

Blanching effects of radio frequency heating on enzyme inactivation, physiochemical properties of green peas (*Pisum sativum* L.) and the underlying mechanism in relation to cellular microstructure

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ABSTRACT

Fresh green peas require blanching to terminate enzymatic reaction induced quality deterioration before frozen storage. Radio frequency (RF) heating is a novel way of dry blanching for fruits and vegetables with high processing efficiency. In this study, blanching effects of RF heating on relative activities of lipoxygenase (LOX) and peroxidase (POD), physiochemical properties as well as cellular morphology changes of green peas were investigated. Results showed relative activities of pea LOX and POD reduced to $0.90 \pm 0.78\%$ and $1.10 \pm 0.71\%$, respectively at 85 °C by RF heating with an electrode gap 105 mm. Weight loss, color, texture and electrolyte leakage of peas changed significantly with increasing temperature (60–85 °C). Ascorbic acid, chlorophyll and mineral contents had different loss after RF processing and long term heating at 115 mm exacerbated the loss of nutrients. Microstructure features showed the deconstruction of pea cell wall and starch granule gelatinization.

1. Introduction

Green pea (*Pisum sativum* L.) is one important grain legume cultivated worldwide. It is consumed in multiple ways such as frozen peas, canned peas and puree for its high nutritional values such as lowering blood pressure, improving cardiovascular disease, postprandial blood glucose control and regulating gastrointestinal function (Roy, Boye, & Simpson, 2010; Würsch, Vedovo, & Koellreutter, 1986). In the market, quick-frozen green peas are the most common processed pea product while the supply period for fresh peas is quite short due to its cultivation and storage requirements. Green peas are highly seasonal crops with a preference of mild humid climate and no tolerance to hot and drought. After harvesting, fresh green peas with high moisture content still maintain intensive respiration, and endogenous enzymes proceed active biochemical reactions. Nutrient loss and spoilage always occur in green peas stored at high temperature. Although low-temperature storage can slow these processes, enzyme reaction would still cause undesired changes. For long-term preservation, green peas are usually frozen or dried after blanching.

Blanching is a common practice applied to fruits and vegetables prior to processing such as freezing, drying and baking in food production. It

is carried out to inactivate enzymes that lead to texture deterioration, nutrient loss, off-flavors, and color change etc. (Xiao et al., 2017). Blanching also facilitates subsequent processing by removing air in tissue, destroying cell microstructure and softening texture. Lipoxygenase (LOX) and peroxidase (POD) are both enzymes indicating the inactivation efficiency of blanching. LOX is a type of iron-containing dioxygenase which is the main source of off-flavor for legume products. LOX catalyzes the oxidation reaction of polyunsaturated fatty acids containing one or more 1Z, 4Z-pentadiene structures to generate corresponding hydroperoxide. The oxidation products are further metabolized into volatile micromolecule substances such as aldehydes, ketones, acids, alcohols, and furans etc. causing undesired odors and tissue deterioration (Xu, Jin, Gu, Rao, & Chen, 2020; Andreou & Feussner, 2009). Linoleic acid and linolenic acid are the most common liquid oxygen substrates in plants and pea LOX reacts with linoleic acid salts (Siedow, 1991). The loss of chlorophyll and lipid oxidation in frozen peas is related to LOX and decomposition of lipoperoxide (Buckle & Edwards, 1970). POD can lead to enzymatic browning of fruits and vegetables. Phenols in fruits and vegetables are oxidized to quinones under the action of polyphenol oxidase (PPO) and then quinones are further oxidized and polymerized to form browning melanin. POD is

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able to catalyze the oxidation and polymerization of phenols with H_2O_2 produced by PPO oxidation of phenols and participate in the browning reaction (Hamid & Khalil-ur-Rehman, 2009).

Radio frequency (RF) is electromagnetic wave with a frequency ranging from 10 to 300 MHz. The polarity of RF electromagnetic field changes at high frequency which leads to movement and fraction of ions and molecules in food materials. The fractional interaction ultimately results in volumetric heating and temperature increase (Piayseña, Dussault, Koutchma, Ramaswamy, & Awuah, 2003). The effects of RF heating on food drying, pasteurization and insect control have been studied for its advantages of great heating efficiency and large penetration depth (Wang, Zhang, Johnson, & Gao, 2014; Li, Kou, Cheng, Zheng, & Wang, 2017; Wang, Tang, Sun, Mitcham, Koral, & Birla, 2006). However, the application of RF heating technology to blanching is limited. It has been reported that exposure to 60 MHz RF could inactivate POD, PPO, pectinesterase, catalase and α -amylase (Lopez & Bagánis, 1971). Compared with conventional heating, RF treatment was more productive in soybean LOX inactivation (Jiang et al., 2018). RF electric field intensity also exerted influences to fruit enzyme inactivation efficiency (Manzocco, Anese, & Nicoli, 2008). Zhang et al. (2018) found that the relative activity of potato PPO was 3.24% when the central temperature of potato cuboids reached 85 °C by RF with a 120 mm electrode gap. Hardness, color and ascorbic acid content of RF heated carrot cubes were better than those of hot water blanched samples (Gong, Zhao, Zhang, Yue, Miao, & Jiao, 2019). RF treatment in blanching could ensure product quality, improve production efficiency and reduce water waste. Therefore it is meaningful to study the heating effect of RF on quick-frozen vegetables.

This study included three objectives: (1) to investigate the enzyme inactivation effects of RF heating on LOX and POD of green peas, (2) to evaluate physiochemical changes in weight loss, color, texture characteristics, electrolyte leakage and chemical contents of green peas after RF heating, (3) to analyze the mechanism of physiochemical changes after RF blanching in cell level.

2. Materials and methods

2.1. Sample preparation

Fresh peas were cultivated in Panzhihua, Sichuan, China and stored in 4 °C after harvest. Before testing, samples with the same maturity were peeled by hand and injured grains were screened out. Pea grains were then sifted by a two-mesh sieve (12.5 mm) and a three-mesh sieve (8 mm) in sequence to exclude oversized and undersized peas. Prepared samples and polypropylene (PP) sample containers were then equilibrated in 25 °C for 12 h.

2.2. RF heating system

A 6 KW, 27.12 MHz pilot-scale free running oscillator RF system (GJJG-2.1-10A-JY; Hebei Huashijiyuan High Frequency Equipment Co., Ltd, Hebei, China) with two parallel electrodes was employed in this study. The PP container was connected to a rotation system by a round buckle structure attached to the end of a polycarbonate shaft (diameter = 25 mm, length = 950 mm). The rotation system comprised a right-angle geared motor (6GU-2ORT-K, Shanghai Yany Heavy Machinery Co., Ltd., Shanghai, China), a coupling, two ball bearings and the shaft. For the sample container, a cylindrical PP plastic cup with sealed top and bottom side surfaces was divided into two identical halves along the side generatrix to hold samples. One side of the container was fixed by a rubber string and the other side was connected by the buckle. The sample container was located 15 mm above the center of the bottom electrode. The schematic of RF heating system and rotation system was shown in Fig. 1.

2.3. RF blanching treatment of pea samples

For heating profiles, 80 g of fresh peas were loaded into the container and placed in the RF cavity and the rotation rate of motor was 30 rpm. The motor was started 10 s before the initiation of RF to assure the steady motion of pea grains. After the first 10 s of heating, RF was stopped and sample container was taken out and placed horizontally under an infrared thermography camera (FLIR A300; FLIR Systems Inc.,

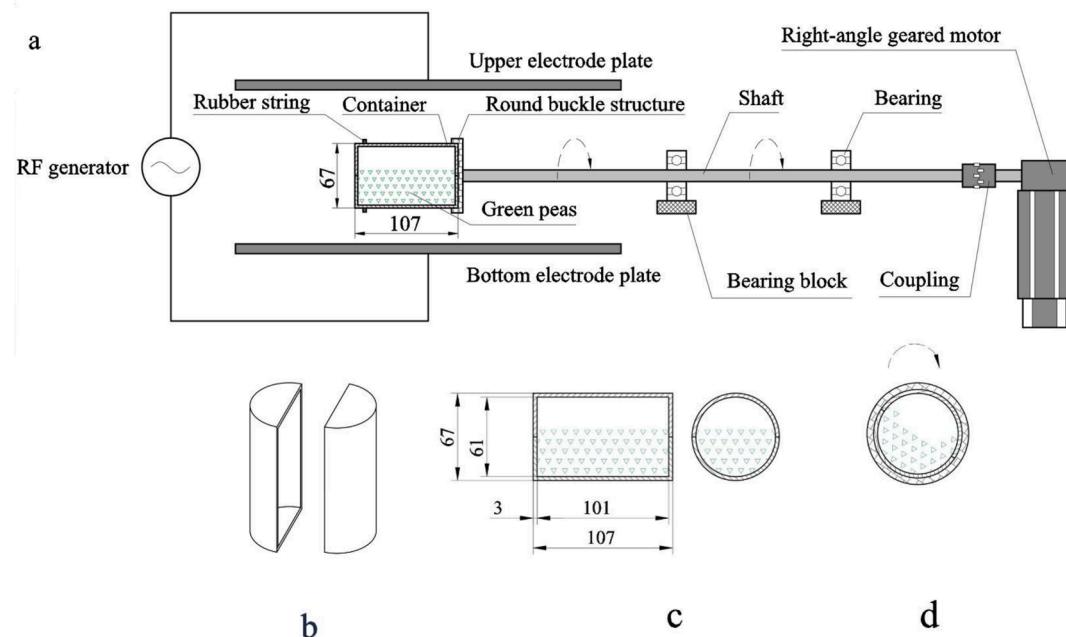


Fig. 1. Scheme of RF heating system and rotating system (a), structure of the container (b), container with pea samples (c) and rotation movement of the sample container (d) (all dimensions are in mm).

Wilsonville, OR, USA) to make the surface of pea samples parallel to the camera lens. The container was opened immediately to get the temperature distribution figures of surface pea samples. This photographing procedure took <15 s and temperature decreased <1 °C. For the average temperature of pea samples, 22,244 ~ 28,634 individual surface temperature data points were collected and calculated by the supporting software (BM_IR V7.4). Untreated fresh peas in a new container were put into RF cavity replacing the processed samples. Operations narrated above were repeated and temperature distribution was acquired after RF heating of 20 s. RF processing operations were duplicated with a 10 s extension for each heating duration until the average surface temperature reached 85 °C. Heating profiles of RF at electrode gap distance of 105, 110, 115 mm were recorded. All blanching experiments were repeated three times.

For blanching treatment, RF heating was stopped when samples reached the target temperature according to the heating profiles in Fig. 2a. Peas were quickly removed and transferred to a polypropylene bag, and cooled to room temperature in 0 °C water bath.

2.4. Extraction and assay of LOX and POD

2.4.1. LOX

Crude pea LOX was extracted by homogenizing 5.00 g pea grains with 50.00 mL of phosphate buffer solution (PBS) (0.1 M, pH 6.5, 4 °C) using a high-speed dispersing homogenizer (FJ200-S, Shanghai Suoying Instruments Co., Ltd, China) at 10,000 rpm for 3 min. The slurry was then centrifuged (12,000 g, 4 °C) for 30 min and supernatant was collected as crude enzyme extraction. The reagents used in this study were all analytical grade and all tests were repeated three times.

LOX activity of peas was determined spectrophotometrically at 234 nm (Szymanowska, Jakubczyk, Baraniak, & Kur, 2009). Before the measurement, crude enzyme solution of LOX was incubated in a water bath at 25 °C for 10 min. The reaction system was 3.0 mL consisting 2.825 mL of PBS (0.1 M, pH 6.5), 0.150 mL of crude LOX solution and 0.025 mL of 5 mM substrate solution. The absorbance value at 234 nm was recorded every 30 s over 2 min and 0.001 increase of absorbance per minute at 234 nm was defined as one unit of pea LOX activity under these conditions. In this study, relative enzyme activity (REA) was used to represent the residual enzyme activity of LOX for the pea samples treated by RF heating and the REA was calculated as Eq. (1):

$$REA = (enzyme\ activity_{treated}/enzyme\ activity_{fresh}) \times 100\% \quad (1)$$

The substrate solution of sodium linoleate was prepared by dissolving 0.5 mmol linoleic acid sodium (Aladdin, Shanghai) with 5 mL of 0.1 M NaOH solution and adjusting the ultimate concentration of sodium linoleate to 5.0 mM by deionized water. The substrate solution needed to be re-prepared before every test.

2.4.2. POD

Crude POD of pea was obtained by homogenizing 10.00 g pea grains with 50.00 mL PBS (0.1 M, pH 6.0, 4 °C) by the homogenizer at 10,000 rpm for 3 min. The pulp was centrifuged (12,000 g, 4 °C) for 30 min and the supernatant was crude pea POD solution.

Before the determination of activity, POD solution was also incubated under the same conditions of LOX. The 3.0 mL reaction system of POD included 2.25 mL PBS (0.1 M, pH 6.0), 0.050 mL crude POD solution, 0.5 mL 1.5% guaiacol solution and 0.25 mL 0.3% H₂O₂ solution (Sheu & Chen, 1991). The absorbance changes at 420 nm were recorded every 30 s over 3 min. The one unit of POD activity was defined as the 0.001 increase in absorbance per minute under these conditions and REA of POD was calculated as Eq. (1).

2.5. Weight loss

Before RF heating, the weight of unprocessed peas (W_0) was

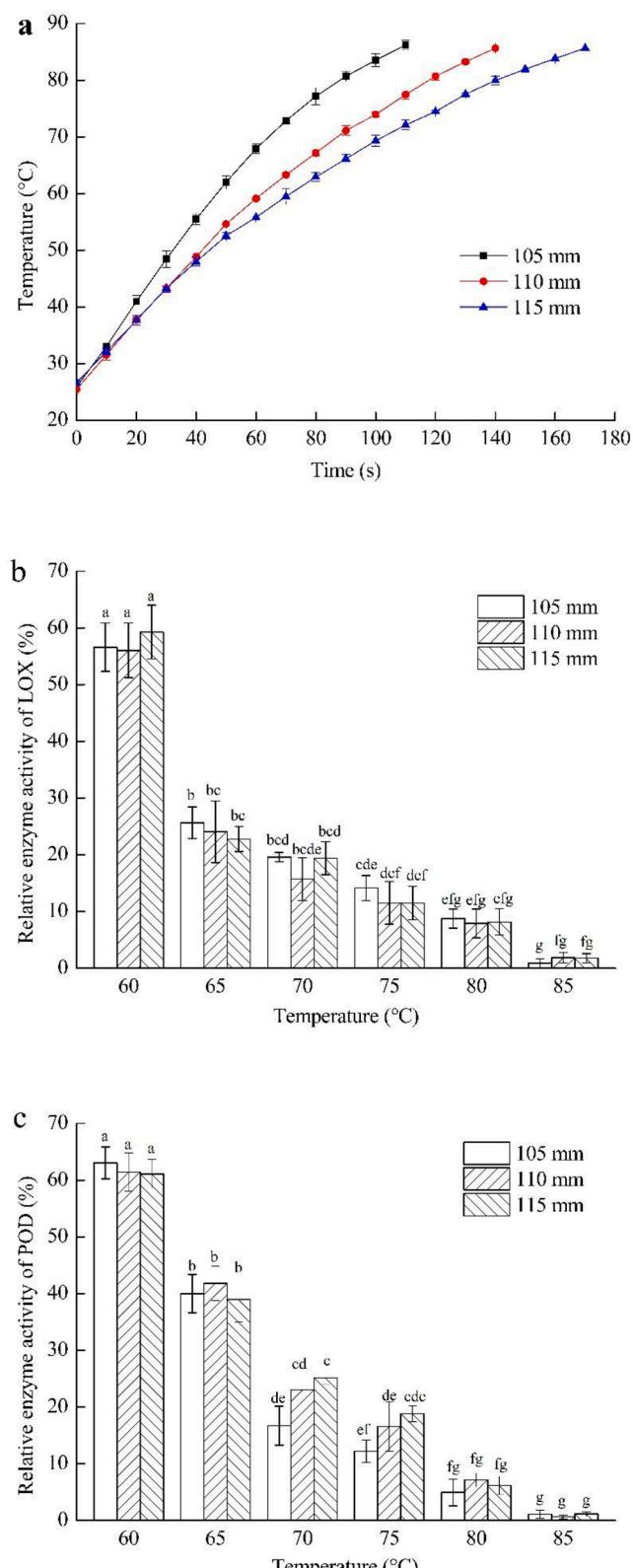


Fig. 2. Time-temperature heating profiles of peas subjected to RF heating under three electrode gaps 105, 110 and 115 mm (a), and the relative enzyme activity of pea LOX (b) and POD (c) at different temperatures under three electrode gaps. (Mean and standard deviation values were obtained by triplicate test. Values with different letter indicate significant differences $P < 0.05$ among treatments.)

recorded. After blanching treatment, the peas were cooled 25 °C and then the moisture on sample surface was wiped and dried with absorbent paper. The processed peas were weighed (W_1) again. The calculation of weight loss degree was shown in Eq. (2):

$$\text{Weight loss}(\%) = (W_0 - W_1)/W_0 \times 100\% \quad (2)$$

2.6. Color

A transmission kit (Ci7600, X-Rite Inc., USA) was used to measure the surface color of peas with and without RF heating treatment. The sampling point located at the equator of one cotyledon side of green peas. CIE spectral values were expressed in terms of L^* (lightness), a^* (redness ± greenness), and b^* (yellowness ± blueness). The total color difference (ΔE) was used to evaluate the overall change of sample color as shown in Eq. (3):

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (3)$$

where L^* , a^* , and b^* were the color of processed peas and L_0^* , a_0^* , and b_0^* were the color of fresh peas.

2.7. Texture

The texture characteristics of green peas before and after RF heating were measured by a texture analyzer (TA.XT Plus, Stable Micro system Ltd., Britain) fitted out with a 50 kg load cell. Operating parameters were as follows: a 2 mm diameter cylinder probe, compression ratio of 70% and the pre-test rate, test rate as well as post-test rate all the same of 1 mm/s. The properties of hardness, adhesion and cohesion of the pea samples were obtained.

2.8. Electrolyte leakage

The electrolyte leakage rate was measured based on the method of Fan & Sokorai (2005) with some modifications. Five grams of pea grains were incubated in 20 mL 0.35 M mannitol solution at 25 °C and gently stirred for 2 h. The electric conductivity of the bathing solution at 2 h (C_1) was measured by a conductivity meter (LE703, METTLER TOLEDO, Switzerland). Then samples were homogenized at 10,000 rpm for 3 min with the high-speed homogenizer, and the supernatant was centrifuged (12,000 g, 25 °C) to measure the total conductivity (C_0) of the sample. Electrolyte leakage was calculated as eq. (4):

$$\text{Electrolyte leakage}(\%) = C_1/C_0 \times 100\% \quad (4)$$

2.9. Ascorbic acid analysis

The ascorbic acid (AA) content in samples was calculated by the 2,6-dichloroindophenol titration method as stated in AOAC (1990). Ten grams of peas were homogenized with 40 mL oxalic acid solution (20 g/L, 4 °C). The slurry was then fixed to 50 mL by oxalic acid solution and centrifuged (12,000 g, 4 °C) for 20 min. The supernatant was collected to the dilution and all operations were carried out avoiding light.

2.10. Chlorophyll determination

Two grams of samples were ground with 20 mL ethanol:acetone (1:1 v/v) solution and stored at 25 °C for 5 h with gently shaking. The samples were then centrifuged (8,000 g, 4 °C) for 10 min, and supernatant was collected to measure the absorbance at 663 nm and 645 nm. Absorbance values of chlorophylls including chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) were extracted and calculated according to Arnon formula with coefficients given by Marr, Suryana, Lukulay, and Marr (1995) in eq. (5) and eq. (6):

$$\text{Chl } a = 12.72 \times A_{663} - 2.59 \times A_{645} \quad (5)$$

$$\text{Chl } b = 22.88 \times A_{645} - 4.67 \times A_{663} \quad (6)$$

All operations were completed in dark conditions.

2.11. Mineral analysis

Fresh and RF treated green peas were first dried to remove moisture and powdered to ensure complete digestion. Dry samples were digested by microwave digestion and then the contents of calcium (Ca^{2+}), iron (Fe^{2+}), magnesium (Mg^{2+}) and zinc (Zn^{2+}) were determined by a flame atomic absorption spectrometer (PinAAciee 900F, PE, USA) by standard curve method (Llorent-Martínez, Ortega-Vidal, Ruiz-Riaguas, Ortega-Barrales, & Fernández-de Córdova, 2020; Ikanone & Oyekan, 2014). The results were then converted to wet base.

2.12. Microstructure change

2.12.1. Cotyledon cell change

For the cell structure change of green peas before and after RF treatment, the central medulla tissue of the pea cotyledons was cut into $3 \times 1 \times 1 \text{ mm}^3$ slices and fixed in a 4% (w/v) glutaraldehyde solution for 12 h. The samples were then washed four times with PBS (0.1 M, pH 7.2) 10 min each time. The samples were immersed in 1% (w/v) OsO₄ solution for 2 h at room temperature and washed with the same PBS for three times. The samples were then subjected to gradient dehydration in 30%, 50%, 70%, 80%, and 90% (v/v) ethanol solutions in sequence with each gradient time being 10 min, and dehydration in 100% ethanol for 2 h. The samples were embedded in the 3:1, 1:1, 1:3 (v/v) ethanol: LR white mixture for 2, 8, and 12 h, respectively. Then samples were embedded in pure LR white 4 h for 3 times. Finally, the samples were embedded in LR white and fixed in an oven at 55 °C for 48 h. Fixed samples were sliced into sections (1 μm) by a microtome (Leica EM UC7, Leica Microsystems, Germany). Sections were stained in 0.05% Toluidine Blue solution and observed under a light microscope (Leica DM6 B, Leica Microsystems, Germany) with a magnification of 400X.

2.12.2. Microstructure of starch granule

The change of pea starch grains in cotyledon cell situ was observed by a scanning electron microscopy (SEM) (S-4800, Hitachi, Ltd., Japan). The medulla part of pea cotyledons was cut into $5 \times 5 \times 3 \text{ mm}^3$ slices and fixed in a 4% (w/v) glutaraldehyde solution for 12 h. The samples were then washed four times with PBS (0.1 M, pH 6.8) for 10 min each time. The samples were then sequentially dehydrated in 30%, 50%, 70%, 80%, and 90% (v/v) ethanol solutions for 15 min per gradient and three times in 100% ethanol for 30 min each time. After dehydration, vacuum drying was performed and the surface of the sample was sputter coated with gold. Samples were observed with a 10.0 kV acceleration voltage under high vacuum at a magnification of 6,000X.

2.13. Data analysis

The results reported as means and standard deviations (SD) were analyzed by ANOVA through Tukey test at a significant level of 95% using SPSS (IBM SPSS Statistics 20, IBM Inc., New York, USA).

3. Results and discussion

3.1. Effects of RF heating on pea LOX and POD activity

3.1.1. LOX activity

Fig. 2b shows changes in relative activity of pea LOX at different temperatures (60, 65, 70, 75, 80 and 85 °C) under three different electrode gaps (105, 110 and 115 mm). Temperature had significant effects ($P < 0.05$) on pea LOX activity while electrode gap exerted no significant influence ($P > 0.05$) on residual LOX activities at the same temperature. The activity of LOX decreased with increasing temperature under the

same electrode gap, and the changing pattern of LOX activity under three electrode gaps showed similarity. At 65 to 80 °C, most of pea LOX had lost vitality while residual LOX activity dropped slowly. The relative LOX activities under 105, 110 and 115 mm electrode gaps reduced to $8.76 \pm 1.66\%$, $7.90 \pm 2.54\%$, $8.16 \pm 2.31\%$ at 80 °C within 110, 140 and 170 s, respectively. The relative activity of corresponding enzymes in fruits and vegetables should generally be reduced to below 10% in order to alleviate quality deterioration during frozen storage (Bahçeci, Serpen,

Gökmen, & Acar, 2005). When heated to 85 °C, the relative activity of pea LOX decreased sharply and reduced to $0.90 \pm 0.78\%$, $1.86 \pm 0.78\%$ and $1.23 \pm 0.59\%$ at 105, 110 and 115 mm, respectively. At this temperature, LOX activity was quite low, and there was no significant difference between the three electrode gaps.

3.1.2. POD activity

The REA of pea POD after RF treatment under different electrode

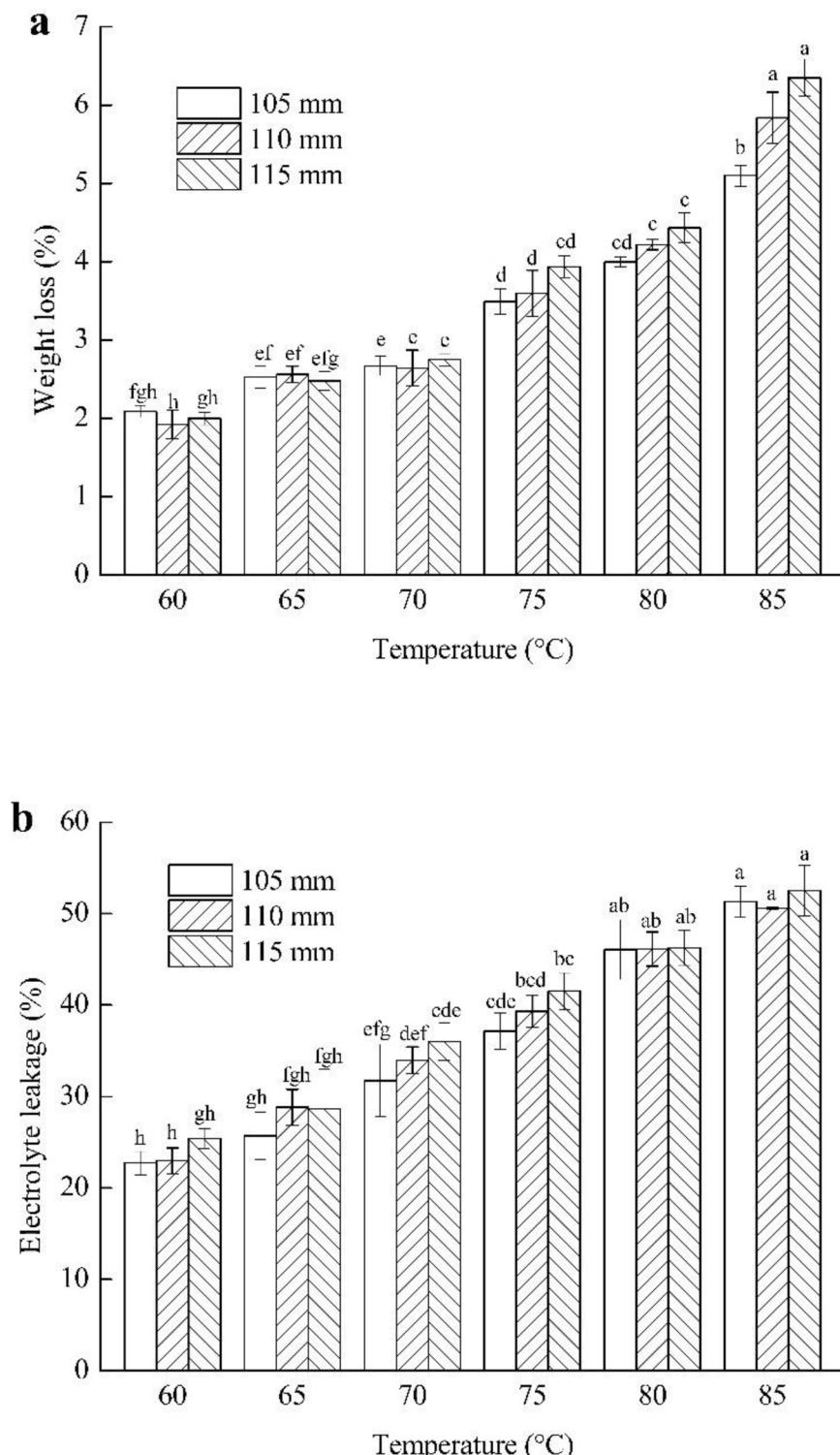


Fig. 3. Weight loss (a) and electrolyte leakage (b) of green peas at different temperatures by RF heating under three electrode gaps. (Mean and standard deviation values were obtained by triplicate test. Values with different letter indicate significant differences $P < 0.05$ among treatments.)

gaps were shown in Fig. 2c. Temperature had significant influences ($P < 0.05$) on pea POD activity and electrode gap exerted significant effect ($P < 0.05$) on POD activity at 70 °C. The residual activity of POD at 60 °C, 105 mm was $63.05 \pm 2.81\%$ and reduced to $4.97 \pm 2.36\%$ at 80 °C. When the average surface temperature got 85 °C, the residual activity of pea POD was only $1.10 \pm 0.71\%$ under 105 mm. The REA reduction degree of POD was greater under small electrode gap (105 mm). LOX and POD possessed diverse thermal resistant behaviors in different vegetables (Morales-Blancas, Chandia, & Cisneros-Zevallos, 2002; Ganthavorn, Nagel, & Powers, 1991; Güneş & Bayindirli, 1993). Bahçeci et al. (2005) found that POD in green beans was more heat resistant than LOX during water blanching and a blanching treatment at 90 °C for 3 min was needed to obtain a 90% reduction in green bean POD. It was observed in Fig. 2a that as the RF treatment continued, heating time required for a 5 °C increase in average surface temperature extended. Between 65 and 80 °C, REA of pea POD exhibited greater temperature sensitivity than LOX. The decrease of relative enzyme activity after RF heating was the result of structural damage as the thermal stability of the enzymes were destroyed by high temperature and long-term heating. At 85 °C, the relative activities of pea POD decreased to $1.10 \pm 0.78\%$, $0.63 \pm 0.35\%$ and $1.14 \pm 0.39\%$ under 105, 110 and 115 mm without significant difference.

3.2. Weight loss

Weight loss always exists in fruit and vegetable processing especially in dry blanching. The loss of material weight is mainly due to water

evaporation. The loss of weight would lead to changes in important sensory characteristics, such as shrinkage, softening or hardening of texture, and the change of color. The degree of weight loss exacerbated with increasing temperature by RF heating under three electrode gaps as shown in Fig. 3a. Both temperature and electrode gap had significant influences ($P < 0.05$) on weight loss. The weight loss of peas increased rapidly from 75 °C and reached the maximum at 85 °C. At 85 °C, the sample weight loss under electrode gap 105 mm were significantly lower than that of the other two electrode gaps and maximum weight loss $6.35 \pm 0.24\%$ was obtained under 115 mm. Electrode gap affected weight loss by prolonging or shortening processing time. Ruiz-Ojeda and Peñas (2013) considered that weight loss up to 10–15% during blanching was acceptable for industrial profitability. According to pilot study, weight loss still existed in conventional blanching due to moisture loss through cooling process and a hot water treatment of 95 °C for 70 s could result in a $3.51 \pm 0.27\%$ (triplicate) weight loss in green peas.

3.3. Color change

Color is one of the most important sensory characteristics attributing to the quality of fruits and vegetables, which directly reflects degrees of maturity, freshness and nutritional value. Proper blanching treatment could bright sample color and slow pigment deterioration of products during storage. The color values and total color difference of fresh peas and RF processed samples were shown in Table 1. The L^* values of pea samples with RF heating was lower than that of untreated samples, which meant the brightness of pea grains abated after RF treatment.

Table 1

Color and texture values (mean \pm SD) of fresh and processed pea samples treated by RF heating at three electrode gaps (105, 110, and 115 mm) and six temperatures (60, 65, 70, 75, 80, and 85 °C).

Electrode gap (mm)	Temperature (°C)	Color				Texture		
		L^*	a^*	b^*	ΔE	Hardness (g)	Adhesiveness	Cohesiveness
Control 105	25	85.67 ± 0.84^a	-14.84 ± 1.00^a	51.34 ± 1.18^{ef}	—	925.25 ± 72.73^a	-362.42 ± 51.45^e	0.30 ± 0.03^a
	60	77.97 ± 1.28^{cde}	-18.18 ± 1.08^{bc}	51.74 ± 2.08^{def}	8.62 ± 1.59^{ik}	762.61 ± 88.97^{ab}	-76.03 ± 10.11^{cd}	0.27 ± 0.05^{ab}
	65	78.97 ± 2.61^{bcde}	-20.61 ± 1.22^d	53.32 ± 2.27^{def}	9.59 ± 1.00^{ijk}	657.50 ± 43.91^{bc}	-42.96 ± 13.05^{abcd}	0.21 ± 0.05^{abc}
	70	79.65 ± 1.26^{bcde}	-24.32 ± 1.31^{ef}	54.77 ± 2.50^{cdef}	12.00 ± 1.53^{fghi}	517.96 ± 7.60^{cdef}	-21.91 ± 1.69^{abcd}	0.21 ± 0.03^{abc}
	75	78.82 ± 1.59^{bcde}	-25.04 ± 0.99^{fgh}	56.80 ± 3.21^{cd}	13.77 ± 1.72^{defg}	406.18 ± 50.67^{fg}	-14.73 ± 8.42^{abc}	0.19 ± 0.07^{abc}
	80	79.53 ± 3.20^{bcde}	-25.31 ± 1.12^{fgh}	58.57 ± 3.81^{bc}	14.84 ± 1.27^{bcdef}	385.45 ± 22.03^{fg}	-10.13 ± 1.68^{ab}	0.17 ± 0.05^{bc}
110	85	81.47 ± 2.14^{abc}	-25.94 ± 1.04^{fgh}	64.37 ± 2.21^a	17.81 ± 1.76^{ab}	335.23 ± 9.12^g	-8.66 ± 1.33^a	0.12 ± 0.02^c
	60	78.87 ± 2.33^{bcde}	-16.07 ± 0.60^{ab}	51.89 ± 2.42^{def}	7.46 ± 1.59^j	760.33 ± 106.32^{ab}	-83.94 ± 12.42^d	0.26 ± 0.01^{ab}
	65	77.56 ± 2.36^{cde}	-19.14 ± 0.65^{cd}	52.62 ± 3.13^{def}	9.64 ± 1.28^{ijk}	660.48 ± 67.00^{bc}	-72.31 ± 3.98^{bcd}	0.26 ± 0.01^{ab}
	70	78.53 ± 1.77^{bcde}	-20.56 ± 0.65^d	54.48 ± 2.70^{cdef}	10.02 ± 1.29^{hijk}	605.63 ± 45.04^{bcde}	-34.33 ± 5.17^{abcd}	0.21 ± 0.06^{abc}
	75	79.51 ± 1.46^{bcde}	-23.29 ± 1.31^e	55.62 ± 1.56^{cde}	11.45 ± 1.44^{ghij}	475.32 ± 87.98^{defg}	-22.24 ± 5.20^{abcd}	0.20 ± 0.08^{abc}
	80	80.49 ± 3.59^{bcd}	-24.83 ± 1.53^{efg}	59.17 ± 3.85^{bc}	14.47 ± 2.11^{cdefg}	453.73 ± 53.58^{efg}	-20.69 ± 2.50^{abc}	0.21 ± 0.07^{abc}
115	85	79.81 ± 2.38^{bcde}	-25.38 ± 0.72^{efgh}	63.04 ± 2.41^{ab}	17.06 ± 1.43^{abc}	397.77 ± 16.50^{fg}	-8.05 ± 1.65^a	0.17 ± 0.01^{bc}
	60	76.47 ± 2.047^{de}	-19.05 ± 1.28^{cd}	50.07 ± 2.02^f	10.48 ± 1.79^{hijk}	747.28 ± 66.01^b	-60.49 ± 7.33^{abcd}	0.24 ± 0.03^{abc}
	65	78.33 ± 2.068^{bcde}	-24.51 ± 0.69^{ef}	54.79 ± 1.83^{cdef}	12.87 ± 0.96^{efgh}	635.26 ± 54.69^{bcd}	-39.19 ± 9.03^{abcd}	0.23 ± 0.06^{abc}
	70	75.92 ± 0.40^{de}	-25.74 ± 0.65^{fgh}	55.50 ± 0.59^{cde}	15.22 ± 0.52^{bcde}	609.24 ± 46.82^{bcde}	-32.07 ± 6.64^{abcd}	0.20 ± 0.03^{abc}
	75	76.00 ± 0.80^{de}	-26.49 ± 0.32^{fgh}	56.44 ± 2.23^{cde}	16.09 ± 0.89^{bed}	411.37 ± 48.09^{fg}	-16.73 ± 3.08^{abc}	0.19 ± 0.03^{abc}
	80	80.21 ± 1.34^{bcde}	-26.95 ± 0.17^{gh}	62.68 ± 1.29^{ab}	17.53 ± 0.55^{abc}	391.43 ± 88.64^{fg}	-8.34 ± 3.82^a	0.18 ± 0.06^{bc}
	85	82.62 ± 2.55^{ab}	-27.27 ± 1.11^h	66.16 ± 2.19^a	19.81 ± 1.47^a	322.93 ± 35.39^g	-7.26 ± 1.85^a	0.17 ± 0.03^{bc}

The mean and standard deviation values were obtained by triplicate test.

Values in the same column with different letter indicate a significant difference ($P < 0.05$) among treatment

Through RF processing, L^* values slightly increased in the later heating stage but were still lower than the fresh samples. With the increase of temperature, a^* values of pea samples decreased rapidly, while b^* value increased. The a^* value represented the red-green color characteristic of samples as reduction in a^* value indicated that green was getting more obvious through the heating process. The b^* value represented the yellow-blue value of the sample color, and the increase in b^* value of the pea samples was related to the composite result of green effect on yellow and blue. The increase in green color values was owing to outflow of chlorophylls caused by the heat damage to cell membrane and matrix structure. Blanching could expel gas between plant tissues and evaporate moisture, and thus would condense the color of peas. In this study, the value of ΔE of all treated samples was higher than 3.0 indicating that total color difference of peas after RF heating compared to untreated fresh samples was distinguishable by eyes (Borneo, Alba, & Aguirre, 2016).

3.4. Texture change

Texture characteristics are important indexes manifesting taste quality of products. Blanching can soften tissue structure and facilitate subsequent processing by destroying cell structure and intercellular substance. Table 1 lists parameters of green pea tissue texture before and after RF blanching treatments. Both temperature and electrode gap exerted significant effects ($P < 0.05$) on hardness, adhesiveness and cohesiveness. Compared with fresh samples, the hardness of peas decreased rapidly with the increase of temperature under the same electrode gap as a result of β -elimination depolymerization of pectins (Ruiz-Ojeda & Peñas 2013). Most of the hardness had decreased when heated to 75 °C and after 75 °C, the rate of hardness changed slowed down. The adhesiveness of peas increased sharply before and after RF treatment, and the increase rate slowed down in following RF heating. The cohesiveness of peas decreased by RF treatment. Both adhesiveness and cohesion changes occurred in the early stage of RF heating. The change of texture characteristics was the result of multiple factors including water loss, cell wall dissociation, calcium-pectin gel dissolution and starch granule swelling etc. (Thybo, Martens, & Lyschede, 1998; García-Segovia, Andrés-Bello, & Martínez-Monzo, 2008).

3.5. Relative electrolyte leakage

The rate of electrolyte leakage was used to assess impairing of RF heating to the cell membrane of peas. Fig. 3b shows the electrolyte leakage of green peas heated to different temperatures under three electrode gaps. Temperature had significant effects ($P < 0.05$) on the leakage of pea electrolyte. The leakage rates of electrolyte increased as temperature rose. When the temperatures reached 85 °C, the leakage rate of electrolyte reached 51.32 ± 1.71%, 50.62 ± 0.11% and 52.55 ± 2.77% at 105, 110 and 115 mm, respectively. Preliminary test showed that conventional blanching of 95 °C for 70 s could result in 50.53 ± 2.67% (triplicate) leakage rate in green peas. Electrode gap had no significant influence ($P > 0.05$) on electrolyte leakages at the same temperature, which indicated that heating rate showed relatively little effect on the cell membrane functionality. In healthy living cells, the cell membrane with certain fluidity could control the inflow of nutrients and outflow of internal substance (Fan & Sokorai, 2005). Lipid and protein were the main components of cell membrane and thermal heating could lead to the change of lipid phase transition, protein conformation, and increase in membrane permeability (Bischof et al., 1995). Moreover, the destruction of cell wall and intercellular substance facilitated the outflow of cell contents and increased the electrolyte leakage.

3.6. Effects of RF heating on ascorbic acid, chlorophyll and mineral contents

Ascorbic acid is an important antioxidant index of fruits and

vegetables. The loss of ascorbic acid content during the blanching process has been used to evaluate heating efficiency. The heating process could lead to destruction of cell membrane and resulted in the degradation of chlorophyll to demethylchlorophyll, causing yellowness and dimness of green vegetables (Koca, Karadeniz, & Burdurlu, 2007). Mineral loss of peas could also indicate the outflow of nutrients and destruction of cell membrane. Table 2 compares ascorbic acid, chlorophyll and mineral element contents in fresh peas and blanched pea samples at the end point of RF heating (85 °C) under three different electrode gaps. RF assisted blanching under all three electrode gaps caused significant influences ($P < 0.05$) in AA content. The AA content suffered the most significant loss at electrode gap 115 mm. Compared with fresh peas, the chlorophyll contents of peas after RF heating decreased slightly, but there was no significant difference ($P > 0.05$) in chlorophyll contents after each treatment indicating RF dry blanching as an efficacious way to preserve chlorophylls and color of green peas. Comparisons of mineral contents including calcium, iron, magnesium and zinc between fresh and RF processed samples demonstrated that RF heating could well retain these four elements. Furthermore, there were slight increases in mineral contents in RF treated samples, which could be owing to the wet weight divergence caused by weight loss. Results in table 2 illustrated that RF dry blanching had good retention for nutrients and electrode gap with fast heating rate and efficiency should be selected for RF blanching.

3.7. Microstructure of pea cotyledon cells

Fig. 4a shows the morphological changes in fresh and RF treated pea cotyledon cells under the electrode gap 110 mm. Cells in fresh pea cotyledon were ellipsoidal and plump stuffed with large starch granules. Cells connected with each other were tightly packed and cell walls were intact with uniform thickness. At 60 °C- 70 °C, cell walls were slightly thinned and fractured, while large starch granules started to disintegrate. As indicated by results of weight loss and electrolyte leakage, cell turbulence which maintained the mechanical morphology of fresh cells with cell wall had been affected. After 70 °C, the starch granules gradually disappeared and starch gelatinized violently causing the cells to be filled with starch paste. At 85 °C, there were still a few small starch particles in the center of gelatinized starch paste, which indicated that the in-situ starch was not fully gelatinized at the end of RF heating. In the meanwhile, huge cracks and reduced thickness in cell walls were clearly observed. The original contour of cells was still intact but intercellular space enlarged. The gluey pectin layer affords major

Table 2

Contents (mean ± SD) of ascorbic acid, chlorophyll (Chl *a* and Chl *b*) and mineral contents (Mg, Ca, Zn) of fresh and RF blanched pea samples under three electrode gaps at 85 °C.

Electrode gap (mm)	Ascorbic acid (mg/100 g)	Chlorophylls (mg/100 g)		Minerals (mg/100 g)			
		Chl <i>a</i>	Chl <i>b</i>	Ca ²⁺	Fe ²⁺	Mg ²⁺	Zn ²⁺
		Control	28.96 ± 0.71 ^a	9.71 ± 0.30	3.73 ± 0.68	29.34 ± 1.94	1.21 ± 0.12
105	25.92 ± 0.39 ^b	9.27 ± 0.56	3.57 ± 0.31	26.03 ± 0.64	1.24 ± 0.01	29.23 ± 0.22	0.89 ± 0.01
		0.31 ^b	0.36	0.06	0.07	30.42 ± 1.26	0.92 ± 0.04
110	24.98 ± 0.31 ^b	9.45 ± 0.36	3.31 ± 1.40	27.07 ± 1.40	1.36 ± 0.07	28.90 ± 1.26	0.88 ± 0.04
		0.31 ^b	0.36	0.06	0.07	30.42 ± 1.26	0.92 ± 0.04
115	22.19 ± 0.76 ^c	9.70 ± 0.18	3.75 ± 0.32	25.33 ± 1.62	1.41 ± 0.02	28.90 ± 0.76	0.88 ± 0.03
		0.76 ^c	0.18	0.32	0.02	28.90 ± 0.76	0.88 ± 0.03

The mean and standard values were obtained by triplicate test.

Values in the same column with different letter indicate a significant difference ($P < 0.05$) among treatment.

No significant change exists in chlorophyll and mineral contents.

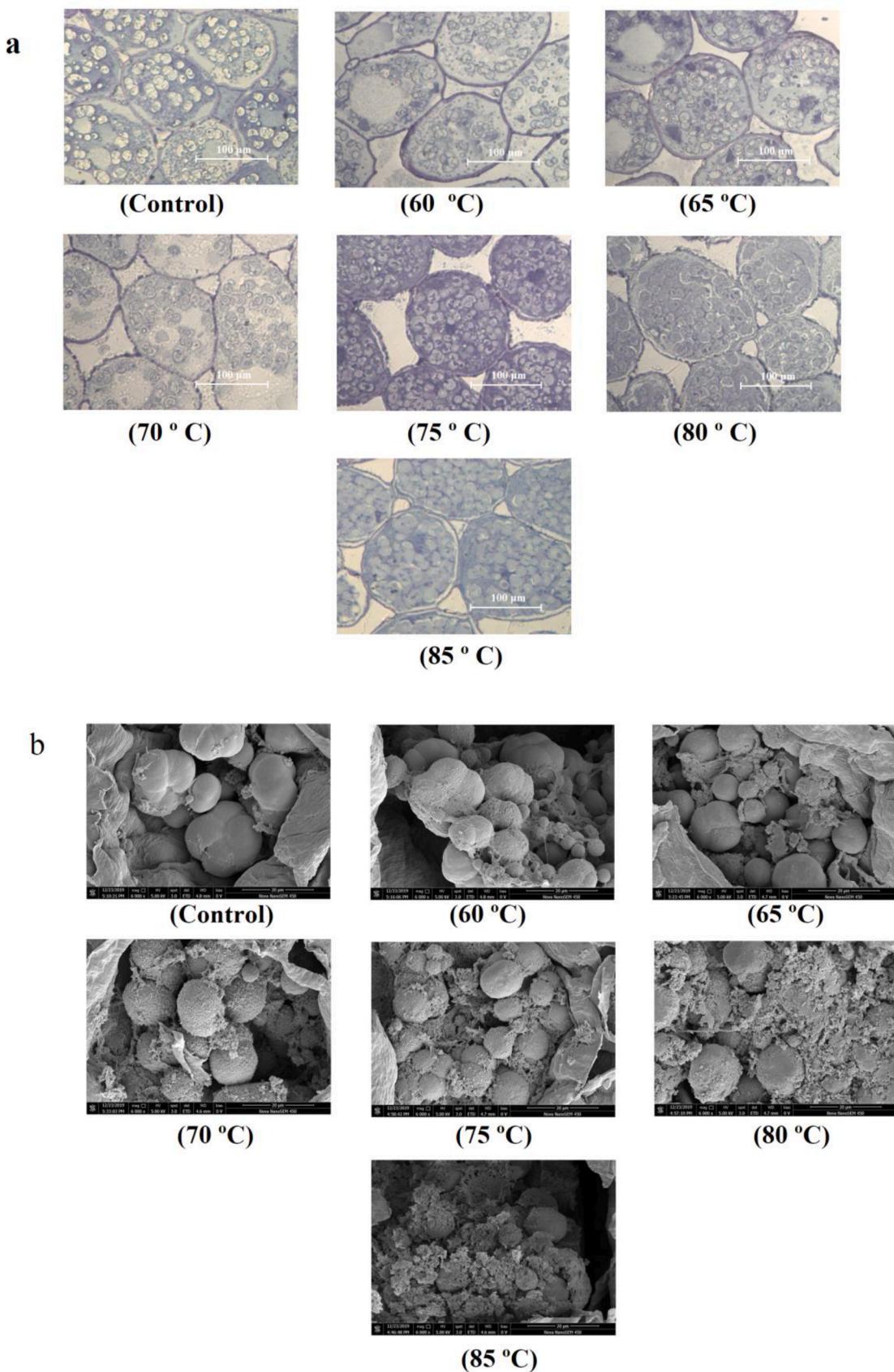


Fig. 4. Light microscope images of cotyledon cells (a) and SEM images of starch granules in-situ (b) of fresh and RF treated green peas at different temperatures under the electrode gap 110 mm.

responsibility for the adhesion of cells with cell wall polysaccharides. Decomposition of pectin reduced intercellular adhesion and destroyed the cell wall which eventually resulted in tissue texture change (Yao et al., 2020). Compared with pectin, hemicellulose and cellulose had less changes in the processing of fruits and vegetables (Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011), but the destruction of hemicellulose-cellulose structure caused cell wall network disruption and cell collapsed. Rupture and dissolving of cell wall fibers together with the degradation of pectin contributed to softness of pea cells. Moreover, swelling pressure of starch granules could also resulted in soft texture during blanching (Liu & Scanlon, 2007). The changes in these microstructures due to RF effect on pea cell wall and protoplast presented further influence on color, texture, and electrolyte leakage rate of green peas.

The SEM images of starch granules in pea cotyledon cells before and after RF treatment at 110 mm were shown in Fig. 4b, which further demonstrated the changes of pea starch granules with increasing temperature. The results were consistent with the characteristic changes observed by optical microscopy. In fresh peas, large starch granules were smooth-surfaced splitting ellipsoid with a small amount of protein chain attached and small spherical starch granules could also be clearly observed. When heated to 65 °C, the small starch granules had gelatinized, and the surface of the large starch granules had a slight swelling change. At 70 °C, the surface of all starch granules disintegrated indicating the trend of swelling and gelatinization begun to aggravate with increasing temperature. Zhang et al. (2018) also found that starch in RF heated potato cuboids began to gelatinize severely after 75 °C. At 85 °C, a small number of small starch granules were wrapped in the gelatinized starch paste. Incomplete gelatinization of pea starch might due to water deficiency or short heat preservation time (Srikaeo, Furst, Ashton, & Hosken, 2006). Compared with the extracted pure starch, the gelatinization temperature of pea starch in situ exhibited a certain level of delay (Ratnayake, Hoover, Shahidi, Perera, & Jane, 2001).

4. Conclusions

Results revealed that pea LOX and POD were inactivated effectively by RF heating. Significant changes happened in weight loss, color, texture, electrolyte leakage, nutrient contents and microstructure of green peas during RF blanching process and change degrees aggravated with the increase of temperature. The relative activity of pea LOX and POD decreased with increasing temperature under all three electrode gaps while LOX and POD exhibited different thermal resistance and heat sensitivity. Different loss levels happened in ascorbic acid, chlorophyll and mineral contents after RF processing with three electrode gaps. Microstructure analysis showed that pea cell still preserved its shape, as cell wall disintegrated and starch granules gelatinized through RF heating. This study provides meaningful date for RF dry blanching treatment of vegetables to inactivate enzymes and slow quality deterioration.

CRediT authorship contribution statement

Caiyue Zhang: Conceptualization, Methodology, Investigation, Software, Data curation, Writing - original draft. **Chenchen Hu:** Data curation, Formal analysis. **Yanan Sun:** . **Xueying Zhang:** Software. **Yequn Wang:** Supervision. **Hongfei Fu:** Supervision. **Xiangwei Chen:** Supervision. **Yunyang Wang:** Writing - review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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