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EFFECT OF FUZZY-CONTROLLED SLOW FREEZING ON PUMPKIN (*CUCURBITA MOSCHATA* DUCH) CELL DISINTEGRATION AND PHENOLICS

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ABSTRACT
Freezing has been widely used to preserve vegetables including seasonal pumpkins. This work aimed to investigate the effects of freezing on pumpkin cell disintegration and phenolics. A fuzzy logic control (FLC) system was built to obtain better temperature control of the freezing system. Changes in cellular disintegration, electrical conductivity and phenolics content were evaluated. The angle measure technique and principal component analysis were used to delineate the surface texture changes of the frozen pumpkin cells. The results showed that FLC offered reliable temperature control performance. Freezing at -18 °C for 7 h caused the highest cell degradation of 0.467 on the disintegration scale. Decomposition was also indicated by an almost double increase in electrical conductivity. The changes in texture were accurately reflected in the mean angle spectra and 81.3% and 7.4% of the variability due to treatments could be explained by two principal components respectively. Freezing pumpkin at -18 °C for 6 h correlated to the maximum increase in total phenolics of 70.44%. The increased phenolics were dominated by caffeic acid, chlorogenic acid and p-coumaric acid. In conclusion, as the freezing system exhibits positive effects on the phenolics content of pumpkin, it may be employed to process seasonal pumpkin to obtain higher value from the produce.

Keywords: pumpkin; freezing; fuzzy; disintegration; phenolic

INTRODUCTION
Pumpkins can be cultivated in warm areas around the world **(Kumar, Rattan and Samnotra, 2016)**, are cheap to grow and provide a high nutrient content **(Provesi and Amante, 2015)**. The valued nutrients of pumpkin include phenolic compounds ranging from 4.44 to 5.65 mg GAE/g dry matter **(Mendelova, et al., 2017)**, flavonoids (4.4 mg/100 g) and anthocyanins (0.14 mg/100 g) **(Oloyede, et al., 2012)**.

The most frequently used preservation method for fresh food from living tissue or cellular food materials is freezing **(Li, Zhu and Sun, 2018)**. Freezing causes plant cells to undergo cellular disintegration related to dehydration and mechanical damage mechanisms. These allow cells to lose water by diffusion, leading to irreversible shrinkage until the cells collapse **(Fellows, 2009)**. The migration of intracellular water to form ice crystals in the extracellular area results in intracellular dehydration and an increase in the ionic strength of the cell. The mechanical damage to cells results from membrane distortion and stress on rigid structures **(Zaritzky, 2011)**. Freezing of plant foods enhances the release of phytochemicals due to the extraction effects induced during the process **(Leong and Oey, 2012)**.

The effect of processing on food structure can be precisely assessed by imaging of the food surface. Within the food image, information about the surface of food and cells is stored as an array of pixels of different intensities to form the image texture **(Quevedo, et al., 2002)**. Using the angle measure technique (AMT), a raw image is converted to a one-dimensional signal, then a number of points are selected along the signal point and the mean angle (MA) at each selected point is calculated. This step is repeated to reach the last point, to generate an MA spectrum of the image. The MA spectra obtained from images of various treatments are then used for principal component analysis (PCA) to obtain image separation **(Fongaro and Kvaal, 2013)**.

There is scarce information on the effect of slow freezing on the cellular integrity and phenolics content of pumpkin. Based on our preliminary study, temperature controls of the currently available household freezers employ a conventional thermostat with which a precise freezing temperature is difficult to achieve. Among other control systems, fuzzy logic control (FLC) is regarded as an updated approach which does not require a precise model to build and would improve refrigeration efficiency **(Saleh and Aly, 2015)**. Furthermore, FLC is considered an intelligent control system that well accommodates the implementation of heuristic knowledge of a system **(Jantzen, 2013)**. This study therefore aimed to elaborate the

effect of fuzzy-controlled slow freezing on pumpkin cellular structures and phenolic compounds.

Scientific hypothesis

Fuzzy-controlled freezing increases pumpkin cell disintegration and phenolics content.

MATERIAL AND METHODOLOGY

Pumpkin samples

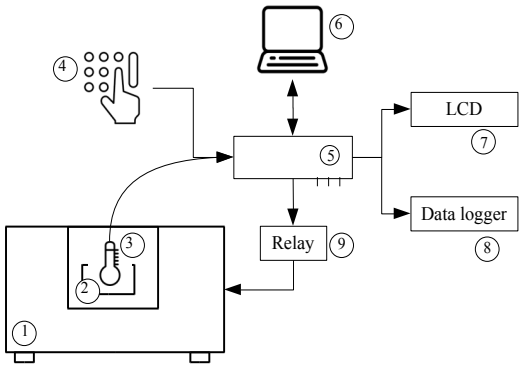
Pumpkin fruits (*Cucurbita moschata* Duch) were obtained from a local market. Confirmation of the correct species was obtained from an expert at the Materia Medica government herbal centre. The fruits were chosen based on the following conditions: fresh, clean, correct maturity stage, free from apparent physical defect and biological spoilage, and weighing approximately between 2.5 and 4.0 kg each. The fruits were kept under controlled conditions within the recommended temperature range of 7 – 13 °C **(Raju, Chauhan and Bawa, 2011)** and RH of 50 – 70% **(Maynard and Hochmuth, 2007)**. A data logger (Extech RHT20, FLIR Systems, Inc., Massachusetts, USA) was used to monitor the humidity and temperature. The actual temperature and RH range during experiments were 10.3 – 10.8 °C and 48 – 68.4% respectively. The fruit exocarp, fibrous strand and seeds were removed, and the pulp was obtained by cutting the fruit following the rib.

For freezing experiments, samples were cut into 2 cm x 2 cm x 3 cm pieces weighing 250 g. For the cell damage experiments, the pumpkins were prepared in the form of a cylinder 1 cm in diameter and 3 cm in length obtained using a stainless steel laboratory borer. Dried pumpkin powder for determining total phenolics content and liquid chromatograph mass spectrometer (LCMS) analyses was prepared by slicing of frozen samples after complete thawing for 2 h at room temperature. The samples were then dried at 55 °C in a hot air-drying oven (FDH6, Maxindo, Indonesia) for 24 h. The samples were finally ground (Philips HR2116, Indonesia) and sieved (Retsch 5657, Germany) at mesh 50 to obtain uniform particles with a maximum size of 300 μm. The powdered samples were vacuum-sealed and stored in a dark container at room temperature for analysis.

Development of fuzzy-controlled freezer

A new household chest freezer (Sansio SAN-153F, China) was used to build the fuzzy-controlled freezing system. The freezer was an air-stagnant type designed for a tropical climate with a capacity of 153 L, R134a cooling medium and cyclopentane insulation. A microcontroller-based FLC was developed to control the temperature and freezing time during the experiment. The design of the freezing system used in the experiment is presented in Figure 1.

The FLC was designed to operate based on two input variables, namely temperature error and delta error, and pulse width modulation (PWM) as the output variable. The temperature error was obtained from the difference between temperature setting and the temperature of food samples. Temperature measurement was carried out by inserting a three-wire WZP-187 PT100 sensor (accurate to ±0.01 °C) into the samples placed on the hanging basket inside the freezer compartment in approximately the geometric centre.



Note: 1. chest freezer, 2. pumpkin sample, 3. temperature sensor, 4. key input, 5. microcontroller, 6. computer, 7. LCD monitor, 8. data logger, 9. solid state relay.

Figure 1 Design of the fuzzy-controlled freezing system.

The temperature difference between two consecutive measurements was assigned to the delta error variable. The input variables were then fuzzified based on their type and range which were determined based on trial-and-error experiments in the initial FLC development. The variables and degree of membership (μ) used to develop the FLC are presented in Figure 2.

The Takagi–Sugeno–Kang method was used during the defuzzification step to obtain PWM values based on 18 rules (Table 1) to produce output values which were then directed to the freezer compressor. A Crydom solid state relay D2450-10 with attached ULN2803A integrated circuit was employed to control the inductive load of the relay. The temperature was read at 1 s intervals and logged using a Deek Robot logging shield.

A computer program was written to implement the developed system and uploaded onto an ATmega2560 Arduino microcontroller using a personal computer. The fuzzy inference system modelling of MATLAB® R2017a was used to help design and simulate the fuzzy logic system. The difference between the PWM output of the modelled and actual systems was expressed as the mean absolute percentage error. Before experiments, the PT100 sensor was calibrated by comparing the resistance of the sensor and the value on the data sheet using 30 temperature points ranging

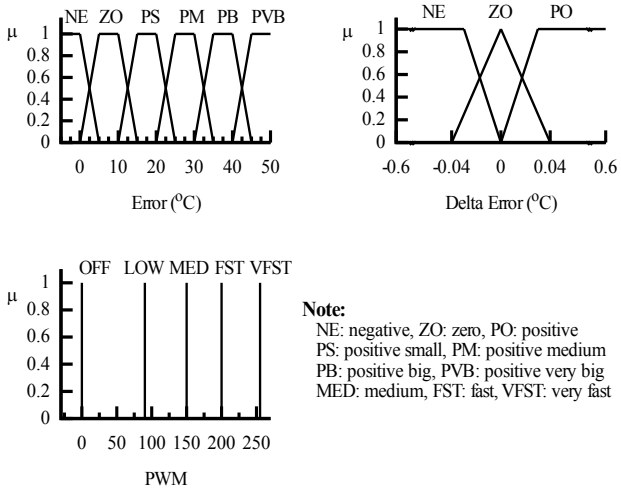


Figure 2 Variables of the fuzzy-controlled freezing system.

Table 1 Rule base of the control system.

Error	Delta error		
	Negative	Zero	Positive
Negative	Off	Off	Off
Zero	Low	Medium	Medium
Positive small	Low	Medium	Medium
Positive medium	Medium	Medium	Fast
Positive big	Medium	Fast	Fast
Positive very big	Fast	Very fast	Very fast

from 3 to -15 °C. The developed system was eventually calibrated using a Fluke 724 Calibrator (Fluke Corporation, USA) and method AS 2853:1986 at the Central Laboratory of Science and Engineering, University of Brawijaya, Indonesia.

Freezing experiments

The effects of freezing on pumpkin tissue were investigated at temperatures starting from -3 °C for 4 to 8 h. The starting freezing temperatures for the experiments were determined based on the initial freezing point of pumpkin which was ascertained during pre-experiments using the cooling curve method **(Rahman, et al., 2009)**. For this purpose, the empty freezer was run at a medium temperature setting to reach a stable temperature for at least 2 h. Then, the temperature sensor was inserted into a 200 g sample approximately at its centre to approach the coolest point. The sample was then placed on a 15 mm thick Styrofoam pad in the freezer compartment and allowed to freeze **(Bainy, Corazza and Lenzi, 2015)**. The changes in temperature were recorded on a personal computer and plotted using Gnuplot version 5.2. The process was stopped when the temperature reached the end freezing point. Based on the logged data, a cooling curve was drawn, then the initial freezing and end freezing temperatures were determined based on the zero slope of the starting plateau and end of the plateau on the curve respectively. The freezing rate was determined based on the time required by the coolest sample area to decrease from 0 to -18 °C **(Pham, 2014)**.

Cell disintegration experiments

The effect of treatment on pumpkin cell damage was estimated by measuring the electrical conductivity (EC) of samples with stirring using a CON 700 meter (Oakton Instruments, Singapore). The cell disintegration index Z was calculated using the formula $Z = (\sigma - \sigma_i)/(\sigma_d - \sigma_i)$, where σ is the EC of the treated sample, and σ_i and σ_d refer to the conductivity of intact and maximally damaged cellular samples respectively **(Vorobiev and Lebovka, 2009)**. The latter value was obtained from the EC of thermally processed samples (85 °C, 15 min) to represent the maximum disintegration of the samples **(Maskooki and Eshtiaghi, 2012)**. The samples (1.0 cm in diameter and 3 cm in length) were vacuum-packed in an embossed polyethylene bag to avoid leaching **(Leong and Oey, 2012)** and immersed in hot water at 60 and 80 °C for 15 min. The EC measurements were carried out at a controlled room temperature of 22 °C. The prepared samples were immersed in 100 mL of distilled water in a beaker after the samples reached room temperature.

Microstructure analysis

Samples for microstructure analysis were prepared using the air-drying preparation method **(Pathan, Bond and Gaskin, 2010)**. Pumpkin samples of 20 mm x 15 mm were cut to approximately 1 mm thick using a B Braun 22 scalpel and allowed to dry slowly at room temperature for 12 – 24 h. Images of the prepared samples were then acquired using a scanning electron microscope (SEM) (FEI™ Inspect S50, USA). Before imaging, the dried samples were sputter-coated (Emitech SC7620 Sputter Coater, UK) with a thin layer of gold–palladium (approximately 10 nm, 5 mA, 180 s) at room temperature and a pressure of 10^{-2} Pa. The samples were observed at an acceleration voltage of 15 kV, and difference magnifications were used to obtain images.

Images obtained from the SEM machine were saved at a resolution of 34 pixels per cm (34 bits per pixel) in tagged image file format (TIFF). The complexities of image textures were characterized using the AMT method **(Kvaal, et al., 2008)**. The image processing was carried out using ImageJ software version 1.52p **(Schneider, Rasband and Eliceiri, 2012)** with AMT plugin. The setting values for ATM algorithms were maximum scale 500 pixels, method of unfolding spiral, unfolded pixels start from 0, and 500 sampling points.

Total phenolics content (TPC)

Approximately 0.5 g of dried pumpkin samples was added to 20 mL of 95% ethanol and macerated for 24 h in the absence of light at room temperature. The extracts were then filtered through a Whatman filter paper and stored in a brown bottle before analysis. TPC was estimated using the Folin–Ciocalteu method **(Alara, Abdurahman and Ukaegbu, 2018)**. The TPC in the pumpkin samples was calculated based on the standard curve of gallic acid constructed over a concentration range of 0 to 50 mg/L. The resulting regression line was y = 0.0044x + 0.093; R^2 = 0.9876, where y was the absorbance at 765 nm and x was the concentration from the calibration points. Results were reported as gallic acid equivalent in milligrams per gram of dried samples (mg GAE/g dw).

LCMS analysis

LCMS analyses were performed on untreated pumpkin and samples frozen at -18 °C for 6 h which exhibited the highest TPC. The TPC extracts were further filtered (0.45 μm) and analysed **(Zdunic, et al., 2016)**. The analyses were carried out using a Shimadzu LCMS - 8040 LC/MS equipped with a Shimadzu column Shim Pack FC-ODS (2 mm D x 150 mm, 3 μm) (Shimadzu Corporation, Japan), and run with the following settings: injection volume 1 μL; capillary voltage 3.0 kV; column temperature 35 °C; flow gradient 0/0 at 0 min, 15/85 at 5 min, 20/80 at 20 min and 90/10 at 24 min; flow rate 0.5 mL/min; sampling cone 23.0 V; MS ion type [M]+; collision energy 5.0 V; desolvation gas flow of 6 L/h and temperature of 350 °C; low energy CID fragmentation; ESI ionization with scanning of 0.6 s/scan (*m/z*: 10 – 1000); source temperature 100 °C; and 80 min run time. Ethanol (95%) and water were used as solvents. Curve areas on chromatograms of untreated and treated samples were then compared to determine the individual phenolic changes due to freezing.

Statistical analysis

The average means of the response variables were compared using a one-way analysis of variance test. Pearson correlation was used to determine correlation between the disintegration index and EC of samples. PCA was run to identify the variability of surface texture due to freezing. All statistical tests were carried out using the R software environment for statistical computing, version 3.6.2 **(R Core Team, 2018)** at $p < 0.05$.

RESULTS AND DISCUSSION

FLC performance

The system calibration report showed that the temporal and spatial variations of the freezer were within acceptable values of -1.0 and 1.9 °C respectively. During the performance test at a setting temperature of -10 °C, the freezer demonstrated an average rise time of 21.38 ±2.40 min, settling time of 31.36 ±1.29 min, and steady error positive and negative of 1.55 ±0.11 and -1.28 ±0.33 °C respectively. The small temperature errors could be used as evidence for the high performance of fuzzy-controlled refrigeration systems **(Cheung and Kamal, 1997)**. Other related data that support the system's robustness include the strong correlation between actual and theoretical resistance values ($y = 1.0001x - 0.0211$; $R^2 = 0.9996$) and the small mean absolute percentage error value of the system (9.52%).

Based on the freezing rate of pumpkin samples in the experiment (4.2 °C/h) as shown in Table 2, the developed freezing system can be classified as slow freezing. This process is typically characterized by a freezing rate of 1 – 10 °C/h **(Brown, 1991)**. The determination of this freezing rate is important to ensure the correct slow rate applied in the experiment. The initial freezing point of pumpkin samples was -3.06 °C (Table 2). This point is close to freezing temperature of similar vegetables (-0.8 to -2.8 °C) **(Fellows, 2009)**. Differences in the water content and other chemical compounds in the sample and the values used in the literature may have been responsible for the disparities. The observed freezing point of pumpkin was then used as the basis for further freezing experiments to have a correct starting point for temperature assignments for the rest of the experiments.

Table 2 Freezing parameters of pumpkin.

Run number	Freezing point (°C)	End point of freezing (°C)	Time to reach from 0 to -18 °C (min)	Rate of freezing (°C/min)
1	-3.04	-6.09	219	0.08
2	-3.07	-7.60	242	0.07
3	-3.17	-6.62	299	0.06
4	-2.87	-6.02	279	0.06
5	-3.14	-8.53	273	0.07
M	-3.06	-6.97	262.40	0.07
SD	0.12	1.08	31.73	0.01

Cell disintegration

The fuzzy-controlled freezing of pumpkin caused great cell disintegration (Figure 3). There was a tendency for decomposition to increase as the temperature was lowered for a longer process time. This was notable particularly for freezing of less than 7 h and prolonged treatments would eventually have decreased the disintegration. The maximum disintegration of 0.467 ±0.051, as a result of freezing at -18 °C for 7 h, corresponded to an increase of EC from 36.2 µS/cm for untreated samples to 74.75 µS/cm. This ionic elevation could be caused by intracellular water which migrates extracellularly to form ice crystals during the freezing process **(Zaritzky, 2011)** and the presence of damaged cells **(Vorobiev and Lebovka, 2009)**. This result is consistent with other work by **Marra (2013)** which showed that slow freezing increases the EC of potato, carrot and apple, attributable to changes in the structure of the food samples used. Furthermore, that work also indicated a greater increase for quick freezing.

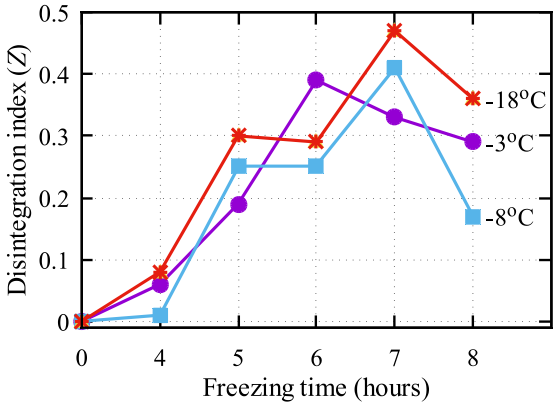


Figure 3 Effect of freezing on disintegration of pumpkin cells.

The degree of cell disruption in the present study could be considered sufficient with respect to heat-sensitive nutrient retention. More severe cell degradation may be achieved by applying a higher temperature during the thermal processing of samples, but this could destroy heat-sensitive polyphenols. **Moreira et al. (2019)** have shown that boiling and steaming of pumpkin accounts for 23.41 − 44.63% and 24.39 − 43.00% losses of total phenolic compounds respectively. Epimerization of polyphenols may occur under high-temperature processing; therefore, low-temperature processing and storage are preferred **(Deng, et al., 2018)**.

The cell disintegration and EC of samples was positively correlated (Pearson's $r = 0.95$; $p < 0.001$). This indicates that EC could be used for rapid assessment of pumpkin cell disintegration during freezing storage. Several studies have also suggested the use of EC to assess cell degradation due to food processing of potato, carrot, apple and sugar beet **(Marra, 2013; Maskooki and Eshtiaghi, 2012)**.

Microstructure changes

Untreated pumpkin tissue consisted of cells which are closely bound together by the cellular structure, roughly circular in shape and notably different in size (Figure 4a). This characteristic of visual structure is similar to the result reported in other work **(Rojas and Augusto, 2018)**. The disintegration of cells after freezing may be conventionally concluded by comparing the images for frozen samples (Figure 4b) and their untreated counterparts. The frozen

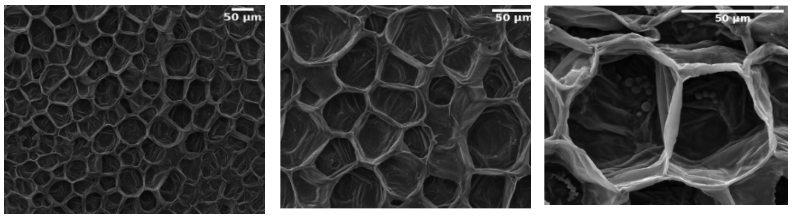


Figure 4a Microstructure of intact, untreated pumpkin cells at different magnifications, from left to right 500x, 1000x and 2.500x; scale bar 50 µm.

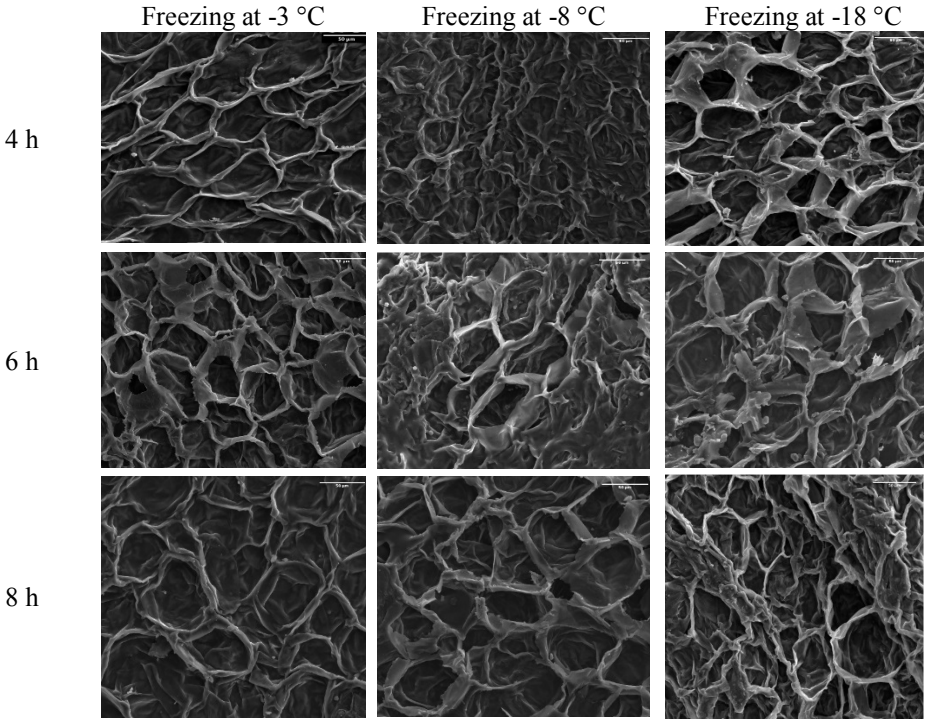


Figure 4b Comparison of microstructure of pumpkin samples after treatment at different temperature (°C) and duration of freezing (h); magnification 1000x, scale bar 50 µm.

tissue became distorted, some cells undergoing rupture and suffering from an increase in irregularity in shape. The cellular disintegration of plant tissue could be a result of water losses from the cell which lead to cell shrinkage **(Fellows, 2009)**. Membrane distortion and stress on rigid cell structures due to ice crystal growth can also cause mechanical damage to cells **(Zaritzky, 2011)**. Generally, the main factors affecting cellular structure during freezing are the formation of ice crystals, migration of water, and the intrinsic characteristics of the cells **(Li, Zhu and Sun, 2018)**.

In the present study, AMT was used to delineate and to conform the changes in surface texture of pumpkin samples as a response to freezing treatment. As the microstructure undergoes changes, the surface texture is expected to change accordingly. Along with other factors such as the illumination and imaging system used during experiments, the surface texture of samples is a determinant characteristic of the visual texture **(Kvaal, et al., 2008)**. Based on the MA spectra obtained, it was evidenced that frozen pumpkin exhibited a higher MA compared to untreated pumpkin (Figure 5). Increases in MA in the MA spectrum correspond to the irregularity, coarseness and intricacy of surfaces **(Huang and Esbensen, 2000)**. The rough texture could be the result of surface texture changes which reflect cellular decomposition following the freezing treatments. Therefore, it is clear that the surface texture of the samples deviated from that of untreated pumpkin, indicating the effects of the treatments.

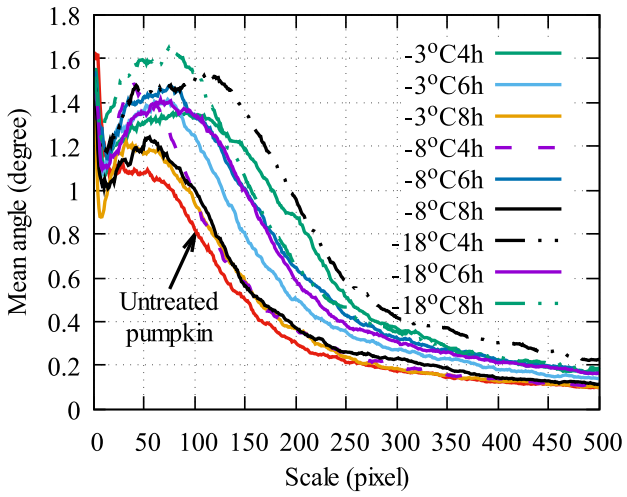


Figure 5 Mean angle spectra of pumpkins after freezing; a higher mean angle value indicates rougher surface texture.

The PCA results provide more specific information on the variation of texture as a result of various freezing treatments. The majority of the variability of sample texture in the projection model can be addressed by two principal components, accounting for 88.2% and 7.4% of variability respectively (Figure 6). The result clearly shows that untreated samples contribute a small degree of variability to the data set as signalled by the AMT spectra. The results also indicate that samples frozen at -18 °C have clearly

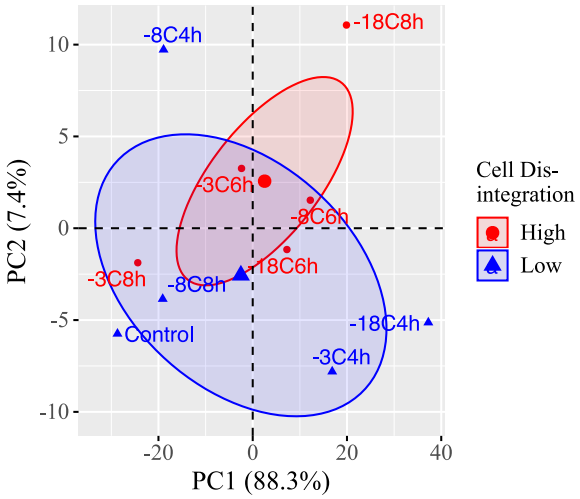


Figure 6 PCA result for pumpkin samples from different freezing treatments.

different texture characteristics compared to those frozen at -8 or -3 °C as they are separated on the PCA graph. Frozen samples which have undergone more severe cell disintegration seem to separate apart from the rest. This could indicate the correlation between cell disintegration and the texture of pumpkins due to freezing. A similar correlation has been demonstrated on red sweet pepper tissue using high hydrostatic pressure and pasteurization treatments **(Hernández-Carrión, et al., 2015)**. Due to the apparent complexity of the image textures, the MA spectra and valuable PCA outputs would substantially increase the accuracy in drawing conclusions compared to solely visual analysis of the sample images.

TPC

The TPC of untreated pumpkin was 8.49 ±0.08 mg GAE/g which is higher than the range of 4.44 – 5.65 mg GAE/g db **(Mendelova, et al., 2017)** reported previously. This may be attributable to differences in variety, environment and agricultural practices. The freezing treatments substantially increased the TPC of pumpkin samples, ranging from 9.02 ±0.03 to 14.47 ±0.05 mg GAE/g. The most notable increase resulted from freezing at -18 °C for 6 h (Figure 7). The effect of lowering temperature on the TPC increase was statistically significant (p <0.05). Although the freezing time appeared to increase the TPC, the effect was not statistically significant (p >0.05).

The increase in TPC can be attributed to the extraction effect of slow freezing on food **(Leong and Oey, 2012)**. The maximum gain of TPC in the present research (70.44%) is higher compared to other findings. The increase in TPC of strawberries frozen in an air-stagnant household freezer at -

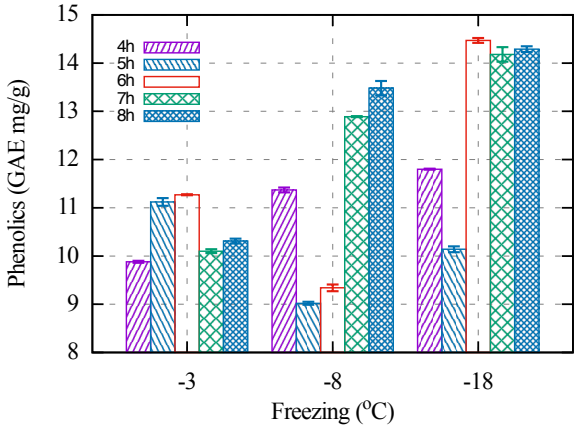


Figure 7 Total phenolics content of frozen pumpkins.

Note: Different notations within values in respective group indicate difference values at p <0.001.

27 °C for 1 week reported by **Bulut et al. (2018)** is 3.01%. An increase in TPC of 27.34% has been also reported for cagita fruit following freezing at -40 °C **(Santos, et al., 2018)** and of 17.15% for 'Hudson' spinach after 3 months of freezing **(Bystrická, et al., 2015)**. The extent to which these increases vary could be related to different influencing factors including the food matrix **(Leong and Oey, 2012)** and process parameters used in the experiments. The use of FLC to achieve better control of temperature is an interesting independent variable to which attention may be drawn. The scarcity of published data on the type of freezer temperature controller and on performance tests before systems are used in experiments makes comparison of any results difficult to attain. A precise freezing temperature of a desired point for experimental purposes is difficult to achieve when an air-stagnant household freezer with an analogue thermostat controller is employed. As **Cheung and Kamal (1997)** showed that the performance of a refrigeration system controlled by fuzzy logic is better than that controlled by a proportional derivative method, the small temperature errors observed during the freezer performance test in the present research could serve as evidence for better temperature control or process performance.

LCMS results

The LCMS test showed that slow freezing increased phenolic compounds, primarily caffeic acid, chlorogenic acid and p-coumaric acid (Table 3). Significant increases in other phenolics such as isorhamnetin-3-O-rutinoside, ferulic acid and naringenin were also detected. The increase in phenolic compounds such as anthocyanins and flavonol glycosides due to the extractability effect of freezing has also been demonstrated for grapes **(Tomaz, et al., 2017)**. LCMS provides individual changes in phenolics which cannot be observed by Folin–Ciocalteu assay alone. LCMS tests are also more precise. Ascorbic acid in pumpkin flour samples may act as a reducing agent and interfere with the Folin–Ciocalteu assay, hence reducing test specificity **(Sánchez-Rangel, et al., 2013)**. Dried pumpkin samples used for LCMS analysis may contain ascorbic acid as revealed by related work that pumpkin powder produced by fluidized drying still contains as much as 21.16 mg vitamin C/100 g **(Khan, et al., 2019)**.

Table 3 Increase in pumpkin phenolics due to freezing as detected by LCMS.

Retention time (min)	Compound	Formula	Exact mass	Curve area increase
1.84	p-Coumaric acid	$C_9H_8O_3$	164.0473	1158.7066
3.28	Esculetin	$C_9H_6O_4$	178.0266	839.7814
4.64	Caffeic acid	$C_9H_8O_4$	180.0423	1374.3696
5.01	Scopoletin	$C_{10}H_8O_4$	192.0423	928.1558
5.04	Ferulic acid	$C_{10}H_{10}O_4$	194.0579	1059.2264
9.73	Naringenin	$C_{15}H_{12}O_5$	272.0685	1054.8360
10.32	Kaempferol	$C_{15}H_{10}O_6$	286.0477	774.9496
10.50	Catechin	$C_{15}H_{14}O_6$	290.0790	468.7586
12.42	Chlorogenic acid	$C_{16}H_{18}O_9$	354.0951	1220.7587
14.43	Kaempferol 3-arabinoside	$C_{20}H_{18}O_{10}$	418.0900	884.5015
21.39	Vitexin	$C_{21}H_{20}O_{10}$	432.1056	502.27583
21.43	Kaempferol-3-O-rhamnoside	$C_{21}H_{19}O_{10}$	431.0984	793.8315
22.62	Kaempferol-3-O-D glucoside	$C_{21}H_{20}O_{11}$	448.1006	869.5909
23.19	Astilbin	$C_{21}H_{22}O_{11}$	450.1162	164.3881
24.02	Isoquercitrin	$C_{21}H_{20}O_{12}$	464.0955	929.7765
36.85	Isorhamnetin-3-O-rutinoside	$C_{28}H_{32}O_{16}$	624.1690	1059.9496
37.06	Rhamnazin 3-rutinoside	$C_{29}H_{34}O_{16}$	638.1847	879.9009
46.91	Isorhamnetin 3 rutinoside 4' rhamnoside	$C_{34}H_{42}O_{20}$	770.2269	979.3314

CONCLUSION

In the present study, a slow freezing system controlled by a fuzzy logic algorithm was developed to study pumpkin cell disintegration. Freezing causes severe cell degradation which can be clearly observed by an increase in disintegration index and surface texture changes. Frozen pumpkins exhibit rougher texture or a higher MA at the respective scale as compared to untreated samples. As a result of cell damage, increases in EC and phenolic compounds are also detected. The maximum increase in TPC of 70.44% resulted from freezing at -18 °C for 6 h. The increased phenolics compounds are mainly caffeic acid, chlorogenic acid and p-coumaric acid. Information obtained from cell damage, texture analysis and phenolics content are all in agreement to support the favourable effects of fuzzy-controlled slow freezing on phenolics from pumpkin.

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