany) at 80 °C for 2 days. The dried fruit was ground with a ceramic mortar, and samples (0.20 to 0.25 g) were treated with 2 ml of H_2O_2 (density 1.11 g cm^{-3}) and 2 ml of HNO_3 (density 1.42 g cm^{-3}). This solution was processed in a microwave digester (Model Discover SPD, CEM Corporation F.R. Mattews N.C.) at 200 °C, 300 W, with a ramp time of 5 min, and a pressure of 400 psi. The digested solution was diluted with 100 ml DI water. This solution was passed through a 0.45 μm filter and the filtrate was employed for calcium determination. An atomic absorption spectrometer was set to a wavelength of 422.7 nm and a slit width of 0.5 nm was used (Model Spectr AA 220 Varian Palo Alto, CA). From a standard solution of 100 ppm calcium, dilutions of 0.25, 0.5, 1 and 2.5 ppm were prepared. Samples of unknown calcium concentration were diluted by a factor of 5 and aspirated into the equipment and the absorbance measured. The concentration of calcium was directly read from the spectrometer. Each treatment of infused and control samples was conducted in triplicate.

Statistical analysis

The data analysis was performed using SAS 9.2. A completely randomized factorial design with three factors was used, including vacuum level, processing time, and calcium uptake, and three replicates. Each replicate involved 10 raspberries. An analysis of variance (ANOVA) was used to determine the difference between the means of vacuum level, processing time, and calcium uptake. After each response variable was analyzed separately, a combined analysis of all three variables was performed using desirability functions. In this approach, the response of each replicate was subtracted from the minimum of all replicates and divided by that same minimum. This calculation placed each response on a 0 to 1 scale, in which scores closer to 1 indicated more desirable responses and scores near 0 indicated poor outcomes. Then the geometric mean of the three desirability scores was calculated, one for each response variable. This geometric mean was the product of all three scores, raised to the 1/3 power. This overall desirability score was then analyzed using ANOVA.

RESULTS AND DISCUSSION

Effect of vacuum level and time

Preliminary results suggested that an increase in restoration time from 5 to 10 min had no significant effect on firmness when fruit was infused with LMP-calcium and PME-calcium. Experiments also indicated that vacuum times beyond 15 min at intermediate and higher levels of vacuum damaged the fruit. For both LMP-calcium and PME-calcium solutions at 20 °C, the intermediate vacuum level (50.8 kPa) showed the highest F_M and G_{C3}. In general, increasing the vacuum level from 33.9 to 50.8 kPa increased F_M (Figs 2a and 2b) and G_{C3} (Tables 1 and 2), while further increasing the vacuum to 67.8 kPa decreased F_M and G_{C3}. At a higher vacuum level (67.8 kPa), F_M and G_{C3} values were similar to or less than values obtained at a lower vacuum level (33.9 kPa). In general, the firmness (F_M and G_{C3} values) of LMP-calcium infused raspberries was higher than that from vacuum-treated samples in DI water. In PME-calcium infused solutions, F_M values were higher than

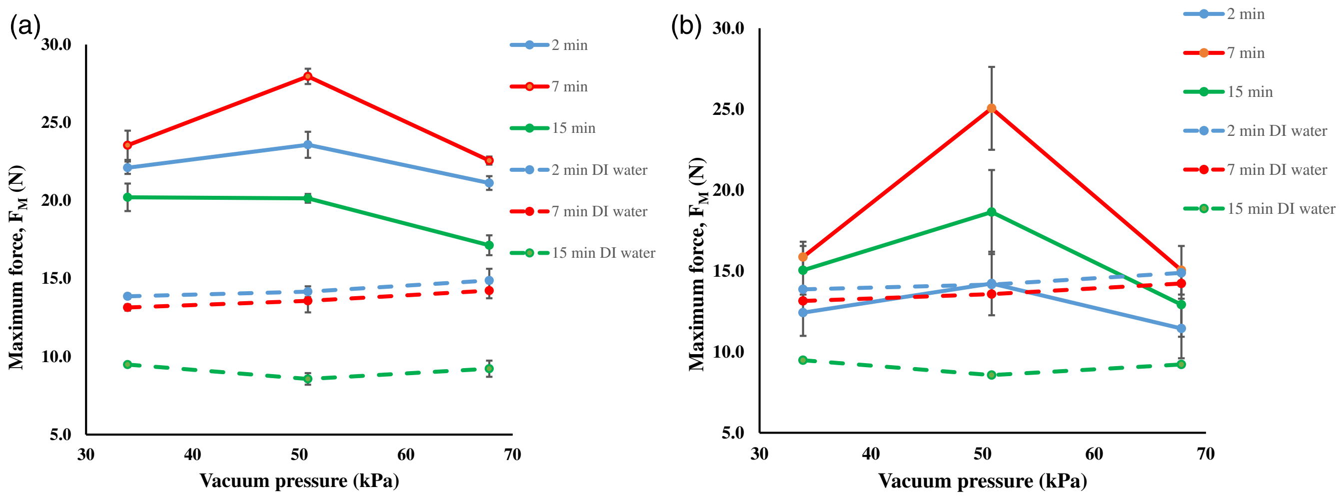

Figure 2. a. Effect of the vacuum level on the firmness of infused raspberries with LMP and calcium at 20 °C. Firmness was measured as the maximum force (F_M). Solid lines represent infused fruit. Dashed lines represent vacuum treated fruit in DI water. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries. b. Effect of the vacuum level on the firmness of infused raspberries with PME and calcium at 20 °C. Firmness was measured as the maximum force (F_M). Solid lines represent infused fruit. Dashed lines represent vacuum treated fruit in DI water. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries.

Table 1. Effect of the infusion (LMP) solutions at 20 °C on the firmness of raspberries

Vacuum pressure (kPa)	Vacuum processing time (min)	Maximum force F_M (N)			Gradient G_{C3} (N mm^{-1})		
		LMP-calcium in DI water	LMP-calcium in isotonic solution	LMP-calcium in hypertonic solution	LMP-calcium in DI water	LMP-calcium in isotonic solution	LMP-calcium in hypertonic solution
33.9	2	22.1 ± 0.39^{cd}	10.8 ± 1.67^{bcd}	9.61 ± 0.78^{a}	5.40 ± 0.39^{d}	2.16 ± 0.59^{a}	1.96 ± 0.49^{a}
	7	23.5 ± 0.98^{b}	9.81 ± 1.67^{abc}	12.0 ± 0.39^{b}	6.87 ± 0.78^{bc}	1.67 ± 0.49^{a}	3.43 ± 0.88^{bcd}
	15	20.2 ± 0.88^{d}	10.4 ± 0.78^{bc}	14.8 ± 0.88^{c}	6.38 ± 0.29^{c}	2.06 ± 0.88^{a}	3.92 ± 0.59^{d}
50.8	2	23.5 ± 0.88^{b}	13.1 ± 0.88^{d}	10.3 ± 0.29^{a}	7.55 ± 0.88^{ab}	2.26 ± 1.37^{a}	1.96 ± 0.78^{ab}
	7	28.0 ± 0.49^{a}	9.12 ± 0.88^{ab}	12.3 ± 0.69^{b}	8.44 ± 0.59^{a}	1.77 ± 0.20^{a}	3.73 ± 0.98^{cd}
	15	20.1 ± 0.29^{bc}	11.8 ± 1.67^{cd}	16.2 ± 0.78^{d}	6.77 ± 0.10^{bc}	1.86 ± 0.10^{a}	4.22 ± 0.69^{d}
67.8	2	21.1 ± 0.49^{e}	7.55 ± 1.86^{a}	11.7 ± 0.20^{b}	5.89 ± 0.59^{cd}	1.67 ± 0.78^{a}	2.65 ± 0.59^{abc}
	7	22.6 ± 0.29^{e}	9.12 ± 0.59^{ab}	14.9 ± 0.69^{c}	6.18 ± 0.29^{cd}	1.86 ± 0.29^{a}	4.12 ± 0.49^{d}
	15	17.2 ± 0.69^{f}	7.65 ± 1.77^{a}	18.3 ± 1.08^{e}	5.89 ± 0.49^{cd}	1.86 ± 0.10^{a}	4.51 ± 0.69^{d}

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries.

vacuum-treated samples in DI water at 7 and 15 min of vacuum times (Figure 2b), while no significant ($P \leq 0.05$) difference was observed for G_{C3} obtained at a lower vacuum level. In general, as the level of vacuum and the release of trapped gas increased, the addition of the firming agent increased. However, the highest vacuum level and the time period damaged the fruit structure.

The intermediate vacuum time (7 min) showed a peak in terms of fruit firmness (F_M and G_{C3}) similar to the effects of the vacuum level. That is, an increase in vacuum time from 2 to 7 min increased fruit firmness, while a further increase in vacuum time to 15 min decreased fruit firmness for both LMP-calcium and PME-calcium infused berries. In all cases, LMP-calcium infused red raspberries were firmer (F_M and G_{C3}) than PME-calcium infused berries.

The application of VI to inhibit softening in fruits and vegetables has been widely used. Firmness in infused eggplants with commercial fungal (*Aspergillus niger*) and citrus PME with calcium chloride (4000 ppm) at different vacuum levels and times significantly increases firmness compared to fresh non-infused and water-infused controls, after processing and during storage for 7 days at 4 °C.[19] Similar results were obtained during vacuum impregnation of polyamines into strawberry slices.[20] Polyamines have similar effects to calcium in terms of improving fruit firmness. Polyamines have been found to increase the firmness of strawberries after storage at 1 °C. The role of polyamines or calcium suggest that they can be bound to the cell wall, promoting cross-linking between ions and pectin in the middle lamella, and this effect increases with storage time, increasing cell-wall rigidity.[21,22]

Effect of the isotonic and hypertonic solutions

Our experiments with isotonic solution allowed us to assess the effect of pectin and calcium solution on fruit firmness due to vacuum impregnation process alone. This does not take into account the difference in solid concentration between the infusion solution and the fruit as driving force. No clear trends were observed for fruit firmness parameters (F_M and G_{C3}) between LMP or PME-calcium in DI water (control) and LMP or PME-calcium in sucrose solutions (Tables 1 and 2). Furthermore, when berries were infused with hypertonic solutions, no definite trend was found for

Table 2. Effect of the infusion (PME) solutions at 20 °C on the firmness of raspberries

Vacuum pressure (kPa)	Vacuum processing time (min)	Maximum force F_M (N)			Gradient G_{C3} (N mm^{-1})		
		PME-calcium in DI water	PME-calcium in isotonic solution	PME-calcium in hypertonic solution	PME-calcium in DI water	PME-calcium in isotonic solution	PME-calcium in hypertonic solution
33.9	2	12.5 ± 1.47^{ab}	11.3 ± 2.94^{a}	15.4 ± 3.43^{cde}	2.16 ± 0.49^{c}	2.65 ± 1.96^{a}	3.53 ± 2.16^{bcd}
	7	15.9 ± 0.98^{cd}	8.63 ± 1.28^{a}	12.1 ± 1.18^{bcd}	3.53 ± 0.98^{b}	1.37 ± 0.59^{a}	5.20 ± 1.18^{de}
	15	15.0 ± 1.47^{bc}	9.91 ± 2.94^{a}	10.6 ± 0.29^{abc}	2.65 ± 0.20^{c}	1.96 ± 0.98^{a}	2.35 ± 0.29^{ab}
50.8	2	14.2 ± 1.96^{abc}	9.52 ± 0.59^{a}	16.7 ± 6.08^{de}	2.35 ± 0.20^{c}	2.35 ± 0.78^{a}	4.71 ± 0.78^{cde}
	7	25.0 ± 2.55^{e}	7.95 ± 2.65^{a}	18.2 ± 0.49^{e}	4.91 ± 0.10^{a}	1.18 ± 0.88^{a}	5.69 ± 0.78^{e}
	15	18.6 ± 2.55^{d}	8.53 ± 0.59^{a}	13.7 ± 0.98^{bcde}	3.92 ± 0.29^{b}	1.77 ± 0.29^{a}	2.94 ± 0.69^{abc}
67.8	2	11.5 ± 1.86^{a}	7.36 ± 0.98^{a}	9.52 ± 1.47^{ab}	2.06 ± 0.10^{c}	1.77 ± 0.20^{a}	1.67 ± 0.69^{a}
	7	15.0 ± 1.47^{bc}	9.52 ± 3.24^{a}	12.5 ± 3.14^{bcd}	4.71 ± 0.10^{a}	2.35 ± 0.78^{a}	5.10 ± 0.29^{de}
	15	13.0 ± 1.96^{abc}	7.85 ± 2.94^{a}	6.87 ± 3.53^{a}	2.26 ± 0.10^{c}	1.67 ± 0.29^{a}	1.96 ± 0.98^{ab}

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries.

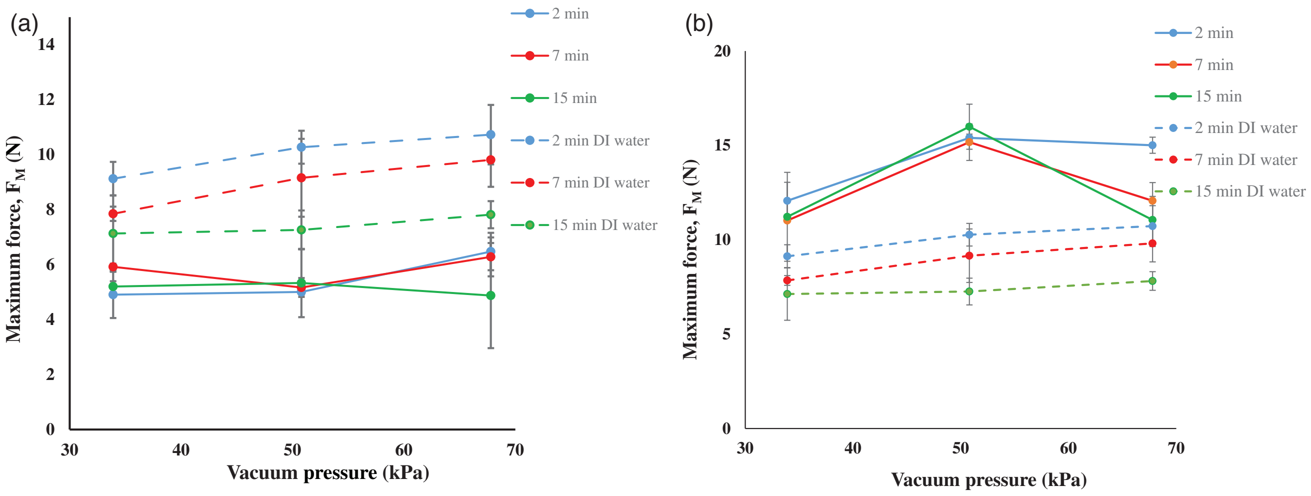

Figure 3. a. Effect of the vacuum level on the firmness of infused raspberries with LMP and calcium at 40 °C. Firmness was measured as the maximum force (F_M). Solid lines represent infused fruit. Dashed lines represent vacuum treated fruit in DI water. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries. b. Effect of the vacuum level on the firmness of infused raspberries with PME and calcium at 40 °C. Firmness was measured as the maximum force (F_M). Solid lines represent infused fruit. Dashed lines represent vacuum treated fruit in DI water. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries.

Table 3. Effect of temperature on G_{C3} (N mm^{-1})

Vacuum pressure (kPa)	Vacuum processing time (min)	DI water		LMP-calcium in DI water		PME-calcium in DI water	
		20 °C	40 °C	20 °C	40 °C	20 °C	40 °C
33.9	2	3.24 ± 0.20^{dc}	2.84 ± 0.49^{a}	5.40 ± 0.39^{d}	0.98 ± 0.29^{c}	2.16 ± 0.49^{c}	4.81 ± 0.29^{abc}
	7	3.63 ± 0.20^{cb}	2.45 ± 0.98^{ab}	6.87 ± 0.78^{bc}	1.57 ± 0.10^{ab}	3.53 ± 0.98^{b}	3.53 ± 0.20^{d}
	15	3.24 ± 0.39^{dc}	1.47 ± 0.88^{b}	6.38 ± 0.29^{c}	1.37 ± 0.20^{abc}	2.65 ± 0.20^{c}	4.41 ± 0.10^{c}
50.8	2	4.32 ± 0.10^{a}	2.75 ± 0.20^{ab}	7.55 ± 0.88^{ab}	1.47 ± 0.59^{b}	2.35 ± 0.20^{c}	5.49 ± 0.39^{a}
	7	4.22 ± 0.20^{ab}	2.06 ± 0.98^{ab}	8.44 ± 0.59^{a}	1.77 ± 0.10^{a}	4.91 ± 0.10^{a}	5.20 ± 0.49^{ab}
	15	2.45 ± 0.29^{e}	1.96 ± 0.49^{ab}	6.77 ± 0.10^{bc}	1.28 ± 0.20^{bc}	3.92 ± 0.29^{b}	5.40 ± 0.10^{a}
67.8	2	4.02 ± 0.20^{ab}	2.06 ± 0.10^{ab}	5.89 ± 0.59^{cd}	1.77 ± 0.10^{a}	2.06 ± 0.10^{c}	4.41 ± 0.49^{c}
	7	4.12 ± 0.69^{ab}	1.96 ± 0.88^{ab}	6.18 ± 0.29^{cd}	1.47 ± 0.10^{abc}	4.71 ± 0.10^{a}	4.51 ± 0.10^{bc}
	15	2.84 ± 0.69^{ed}	1.96 ± 0.49^{ab}	5.89 ± 0.49^{cd}	1.18 ± 0.10^{bc}	2.26 ± 0.10^{c}	4.32 ± 0.88^{c}

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries.

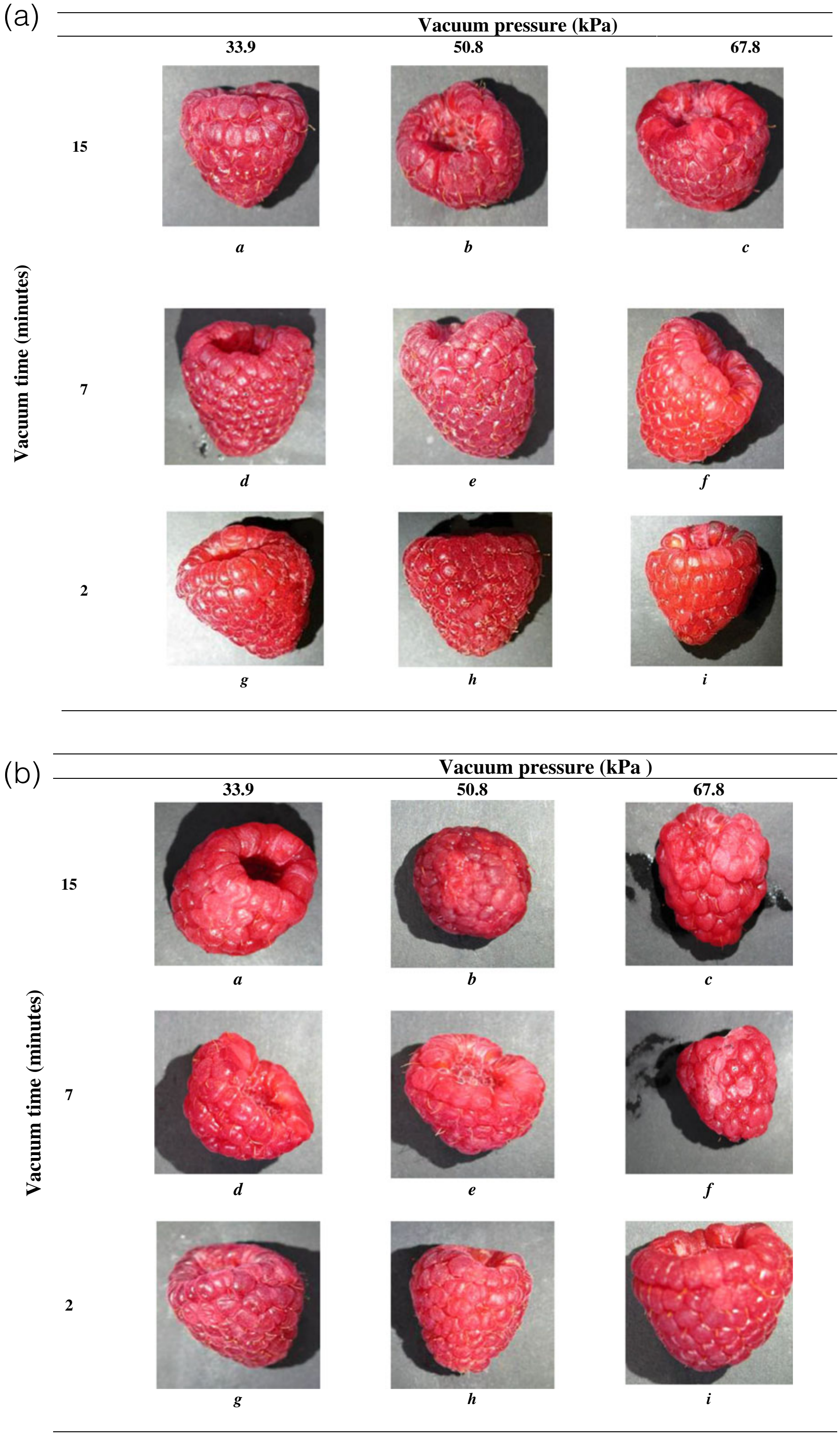

Figure 4. a. Red raspberries infused with LMP and calcium at different vacuum levels and times, and at a solution temperature of 20 °C. Evidence of drupelet collapse (c) can be observed. b. Red raspberries infused with LMP and calcium at different vacuum levels and times, and solution temperature of 40 °C. Evidence bleeding (a, b, c, f), drupelet collapse (d, e, and i), and minor injuries (g and h) can be observed.

F_M and G_{C3} values of control and treated berries. Moreover, when sucrose was used, a sticky condition made the fruit extremely difficult to handle. Consequently, hypertonic and isotonic solutions were not considered in later experiments. The visual appearance of raspberries infused with isotonic and hypertonic solutions after combining pectin or enzyme at different vacuum levels and processing times at 20 °C was evaluated as negative.

The combination of VI and osmotic dehydration of mango fruit was studied to improve the firmness of the dried products.[23] For example, when mango was immersed in hypertonic solution

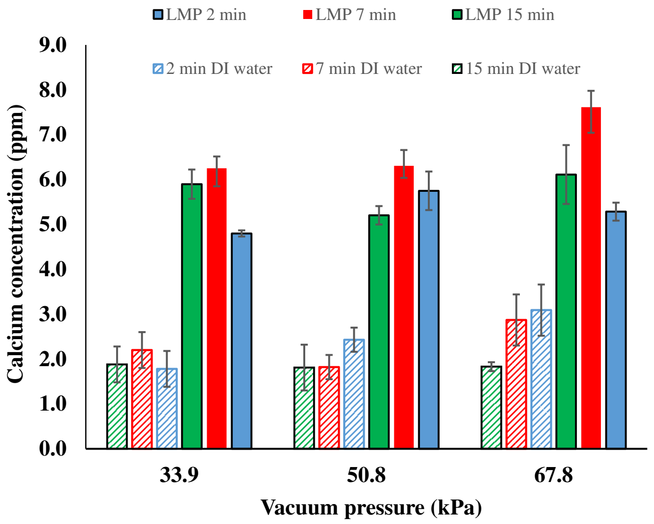

Figure 5. Comparison in calcium intake between infused raspberries with LMP and calcium and vacuum treated fruit in DI water at 20 °C. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries.

at room temperature, VI facilitated water loss and solids gain. However, compared with fresh mango samples, the firmness of the fruit subjected to VI decreased significantly. Degradation of the middle lamella may lead to a decrease in fruit firmness. Zhao and Xie[24] infused strawberries with high fructose corn syrup or high methoxyl pectin (cryoprotectants) solutions containing calcium and/ or zinc before freezing to improve the textural quality and reduce drip loss in frozen–thawed strawberries. Results show that using VI with cryoprotectants improved firmness and reduced drip loss of frozen–thawed strawberries compared to untreated fruit.

Effect of solution temperature

In most cases, a higher solution temperature (40 °C) compromises fruit firmness. For LMP-calcium infused berries, F_M and G_{C3} values at 20 °C are higher than at 40 °C (Fig. 3a and Table 3). However, for PME-calcium infused berries, G_{C3} in a 40 °C solution is generally higher than at 20 °C (Table 3), while F_M values show no clear trend (Fig. 3b). Paes *et al.*[25] studied the effect of VI temperature on the mechanical properties of sliced cylindrical samples from apples (*Fuji variety*). Treated samples at 10 °C were firmer than those at 50 °C due to the softening of fruit tissue at higher temperatures. Lovera *et al.*[26] studied the effect of process temperature, calcium gluconate, and calcium lactate utilization as a source of calcium. They examined different concentrations of these chemical reagents, as well as pH, to assess the firmness of papayas in syrup. The results show that when the temperature increased from 30 °C to 45 °C with calcium lactate at 0.5% (w/w), an increase in firmness was observed. Therefore, the impregnation of calcium was favored by higher temperatures and by increased calcium concentration.

Overall, firmness values for PME-calcium infused berries were lower than LMP-calcium infused berries. The negative effect of temperature on fruit integrity was a key factor. Hence, LMP-calcium infused berries at 20 °C were selected for the calcium uptake study (Figs 4a and 4b).

Calcium uptake and its relation to fruit firmness

In this study, we analyzed raspberries infused with LMP under different operating conditions for calcium uptake. This is an important factor in improving firmness in fruits. Figure 5 presents our first approach to analyzing the role of calcium content in raspberries. Raspberries infused with LMP solutions showed better calcium intake than control samples.

Our results show no clear relationship between calcium concentration and F_M and G_{C3} (see Table 4). The calcium concentration in raspberries infused at 50.8 kPa at 2 minutes was 5.75 ppm, with F_M and G_{C3} values of 23 N and 7.6 N mm^{-1}, respectively. When pressure was kept constant and time changed to 7 minutes, the calcium concentration increased to 6.31 ppm, and F_M and G_{C3} increased to 28 N and 8.4 N mm^{-1}, respectively. However, when vacuum processing time was increased to 15 min, calcium concentration decreased to 5.2 ppm, and F_M and G_{C3} to 20 N and 7 N mm^{-1}, respectively. The highest calcium uptake occurred at 7 min vacuum processing time within treatments at constant pressure. When calcium content in infused berries was analyzed at a constant time within treatments, the highest calcium uptake occurred at 67.8 kPa, except at the 2 min vacuum processing time. Overall, increasing the vacuum level or processing time did not result in a higher level of calcium intake. However, there is a significant difference when infused fruit is compared with vacuum-treated samples in DI water at a level of significance of $P \leq 0.05$. The formation of

Table 4. Effect of calcium concentration on firmness of infused raspberries with LMP solutions at 20 °C

		LMP-calcium in DI water			DI water		
Vacuum pressure (kPa)	Vacuum processing time (min)	Concentration (ppm Ca^{2+})	Maximum force F_M (N)	Gradient G_{C3} (N mm^{-1})	Concentration (ppm Ca^{2+})	Maximum force F_M (N)	Gradient G_{C3} (N mm^{-1})
33.9	2	4.80 ± 0.07^{d}	22.1 ± 0.39^{cd}	5.40 ± 0.39^{d}	1.78 ± 0.10^{b}	13.8 ± 0.20^{cb}	3.24 ± 0.20^{dc}
	7	6.25 ± 0.26^{b}	23.5 ± 0.98^{b}	6.87 ± 0.78^{bc}	2.20 ± 0.40^{b}	13.2 ± 0.20^{c}	3.63 ± 0.20^{cb}
	15	5.90 ± 0.33^{b}	20.2 ± 0.88^{d}	6.38 ± 0.29^{c}	1.88 ± 0.40^{b}	9.52 ± 0.20^{de}	3.24 ± 0.39^{dc}
50.8	2	5.75 ± 0.43^{bc}	23.5 ± 0.88^{b}	7.55 ± 0.88^{ab}	2.43 ± 0.45^{ab}	14.1 ± 0.39^{ab}	4.32 ± 0.10^{a}
	7	6.31 ± 0.35^{b}	28.0 ± 0.49^{a}	8.44 ± 0.59^{a}	1.82 ± 0.27^{b}	13.5 ± 0.78^{cb}	4.22 ± 0.20^{ab}
	15	5.20 ± 0.21^{cd}	20.1 ± 0.29^{bc}	6.77 ± 0.10^{bc}	1.81 ± 0.51^{b}	8.53 ± 0.39^{f}	2.45 ± 0.29^{e}
67.8	2	5.29 ± 0.20^{cd}	21.1 ± 0.49^{e}	5.89 ± 0.59^{cd}	3.09 ± 0.39^{a}	14.7 ± 0.78^{a}	4.02 ± 0.20^{ab}
	7	7.61 ± 0.37^{a}	22.6 ± 0.29^{e}	6.18 ± 0.29^{cd}	2.87 ± 0.57^{a}	14.2 ± 0.49^{ab}	4.12 ± 0.69^{ab}
	15	6.11 ± 0.66^{b}	17.2 ± 0.69^{f}	5.89 ± 0.49^{cd}	1.83 ± 0.10^{b}	9.22 ± 0.49^{ef}	2.84 ± 0.69^{ed}

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries.

Table 5. Analysis of response using ANOVA in combination with desirability functions (DS_f)

		Variable			
Vacuum pressure (kPa)	Vacuum processing time (min)	Concentration (ppm Ca^{2+})	Maximum force F_M (N)	Gradient G_{C3} (N mm^{-1})	Composite variable
33.9	2	4.80 ± 0.07^{d}	22.1 ± 0.39^{cd}	5.40 ± 0.39^{d}	0.07^{e}
	7	6.25 ± 0.26^{b}	23.5 ± 0.98^{b}	6.87 ± 0.78^{bc}	0.37^{b}
	15	5.90 ± 0.33^{b}	20.2 ± 0.88^{d}	6.38 ± 0.29^{c}	0.25^{c}
	2	5.75 ± 0.43^{bc}	23.5 ± 0.88^{b}	7.55 ± 0.88^{ab}	0.36^{b}
50.8	7	6.31 ± 0.35^{b}	28.0 ± 0.49^{a}	8.44 ± 0.59^{a}	0.54^{a}
	15	5.20 ± 0.21^{cd}	20.1 ± 0.29^{bc}	6.77 ± 0.10^{bc}	0.20^{c}
	2	5.29 ± 0.20^{cd}	21.1 ± 0.49^{e}	5.89 ± 0.59^{cd}	0.18^{cd}
67.8	7	7.61 ± 0.37^{a}	22.6 ± 0.29^{e}	6.18 ± 0.29^{cd}	0.38^{b}
	15	6.11 ± 0.66^{b}	17.2 ± 0.69^{f}	5.89 ± 0.49^{cd}	0.10^{cd}

Means within a column followed by the same letters are not significantly different at $P \leq 0.05$. Results reported are mean ± SD. Three replicates were used. Every replicate involved 10 raspberries.

calcium bridges between galacturonic acid residues may explain this effect, and a threshold in which the mechanical effect of the vacuum on the structure of raspberries inhibits the construction of a stronger wall structure, regardless of how much calcium is added.

Combined effects

Table 5 summarizes calcium concentration, peak force, gradient, and the calculated composite variable (overall desirability score) using the mean and standard deviation. With more than one quality response, we used the desirability function (DS_f) approach to solve this multi-response optimization problem. Higher values close to 1 are considered better. We calculated the best conditions for the independent variables of vacuum level and vacuum processing time. The conditions for optimal values of the response variables F_M, G_{C3} and calcium concentration with a composite variable value equal to 0.54 were 50.8 kPa vacuum level, 7 minutes processing time.

CONCLUSIONS

Our findings reveal that vacuum impregnation conditions including vacuum level and time, and solution type and temperature, affect the firmness of red raspberries. Results show that the infusion of LMP-calcium is more effective than PME-calcium in improving fruit firmness. Increasing the temperature of the impregnation solution reduced fruit firmness. Calcium uptake was positively correlated with fruit firmness for lower vacuum levels (33.9 and 50.8 kPa).

However, a higher level of vacuum (67.8 kPa) damaged the fruit structure. The optimal VI treatment conditions obtained were LMP concentration at 10 g of pectin kg^{-1} of solution and calcium chloride at 30 g calcium kg^{-1} of pectin in DI water, vacuum level 50.8 kPa, 7 min vacuum time, 5 min restoration and solution temperature of 20 °C. Using VI of LMP and calcium in red raspberries can potentially be used to enhance fruit structural integrity.

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