# Processing of green tea pastes by micro wet milling system: Influences on physicochemical and functional properties

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**Abstract**

This study aimed to develop green tea paste using a new micro wet milling (MWM) system. The influence of milling conditions on the particle size, morphology, solubility, and antioxidant properties of Matcha, shaded Yabukita, unshaded Yabukita, and Hojicha pastes were studied. MWM green tea paste retained smaller particles, better color, higher solubility, and antioxidant properties than the dry milled Matcha paste. Storage temperature and time affected the stability of ascorbic acid and visual green color at 20, 4, -18, and -60 °C for four weeks. Kinetic analysis demonstrated that first-order kinetic models could predict the degradation of ascorbic acid and the reduction in visual green color. Temperature-dependent rate constants of ascorbic acid and color of green tea pastes obeyed the Arrhenius relationship. The total viable count revealed that green tea paste could be stored for 7 days at 20 °C and 21 days at 4 °C within the permissible limit.

**Keywords:** green tea paste; micro wet milling; green color; solubility; kinetic study; total viable count

## 1. Introduction

Green tea is the most popular unfermented tea recognized for its distinctive refreshing aroma, taste, and is one of the world's most consumed drinks. As a result of accumulating scientific reports and evidence highlighting its beneficial effect on human health, its consumption is growing. According to the World Green Tea Association (2018), global green tea consumption has risen by about 20 percent over the past ten years. Green tea contains abundant natural phenolic compounds, which show antioxidative, anti-carcinogenic, and anti-microbial properties (Langley-Evans, 2000). Green tea is now not just limited to the beverage but is used in a myriad of ways. Its applications include catechin dyeing technology, bread making, and supplemental products that utilize the active components of tea (Ahmad et al., 2015; Dulloo et al., 1999; Ning, Hou, Sun, Wan, & Dubat, 2017; Yamamoto, 1997). The inclusion of green tea in the processing of tea-based products with functional properties has recently become popular. The present study involves developing green tea paste as a new product from green tea leaves, which allows maximum retention of the nutritional components of the tea leaves and, therefore, more beneficial than tea powder. In general, green tea pastes are produced from green tea powder, which is processed by dry milling tea leaves or spray-drying/freeze-drying tea leaf extract. However, due to the thermal effect during dry milling or drying, the aroma, color, and heat-sensitive compounds such as ascorbic acid, chlorophyll, catechin, and antioxidant capacity of the green teas were degraded (Zaiter, Becker, Karam, & Dicko, 2016). Another challenge was to the addition of the green tea powder into the processed product due to problems of large particle size, strong agglomeration, very poor dispersibility and solubility in cold water.

In contrast, wet milling is a more intensive process to produce micro-level particles (Loh, Samanta, & Sia Heng, 2014). In the food processing and pharmaceutical industry, the wet milling process is widely used to facilitate the physicochemical characteristics of the food matrix, e.g., solubility, particle size, water holding capacity, yield, foamability, and nutrition bioavailability (Aluko, Mofolasayo, & Watts, 2009; Kethireddipalli, 2002; Maphosa & Jideani, 2016). Although wet milling efficiently reduced the particle sizes, some wet milling, e.g., high rotational grinder and ball mill, produced heat that could adversely affect the quality of the product (Loh et al., 2014). We developed a new modified electric stone milling system with a lower rotational speed named micro wet milling (MWM) to avoid heat damage to the product. A rotary disk feeder with a screw conveyor was attached in the new MWM system for efficient feeding of the raw material instead of a conventional pump. Several authors have reported the application of MWM for the development of fine rice slurry (Koyama & Kitamura, 2014), a cheese-like product from micro wet-milled rice milk (Nakamura, Kitamura, & Kokawa, 2016), processing of orange juice with pulp (Islam et al., 2017), processing of komatsuna (*Brassica rapa var. perviridis*) juice with rich phosphatidic acid (Li, Kokawa, & Kitamura, 2018). However, no studies have reported the production of green tea paste by using new MWM, and no reports, which evaluated the quality of the green tea paste during storage at different temperatures, were found.

Therefore, the objective of the study was to produce green tea paste from green tea leaves using a new MWM system (Fig.1). Then the effects of MWM on the physicochemical and antioxidant properties of green tea paste were determined. To predict the kinetic parameters and quality characteristics, green tea pastes were stored at four different temperatures 20, 4, -18, and -60 °C for one month. Then first-order kinetic models were applied to determine the rate of degradation of color and ascorbic acid in the green tea paste. During storage, a microbial study was also conducted to determine the quality and shelf-life of the green tea paste.

## 2. Materials and Methods

### *2.1. Raw materials*

Three kinds of green tea leaves (Sencha), namely shaded Yabukita, unshaded Yabukita, and Hojicha, were collected from the Yabukita cultivar, Nagasaki Prefecture, Japan. Yabukita is known as an excellent breed, and its aroma and taste is generally regarded as one of the best among Japanese green teas.

The green teas grown in the shade to avoid direct sunlight are called shaded green teas whereas the teas grown under direct sunlight are known as unshaded teas. Hojicha is another Japanese green tea, which is distinct from other green teas because it is roasted, while most of the other Japanese teas are steamed. For a comparative study, dry milled green tea called Matcha was also collected from the same cultivar. All green tea leaves, including dry milled Matcha belonged to the Yabukita variety and were harvested in May 2019. Most chemicals and reagents were obtained from Wako (Osaka, Japan)

### *2.2. Preparation of green tea paste by MWM*

For the production of the green tea paste, green tea leaves were blended with a mixer (SBC-1000J, Cuisinart, Japan) at varying ratios of green tea leaves to distilled water, 10:90, 15:85, and 20:80 w/w, respectively. Matcha paste was prepared according to the same ratio of dry milled Matcha and water. The experimental MWM system is shown in Fig. 1. The mixture of green tea and water was stored in the tank and introduced into the milling machine by a rotary disk with a screw conveyor, and the green tea paste was collected in a receiver tank. The milling machine consists of an upper stone and a lower stone having 4 mm grooves (Fig. 1b and 1c) with a total exterior surface area of 416 $cm^2$. An electric motor rotates the lower stone only (Fig. 1b). The large contact surface area and minimum gap between the upper and lower stones produced very fine and uniform particle size. To investigate the effect of MWM operating parameters on the particle size and nutrition content of shaded Yabukita green tea paste, wet milling conditions were set by varying the green tea to water ratio (10:90; 15:85; and 20:80 w/w), feeding rate (10 mL/min to 25 mL/min) and rotational speed (20, 30, 40, and 50 rpm).

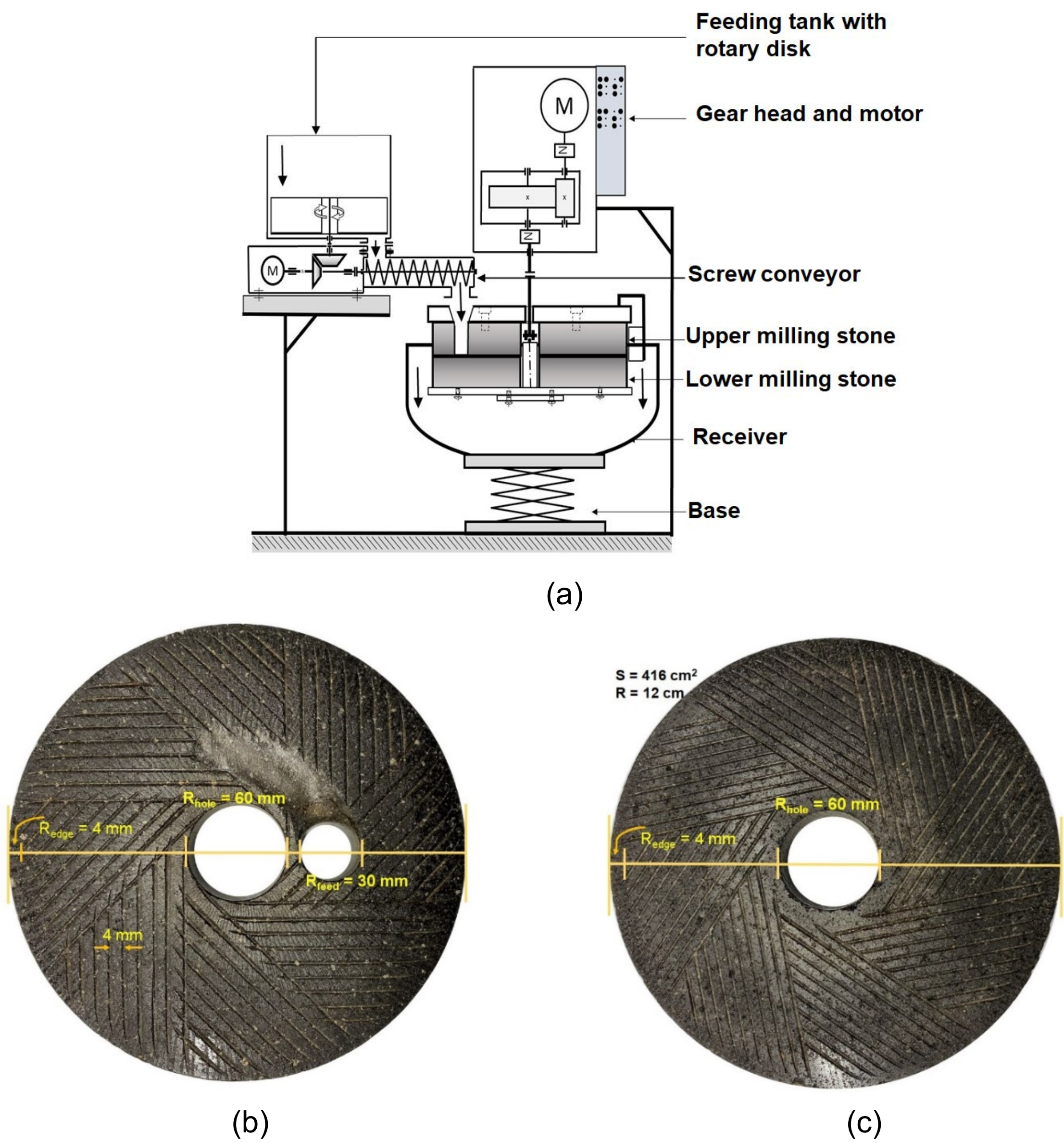



**Fig.1.** Micro wet milling system: (a) schematic diagram of MWM system, (b) Upper milling stone (c) Lower milling stone

### *2.3. Analysis of the physical properties of green tea paste*

#### *2.3.1. Moisture content, total solids, and pH*

The moisture content of the green tea paste was determined by drying in an oven at 105 °C until constant consecutive weights were obtained. Moisture content was expressed as % moisture on a wet basis (AOAC, 2004). The total solids content was calculated as TS% = 100 - moisture content. The pH was determined by a pH meter (F-51, Horiba, Japan)

#### *2.3.2. Water activity*

The water activity of the green tea paste was determined by a water activity meter (Novasina Labmaster-aw, Switzerland).

#### *2.3.3. Viscosity*

The viscosity of the different green tea pastes was determined by a Viscometer (DV-E Brookfield viscometer, USA). Spindle size, rotational speed and torque of the viscometer were selected by trial and error method. In the current measurement, spindle number 63 (rod shape), a rotational speed of 100 rpm and torque 15% were selected. Then 500 mL of green tea paste was transferred into the low form Griffin beaker. Then spindle was submerged into the sample, and the viscometer was powered. The measured viscosity of the sample was recorded as mPa·s. Triplicate measurement was done, and each time the sample temperature was maintained at 25 °C.

#### *2.3.4. Particle size and size distribution*

Particle size (d50 and d90) and its size distribution were determined by a laser diffraction particle size analyzer (SALD-2200, Shimadzu Corporation, Japan) in mode according to the previous report (Koyama & Kitamura, 2014). Working conditions were as follows: measurement principle was the laser diffraction and scattering, measurement ranges was from 0.017 to 2000 µm, wavelength was 680 nm, operating temperature and humidity were 25 °C and 80% respectively, and particle size was taken as D50 and D90 values expressed as μm. D50 is defined as the median diameter, and D90 is the particle size corresponding to 90% of the particles being undersize by mass. The particle size distribution profile was constructed by the particle size and frequency of the distribution.

#### *2.3.5. Scanning electron micrograph*

Images of the particles present in the green tea paste were acquired by Field Emission Scanning Electron Microscope FE-SEM (JSM-6330F, JEOL, Tokyo, Japan). A small amount of green tea paste was placed on a glass slide and dried overnight in an oven at 40 °C to ensure that the sample was completely dry. The samples were coated with platinum-palladium under vacuum in a sputter-coating unit (E-1045, Hitachi, Tokyo, Japan). The images were acquired at 5 kV and 1700-fold magnification.

#### *2.3.6. Water solubility Index*

The water solubility index (WSI) was determined according to the method described by Anderson et al. (Anderson, R.A.; Conway, H.F.; Pfeifer, V.F., Griffin, 1969) with slight modifications. About 2 g of green tea paste and 25 mL of distilled water were vigorously mixed in a 50 mL centrifuge tube, incubated in water baths maintaining three different temperatures, 10 °C, 20 °C and 80 °C for 30 min. Then the sample was centrifuged for 15 min at 5000 rpm (ASONE CN-1050, Osaka, Japan). The supernatant was collected in a pre-weighted beaker and oven-dried at 105±2.0 °C. The WSI was calculated based on the percentage of dried supernatant with respect to the original amount of the sample.

#### *2.3.7. Color parameters*

The color characteristics of the green tea paste were evaluated by using a Colorimeter (CR-200, Minolta Co., Japan). The results were expressed by the Hunter color value $L^*$, $a^*$ and $b^*$ where $L^*$

=100 indicates lightness and L* = 0 denotes darkness, +a* denotes redness and –a* denotes greenness, +b* denotes yellowness and –b* denotes blueness. The color intensity was expressed as Chroma value and calculated by the formula = $(a^{*2}+b^{*2})^{1/2}$. The hue angle (H°) was calculated by $H^o = \tan^{-1}(b^*/a^*)$ and expressed on a scale of 0 to 360°.

### *2.4. Detection of total phenolic content and antioxidant activity*

#### *2.4.1. Extract Preparation*

The green tea paste sample (1 g) was extracted for 5 min with 50 mL distilled water at 10 °C and 80 °C water bath shaker, respectively (Ramírez Aristizabal, ORTÍZ, Restrepo Aristizabal, & Salinas Villada, 2017). Then the mixture was centrifuged for 5 min at 5000 rpm (ASONE CN-1050, Osaka, Japan). The supernatant was collected as an extract. The extraction was repeated five times. All infusions for each temperature were combined for further analysis. The term cold extract (10°C) and hot extract (80 °C) used to denote these two types of infusions.

#### *2.4.2. Determination of ascorbic acid content*

Ascorbic acid content of different green tea pastes was estimated by the Reflectometer (RQFlex-20, USA). First, 1 mL of green tea paste sample was diluted with 5% metaphosphoric acid at a 1:10 ratio. The mixture was centrifuged for 10 min at 5000 rpm (ASONE CN-1050, Osaka, Japan). The supernatant was then collected, the test strip dipped into the supernatant and measured with a reflectometer. Similarly, the ascorbic acid content of the cold and hot extract was also performed. The results were expressed as mg ascorbic acid/100 g dry weight of the green tea paste.

#### *2.4.3. Determination of phenolic content*

Total phenolic content (TPC) of green tea paste samples was measured using the Folin – Ciocalteu spectrophotometric method described by Singleton et al.(Singleton, Orthofer, & Lamuela-

Raventós, 1999). Absorbance was measured at 765 nm. TPC was expressed as Gallic acid equivalents (mg GAE/g dry weight of the green tea paste).

### *2.4.4. DPPH scavenging activity*

Antioxidant analysis of various green tea pastes was carried out based on DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity described by Sharma and Bhat. (O. P. Sharma & Bhat, 2009) with slight modifications. First, 100 μL of the extract was added to 1.4 mL DPPH radical methanolic solution (0.1 mM in methanol). The mixture was left to stand in the dark for 30 min. A blank solution was prepared by mixing 100 μL methanol in 1.4 mL of DPPH radical solution. The absorbance was measured at 517 nm using a UV-Vis Spectrophotometer (Jasco V-630, Germany). For each sample, triplicate measurements were performed. The results were expressed in terms of the percentage of inhibition using the following formula.

$$\text{DPPH radical scavenging activity (\%)} = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right)\times 100 \qquad (1)$$

### *2.5. Storage study of the green tea paste*

Micro wet milled green tea pastes and Matcha paste were packed and sealed in HDPE polyethylene under vacuum. Each sample was stored at a temperature of 20 °C, 4 °C, -18 °C and -60 °C for one month. Observations regarding changes in the level of green color, ascorbic acid amount and total microbial count of different green tea pastes were performed at 7-day intervals.

### *2.6. Kinetic study for color and ascorbic acid content*

The changes in ascorbic acid content and the color of the green tea paste during storage were modeled using first-order kinetics (equation 2) (Burdurlu, Koca, & Karadeniz, 2006).

$$\ln C = \ln C_o - kt \qquad (2)$$

Where C is the concentration at time t and $C_o$ is the initial concentration. For a first-order reaction, ln C is plotted against t, and the rate constant k is represented by the slope.

The temperature dependence of ascorbic acid degradation and color loss was modeled using the Arrhenius equation (Ávila & Silva, 1999)

$$k = k_o \exp(-Ea/RT) \tag{3}$$

where k: rate constant; $k_o$: pre-exponential factor; Ea: activation energy (kJ/mol); R: gas constant ($8.314 \times 10^{-3}$ kJ/mol K); T: temperature in K

Half-life ($t_{1/2}$) corresponds to the time at which ascorbic acid content is reduced by 50% and is calculated by using the equation (5). (Burdurlu et al., 2006).

$$t_{1/2} = \frac{\ln 2}{k} \tag{4}$$

***2.7. Total viable count (TVC)***

The total microbial count was enumerated by using the 3M Petri film described by Ginn et al. (Ginn, Packard, & Fox, 1984). The sample was diluted up to $10^{10}$ with a 0.9% saline solution. Then 1.0 mL of each dilution was placed in the center of the 3M Petrifilm. The top film was gently lowered, and the aliquot spread with the 3M™ Petrifilm™ flat spreader. All plates were incubated at 37 ± 1 °C for 48 ± 2 h. The results were then represented as cfu/g of the sample.

***2.8. Statistical analysis***

The experiments were repeated at least three times, and the results are expressed as mean ± standard deviation. Data were analyzed by the analysis of variance (ANOVA). The mean comparison was determined by post-hoc Tukey's test. The significant level was set at $p \leq 0.05$. The first order and Arrhenius model parameters were estimated through linear regression analysis by using the statistical software Origin Pro 8.5 (Origin Lab Corporation, Northampton, USA). The accuracy of the model fitting to experimental data was evaluated by the coefficient of determination ($R^2$) and root mean square error (RMSE).

$$\mathrm{RMSE} = \sqrt{\frac{1}{(n-p)}\sum_{i=1}^{n}(X_{t,M} - X_{t,E})^2} \tag{5}$$

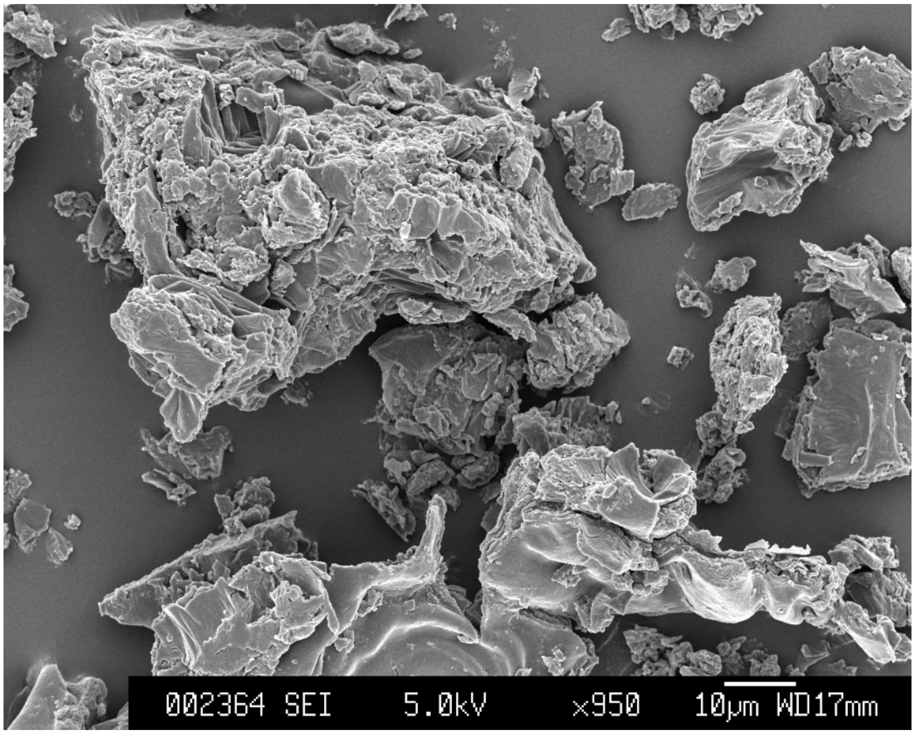
Where, $X_{t,M}$ is the value predicted by the model, $X_{t,E}$ is the experimental value, n is the number of data and p is the number of parameters.

## 3. Results and discussion

### *3.1. Optimized MWM conditions*

The effects of MWM operational parameters on the particle size of the green tea paste is summarized in Fig. 2. The SEM images revealed that the Matcha paste had a greater number of particles with high fibrous content (Fig. 2a), while MWM effectively split these particles down to a smaller diameter as shown in Fig. 2b. The median diameter (d50) of the particles was used as an indicator to determine the best MWM conditions. The incorporation of water into the green teas had a significant effect on the particle size of the MWM paste. When the water ratio was increased from 80% to 90%, particle sizes were also increased, as shown in Fig. 2c. The sample with a higher water ratio passed through the milling zone quickly without adequate particle exposure; as a result, larger particles were produced. On the other hand, a higher ratio of green teas increased the volume of the milling particles in the milling zone, resulting in increased friction and collisions between the particles-millstone and inter-particle collisions, finally breaking up the particles to a smaller size. The best water-to-green tea ratio was 80:20 w/w to produce green tea paste containing uniform and small particle sizes, as shown in Fig. 2c. The feeding rate and rotational speed of the MWM system also affected the particle sizes in the green tea paste. The feeding rate was set to 10, 15, 20, and 25 mL/min, and rotation speed were varied from 20 to 50 rpm. The higher feeding rate increased the product yield, but when the feeding rate was increased beyond 25 mL/min, an overflow occurred at the inlet. The particle sizes of the green tea paste were larger at higher feeding rates and lower rotational speeds, as shown in Fig. 2d. This can be attributed to the particles flowing out of the milling zone before being completely milled (P. Sharma, Chakkaravarthi, Singh, & Subramanian, 2008). The lower feeding rate and higher rotational speed generated stronger friction and collision between the particles-millstone and consequently smaller particles were produced. The particle sizes of the green tea paste at a steady rotational speed of 50 rpm did not vary significantly between feeding rates of 10, 15, and 20 mL/min, as shown in Fig. 2d. The optimal MWM conditions with higher yield were therefore achieved as a water-to-green tea ratio of 80:20, a feed rate of 20 mL/min, and a rotational speed of 50 rpm. Based on these conditions, green tea pastes were produced via the MWM system from three different types of Japanese green teas, namely, shaded Yabukita, unshaded Yabukita, and Hojicha.

(a)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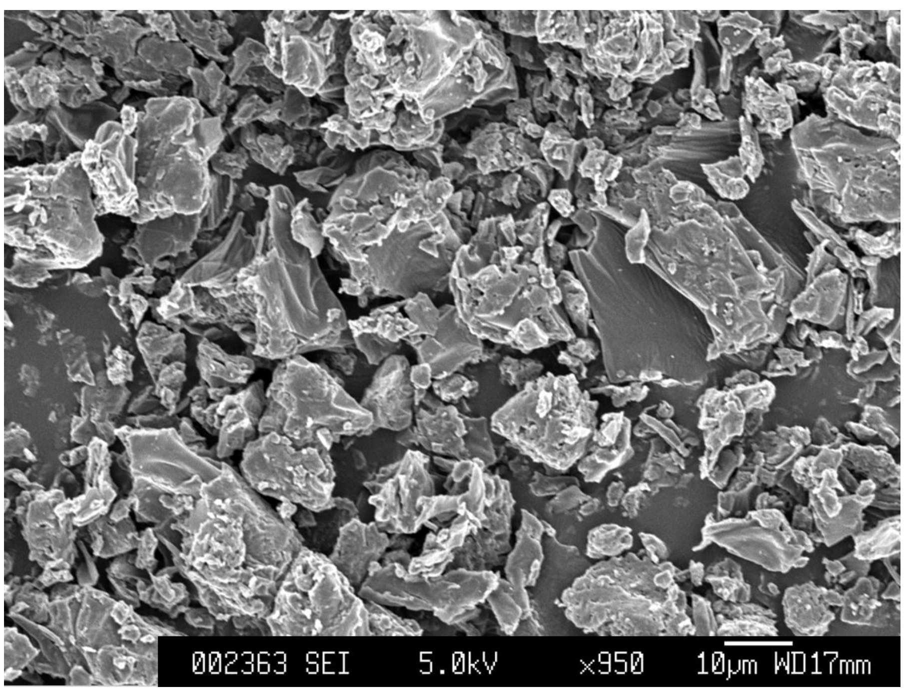
(b)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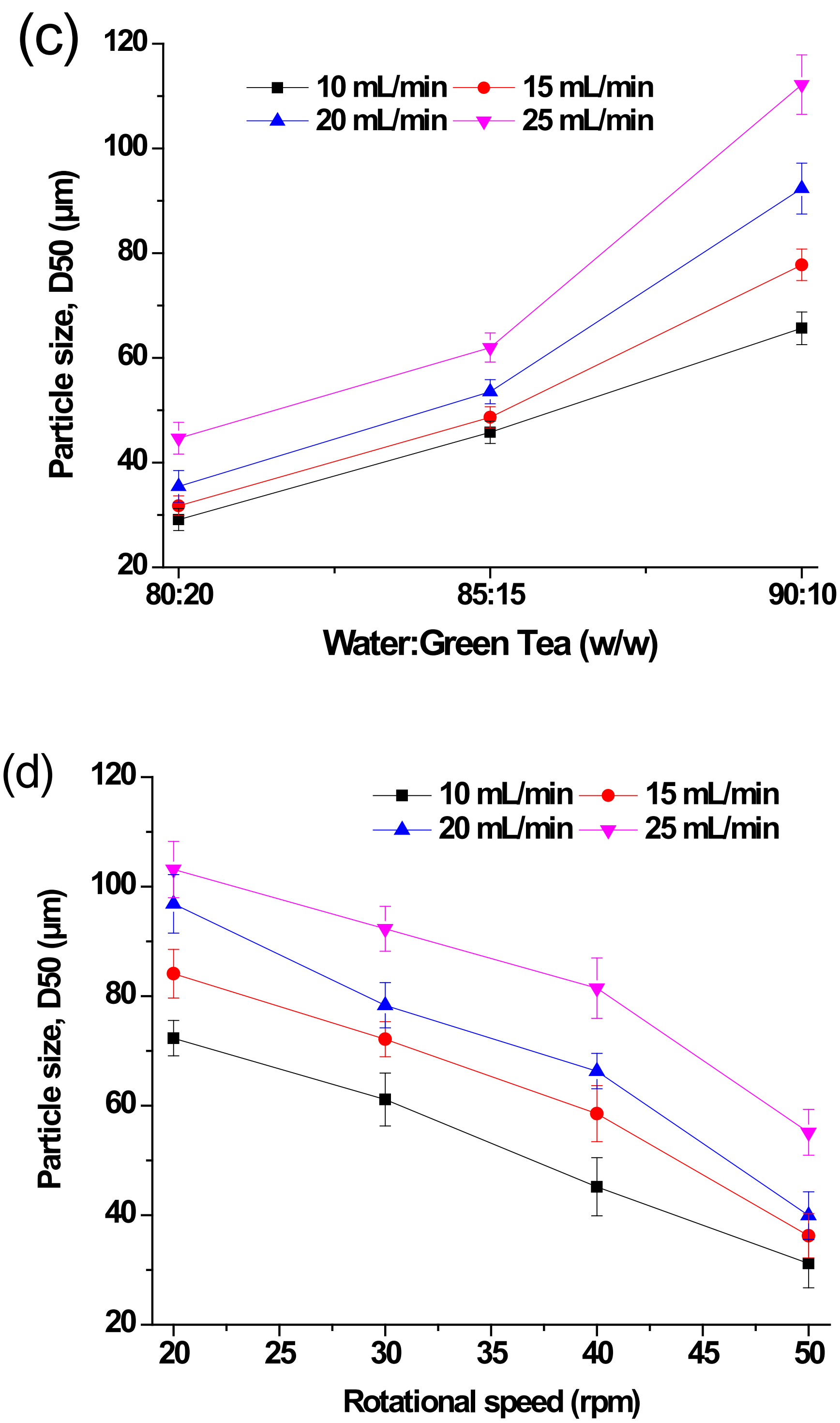



**Fig. 2.** Effect of milling on particle morphology (SEM image) of green tea paste (a) Matcha green tea pasted (b) micro wet milled green tea paste; Effect of MWM operational parameters on particle sizes: (c) water to green tea ratio and feeding rate; (d) rotational speed and feeding rate

### *3.2. Physicochemical properties of green tea paste*

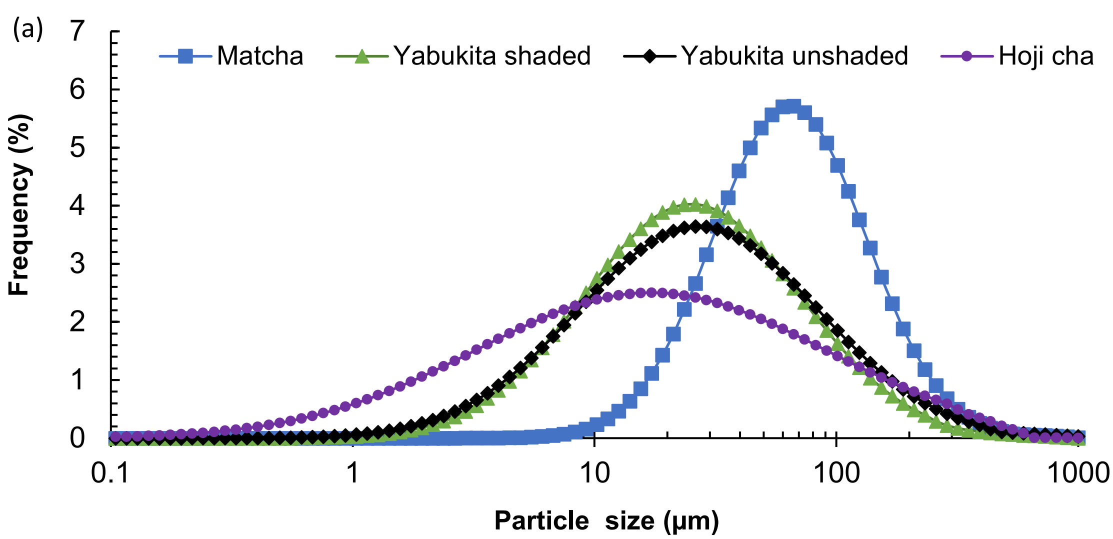
Physicochemical properties are related to the product quality as well as their impact on the processing behavior of foods. To develop new food products and processes, a thorough understanding of the physical structure and properties of the item is necessary. Therefore, the physicochemical properties of the green tea paste were determined, and data are presented in Table 1. The results show that the Hojicha paste contained the lowest moisture content compared to the other green tea pastes. The moisture content of the Matcha, shaded Yabukita and unshaded Yabukita paste were not significantly different and the values were in the range of 81.03 to 84.45%. The total solids content of all the samples followed a similar trend, as the moisture content increased, the amount of dry solids decreased.

The particle size is one of the critical physical parameters that affect the sensory, color attributes, and nutritional quality of the tea. As seen in Fig. 3a, all green tea pastes exhibited a unimodal particle distribution. Among them, Matcha paste showed larger median (d50) particle sizes of 88.56 µm compared to the MWM Yabukita green tea paste and the peak frequency (5.8%) was higher than the other samples (Fig. 3a). The average particle sizes (d50) of the MWM green tea pastes were significantly smaller than the dry milled Matcha powder paste, as shown in Fig. 3b. Our results agree with the findings reported by Kumar et al. (2018) and Nakhon and Ayutthaya (2014) who suggested that wet milling would be the best option which facilitates the reduction of the particle sizes of pharmaceutical ingredients and cereal paste compared to dry milling. Among the MWM green tea pastes, Hojicha contained smaller particle sizes than the Yabukita green tea paste. This may be due to the roasting effect and lower moisture content of the Hojicha that creates greater friction and collision between the particles and the millstone.

The viscosity of the MWM green tea paste and Matcha paste is shown in Table 1. The viscosity of the MWM green tea pastes was higher than the Matcha paste. This can be attributed to the smaller particle sizes of the MWM green tea paste. According to Koca et al. (2018) the viscosity of a product was inversely related to the particle sizes, as particle sizes decreased, the viscosity increased. The pH of all the green tea pastes did not differ significantly and values were in the range between 7.53 ± 0.61 to 8.12 ± 1.05.

The water solubility index is the essential quality parameter of green teas, as higher solubility at lower temperatures provides more functional compounds, better aroma, and color. The water solubility index of all the green tea pastes was determined at three different temperatures, 10, 20, and 80 °C and results are presented in **Table 1**. The water solubility index increased when the temperature increased, and values were not significantly different between the teas at 80 °C. However, at a lower temperature of 10 °C, MWM green tea pastes were more soluble than the dry milled Matcha paste. This could be due to the effect of wet milling that decreased the particle sizes, split the cell present in the particles, and improved the solubility of the green tea paste. Our results were as per the findings reported by Loh et al. (2014), who hypothesized that the wet milling process improves the solubility and ultimately the bioavailability of poorly water-soluble drugs. Similarly, Parada and Aguilera, Parada and Aguilera  (2007) stated that the mechanical or thermal disruption of the cell walls in particles enhanced the solubility and bioavailability of the functional compounds.

The color characteristics of the green tea pastes are presented in Table 1. The MWM green tea paste showed higher lightness L* values in the range of 49.94±1.21 to 56.76±2.34 compared to the Matcha paste (46.74±1.12). Among the green tea pastes, Hojicha had the highest lightness values (56.76 ± 2.34) due to the roasting effect and because the smaller particle sizes of the Hojicha paste scattered more light. A similar phenomenon was observed by Ahmed et al. (Ahmed, Taher, Mulla, Al-Hazza, & Luciano, 2016). The greenness values (-a*) of the shaded Yabukita green tea paste was significantly ($p \leq 0.05$) higher than the other green tea pastes. Because of the shading effect and smaller particle sizes of the Yabukita green tea paste, the greenness values were higher. Several authors mentioned that the shading of green tea before harvesting creates more chlorophyll, making the leaf greener compared to the unshaded tea (Sano, Horie, Matsunaga, & Hirono, 2018). The wet milling reduced the particle sizes and consequently enhanced the accessibility of the color pigment (Islam et al., 2017), whereas, during dry milling, a significant change in the color was observed because of loss of pigment due to the heat generation (Ahmed et al., 2016). The roasted green tea Hojicha displayed the highest yellowness value (b*) among the green tea pastes. The chroma values referred to the total color intensity and shaded Yabukita had higher chroma values of 28.49±2.14 than Matcha (23.57±1.81) and other MWM green tea pastes. The value of the hue angle analysis also indicated that MWM green tea paste demonstrated better color quality than the Matcha paste.



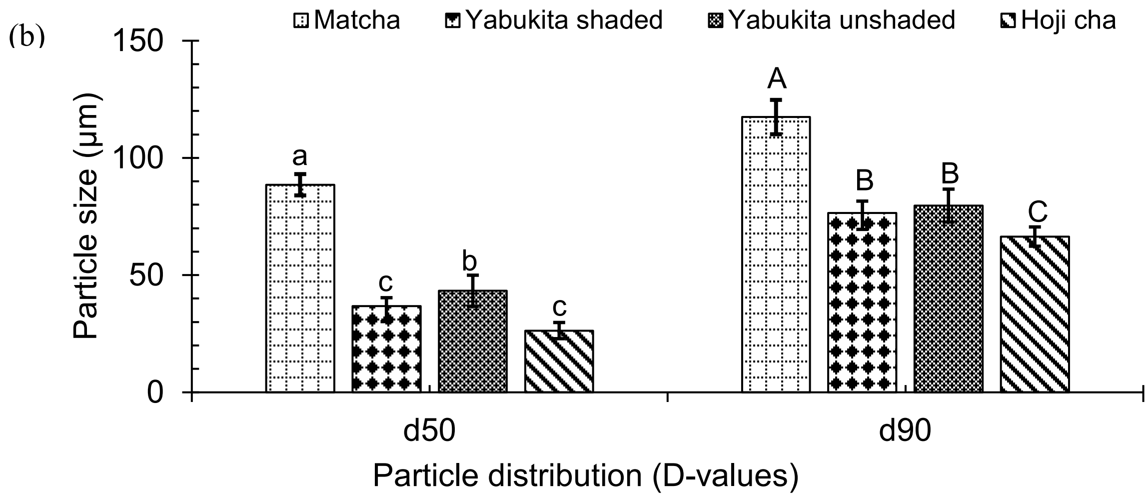



**Fig. 3.** Comparison on the (a) particle size distribution and (b) particle size of the different green tea pastes

Table 1. Physical properties of matcha and micro wet milled different green tea paste

| Parameters | | Matcha paste | MWM green tea paste | | |
|---|---|---|---|---|---|
| | | | shaded Yabukita | unshaded Yabukita | Hoji cha |
| Moisture content (%) | | 84.45±1.54[b] | 83.86±3.11[b] | 83.05±1.67[b] | 81.03±0.02[a] |
| Total solid (%) | | 15.55±1.31[a] | 16.14±2.02[a] | 16.95±1.53[a] | 18.97±2.32[a] |
| Viscosity (mPa·s) | | 205.87±5.34[c] | 221.45±3.38[b] | 218.03±2.87[b] | 232.34±3.21[a] |
| pH | | 7.53±0.61[a] | 7.62±0.73[a] | 7.59±0.54[a] | 8.12±1.05[a] |
| Water solubility index (%) | 10 °C | 81.76±2.04[b] | 90.11±3.01[a] | 89.34±4.11[a] | 76.21±2.76[c] |
| | 20 °C | 84.12±3.11[b] | 92.67±1.76[a] | 91.12±2.09[a] | 79.64±2.12[c] |
| | 80 °C | 94.88±4.34[a] | 96.11±2.13[a] | 97.01±3.31[a] | 89.23±1.52[b] |
| Water activity | | 0.92±0.01[a] | 0.94±0.02[a] | 0.93±0.01[a] | 0.92±0.01[a] |
| Color parameters | L* | 46.74±1.12[d] | 52.74±1.04[b] | 49.94±1.21[c] | 56.76±2.34[a] |
| | a* | -10.61±0.61[b] | -16.31±1.14[a] | -9.49±1.31[c] | 1.56±0.21[d] |
| | b* | 19.72±1.77[c] | 23.36±2.01[b] | 22.81±2.43[b] | 36.34±2.64[a] |
| | Chroma | 23.57±1.81[c] | 28.49±2.14[b] | 24.75±1.92[c] | 36.37±2.32[a] |
| | Hue | 118.23±3.21[b] | 124.93±2.71[a] | 112.54±2.04[c] | 87.54±2.67[d] |

All values are of means of triplicate determination expressed on mean ± standard deviation. Mean values in the following row sharing a common letter are not statistically significant ($p<0.05$).

### *3.3. Antioxidant properties of the green tea paste*

The difference in processing methods change the chemical and antioxidant properties of green tea. The effect of MWM and infusion water temperature on the antioxidant properties of the green tea pastes are presented in Table 2. Under both cold and hot water infusion conditions, wet-milled Yabukita green tea paste contained significantly higher ascorbic acid than the dry milled Matcha paste. The cold-water infusion of the shaded and unshaded Yabukita paste showed ascorbic acid content of 19.57±1.32 and 17.41±2.78 mg/g dry weight, respectively, while that of the Matcha paste contained only 6.24±4.21 mg/g dry weight. This can be explained by the milling operation; wet milling effectively decreases the particle sizes without rising temperature, while in dry milling, the increase in temperature causes a loss of bioactive compounds (Chen, Zhu, Tsang, & Huang, 2001; Kethireddipalli, 2002). The ascorbic acid content of MWM Yabukita green tea paste was much higher than the results reported by Yamamoto (1997), and Caicedo (2015) who were stated that the ascorbic acid content of Japanese tea was 280 mg/100 g and 250 mg/100g dry weight respectively. The ascorbic acid content of MWM Yabukita green tea paste was much higher under cold infusion than hot water infusion. This is because of the nature of the ascorbic acids, as the temperature increases, ascorbic acid levels decrease (Farnworth, Lagacé, Couture, Yaylayan, & Stewart, 2001; Paul & Ghosh, 2012). Among the green tea pastes, Hojicha contained the lowest ascorbic acid levels (0.35±1.31 mg/g dry weight).

Green tea contains much higher polyphenols and antioxidant activity compared to black or oolong tea due to differences in the processing of tea leaves after harvest (Kaur et al., 2015; Langley-Evans, 2000). In the current study found that the total phenolic content of different MWM green pastes were in the ranges of 121 to 321 mg GAE/g of dry weight. The more or less similar results were reported by Y. Zhao et al. (2011), who found the total phenolic content were varied from

174.10 to 252.64 mg GAE/100g dry weight in different green teas. However the MWM green tea paste showed higher TPC value than the results reported by Nibir et al. (2017), who mentioned the TPC content in the green tea extract about 100 mg GAE/g .

MWM Yabukita green tea paste contained higher phenolic and antioxidant activity than the dry milled Matcha paste, as shown in Table 2. The processing method may influence the phenol content of the green tea paste. MWM produced smaller particle sizes in the green tea paste without rising temperature, which enhanced the extraction of the phenolic content. Our results agree with the statement that during extraction, the bioavailability of the bioactive compounds increased as the particle sizes decreased (Li et al., 2018; Loh et al., 2014; Park, Imm, & Ku, 2001; Zaiter et al., 2016). On the other hand, dry milling takes longer and raises the temperature, which leads to a loss of bioactive compounds and antioxidant activity (Chen et al., 2001; Kethireddipalli, 2002). Among the MWM green tea pastes, unshaded Yabukita had significantly higher phenolic content, whereas the roasted Hojicha green tea paste had lower phenolic content than other green tea pastes. Our results were in accordance with Ku et al. (2010), who reported that the shaded green tea contained lower phenolic compounds but higher amino acids than tea are grown without shading. The infusion temperature also affects the extraction of the phenolic content of the green tea paste. In cold infusion, the total phenolic content was lower compared to the hot infusion of the green tea pastes. Our findings were similar to the other authors, who reported that the hot infusion of the tea produces had more phenolic content than cold infusion (Langley-Evans, 2000; Ramírez Aristizabal et al., 2017; Saklar, Ertas, Ozdemir, & Karadeniz, 2015; C.-N. Zhao et al., 2019).

Green tea was thus significantly more potent in terms of antioxidant activity on an equivalent mass basis of soluble solids than all the other teas. MWM green tea paste exhibited the DPPH inhibition in cold extract in the ranges of 55.73 to 56.58 % where as Matcha paste showed the DPPH inhibition of 43.76%. Whereas in hot extract, all MWM green tea paste demonstrated the higher antioxidant activity. Our findings are in accordance with the results reported by Farooq & Sehgal (2018), who reported the DPPH antioxidant activity was 57.48 % in Matcha. Xu et al. (2019) found the higher DPPH antioxidant activity about 75% in green tea extract than MWM green tea paste when the extract was treated with Tannase and ultrasound.

Table 2. Antioxidant properties of matcha and micro wet-milled different green tea paste

| Types of green tea paste | Ascorbic acid (mg/g dw) | | Total phenolic content (mg/g dw) | | Antioxidant activity (% of DPPH inhibition) | |
|---|---|---|---|---|---|---|
| | Cold extract | Hot extract | Cold extract | Hot extract | Cold extract | Hot extract |
| Matcha | 6.24±4.21[c] | 4.34±0.21[b] | 162.76±4.21[c] | 225.42±4.21[c] | 43.76±2.31[b] | 51.19±3.87[c] |
| Shaded Yabukita | 19.57±1.32[a] | 9.83±1.01[a] | 198.13±2.54[b] | 241.76±2.51[b] | 55.73±2.11[a] | 60.78±2.71[b] |
| Unshaded Yabukita | 17.41±2.78[a] | 8.23±0.53[a] | 268.87±4.65[a] | 321.46±5.32[a] | 56.58±2.67[a] | 67.64±2.21[a] |
| Hojicha | 0.35±1.31[d] | 0.21±1.11[c] | 121.75±3.25[d] | 152.88±5.04[d] | 12.67±1.12[c] | 14.51±1.04[d] |

All values are of means of triplicate determination expressed on mean ± standard deviation. Mean values in the following column sharing a common letter are not statistically significant ($p<0.05$). dw = dry weight.

The antioxidant activity of Yabukita green tea showed more significant DPPH inhibition than the other green tea pastes. This was due to the higher ascorbic acid and phenolic content of the Yabukita green tea paste. Tea polyphenols are reported to have strong antioxidant properties and free radical scavenging activity due to the presence of a phenolic hydroxyl group attached to the flavan-3-ol structure (Molan, De, & Meagher, 2009; C.-N. Zhao et al., 2019). The antioxidant activity of green tea polyphenols is mainly due to the combination of aromatic rings and hydroxyl groups that form their chemical structure, thereby binding and neutralizing lipid free-radical hydroxyl groups (Molan et al., 2009; C.-N. Zhao et al., 2019). The infusion temperature also influenced the scavenging activity of the green tea paste, and it is reported that the higher the brewing temperature higher the reducing power of the infusion (Molan et al., 2009; Yadav, Farakte, Patwardhan, & Singh, 2018).

### *3.4. Kinetic analysis of color and ascorbic acid degradation during storage*

The kinetic parameters for the color degradation of different types of green tea paste during storage at various temperatures were predicted by using a first-order kinetic model. The goodness of fit $R^2$ and RMSE values were used to compare the experimental and predicted model data. The degradation kinetics of color was studied during storage at 4, 20, -18, and 60 °C for four weeks. The Hunter color parameter (-a *) of the MWM Yabukita green tea pastes and Matcha paste were recorded each week during storage. The -a * value was used as a physical parameter to represent greenness during color measurement (Weemaes et al., 1999). The experimental data were fitted to first-order kinetic models by plotting $\ln(-a^*/a_o^*)$ versus time, as shown in Fig. 4a-c. The linear relationship indicated that the visual green color degradation of all green tea paste followed first-order kinetics with high $R^2$ values (0.96 - 0.99) and low RMSE values (0.1 - 0.01) as presented in Table 3. Several authors also agree that visual green color degradation during heat treatment and storage of food products follows the first-order kinetic model (Erge, Z, Koca, & Soyer, 2008; Steet & Tong, 1996; Weemaes et al., 1999). The temperature and storage duration adversely affected the -a* values of the green tea pastes. The degradation rate at 20 °C ($kc_1$) was the highest among the different storage temperatures tested for all types of green tea paste (Table 3). Half-life ($t_{1/2}$) of all the samples were calculated from the individual rate loss ($kc_{1\text{-}4}$) values, and data are presented in Table 3. The half-life indicated that, after 8.05±1.42, 9.62±0.56 and 8.15±1.12 weeks at 20 °C for Matcha, shaded, and unshaded Yabukita respectively, the visual green color of will degrade by 50%. The lower storage temperatures kept the maximum greenness values, and data were not significantly differed among the green tea pastes.

Table 3. Rate constants and activation energy values for color and ascorbic acid in green tea pastes, upon storage under different conditions.

| Quality parameters | Storage temperature | Kinetic parameters | Matcha paste | MWM green tea paste | |
|---|---|---|---|---|---|
| | | | | shaded Yabukita | unshaded Yabukita |
| Visual green color (-a*) | 20 °C | $kc_1$ (1/week) | 0.110±0.001[a] | 0.094±0.002[b] | 0.093±0.001[b] |
| | | $t_{1/2}$ (week) | 5.96±1.02[b] | 7.36±0.56[a] | 7.37±1.12[a] |
| | | $R^2$ | 0.97 | 0.98 | 0.96 |
| | | RMSE | 0.11 | 0.06 | 0.01 |
| | 4 °C | $kc_2$ (1/week) | 0.055±0.005[a] | 0.037±0.002[b] | 0.041±0.002[a] |
| | | $t_{1/2}$ (week) | 12.52±2.02[b] | 18.68±1.62[a] | 16.90±2.31[a] |
| | | $R^2$ | 0.96 | 0.95 | 0.98 |
| | | RMSE | 0.06 | 0.01 | 0.01 |
| | -18 °C | $kc_3$ (1/week) | 0.021±0.001[b] | 0.015±0.001[a] | 0.017±0.002[a] |
| | | $t_{1/2}$ (week) | 32.93±3.21[c] | 44.00±2.51[a] | 39.82±2.12[b] |
| | | $R^2$ | 0.99 | 0.98 | 0.99 |
| | | RMSE | 0.01 | 0.01 | 0.01 |
| | -60 °C | $kc_4$ (1/week) | 0.002±0.001[a] | 0.002±0.001[a] | 0.002±0.001[a] |
| | | $t_{1/2}$ (week) | 257.64±3.16[b] | 275.19±4.65[a] | 262.03±3.98[b] |
| | | $R^2$ | 0.99 | 0.97 | 0.98 |
| | | RMSE | 0.01 | 0.01 | 0.01 |
| | Activation energy | Ea (kJ/mol) | 24.09±1.03[a] | 21.37±0.78[b] | 22.57±1.31[b] |
| | | $R^2$ | 0.99 | 0.97 | 0.98 |
| Ascorbic acid | 20 °C | $ka_1$ (1/week) | 0.034±0.001[b] | 0.039±0.002[a] | 0.028±0.001[c] |
| | | $t_{1/2}$ (week) | 22.23±1.31[a] | 17.76±1.02[a] | 24.75±1.24[a] |
| | | $R^2$ | 0.969 | 0.991 | 0.987 |
| | | RMSE | 0.001 | 0.001 | 0.002 |
| | 4 °C | $ka_2$ (1/week) | 0.021±0.003[a] | 0.023±0.001[a] | 0.019±0.001[a] |
| | | $t_{1/2}$ (week) | 33.00±1.11[a] | 28.86±1.21[b] | 30.13±1.23[b] |
| | | $R^2$ | 0.987 | 0.976 | 0.976 |
| | | RMSE | 0.001 | 0.001 | 0.001 |
| | -18 °C | $ka_3$ (1/week) | 0.009±0.001[a] | 0.009±0.002[a] | 0.008±0.001[a] |
| | | $t_{1/2}$ (week) | 63.30±2.05[a] | 77.00±3.67[b] | 86.62±2.87[c] |
| | | $R^2$ | 0.986 | 0.961 | 0.982 |
| | | RMSE | 0.001 | 0.003 | 0.002 |
| | -60 °C | $ka_4$ (1/week) | 0.002±0.001[a] | 0.002±0.001[a] | 0.002±0.001[a] |
| | | $t_{1/2}$ (week) | 173.25±5.89[a] | 173.25±9.67[b] | 173.25±11.02[b] |
| | | $R^2$ | 0.976 | 0.988 | 0.988 |
| | | RMSE | 0.002 | 0.001 | 0.001 |
| | Activation energy | Ea (kJ/mol) | 17.96±2.23[a] | 18.47±1.58[a] | 17.20±3.01[a] |
| | | $R^2$ | 0.98 | 0.98 | 0.98 |

All values are of means of triplicate determination expressed on mean ± standard deviation. Mean values in the following row sharing a common letter are not statistically significant ($p<0.05$).

The Arrhenius model describes the temperature-dependent changes in the visual green color of the green tea pastes. The degradation rate with respect to the different storage temperatures was plotted using the Arrhenius model as shown in Fig. 4d. The activation energy was estimated by using equation 6, and data are presented in Table 3. Higher activation energy implies that the degradation of visual green color in the Matcha paste (Ea = 24.09±1.03 kJ/mol) is more susceptible to temperature elevation than the shaded Yabukita paste (Ea = 21.37±0.78 kJ/ mol) and unshaded Yabukita paste (Ea = 22.57±0.78 kJ/mol). The activation energy of visual green color (-a*) change was reported in the range between 30 and 110 KJ/mol while heating the sample from 70 to 100 °C (Gaur, Shivhare, Sarkar, & Ahmed, 2007; Steet & Tong, 1996).

Ascorbic acid is a highly heat-sensitive compound, and a loss of ascorbic acid is caused by high processing temperature and storage (Farnworth et al., 2001; Righetto & Netto, 2006). The loss of ascorbic acid in green tea paste during storage at different temperatures was determined. The degradation of ascorbic acid in all green tea pastes followed first-order kinetics, as shown in Fig. 5a-c. Ascorbic acid degradation during storage between 4 to 35°C generally follows first-order kinetics as reported in several studies (Bosch et al., 2013; Burdurlu et al., 2006; Sapei & Hwa, 2014). Estimates of the rate constant (k) and their standard errors at each temperature (20, 4, -18, and -60 °C) are presented in Table 3. The highest degradation rate (0.039±0.002 /week) was observed at 20 °C in shaded Yabukita green tea paste while the lowest degradation rate (0.001 to 0.002±0.001/week) was observed at -60 °C in all types of green tea pastes. The results indicated that the ascorbic acid content of shaded Yabukita green paste is more susceptible to higher storage temperatures. This may be due to the smaller particle size of the green tea paste, which accelerate biochemical reactions and as a result, ascorbic acid degrades at a faster rate. The reaction mechanism of decomposition of ascorbic acid in foods has been studied extensively. During storage, ascorbic acid degrades aerobically and anaerobically at different rates, depending on the storage conditions, packaging, and processing method (Kabasakalis, Siopidou, & Moshatou, 2000; Kennedy, Rivera, Lloyd, Warner, & Jumel, 1992). The order of the degradation rate of all types of green tea pastes was 20 °C > 4 °C > -18 °C > -60 °C. A temperature-dependent Arrhenius model was constructed for the degradation of ascorbic acid of the green tea paste.

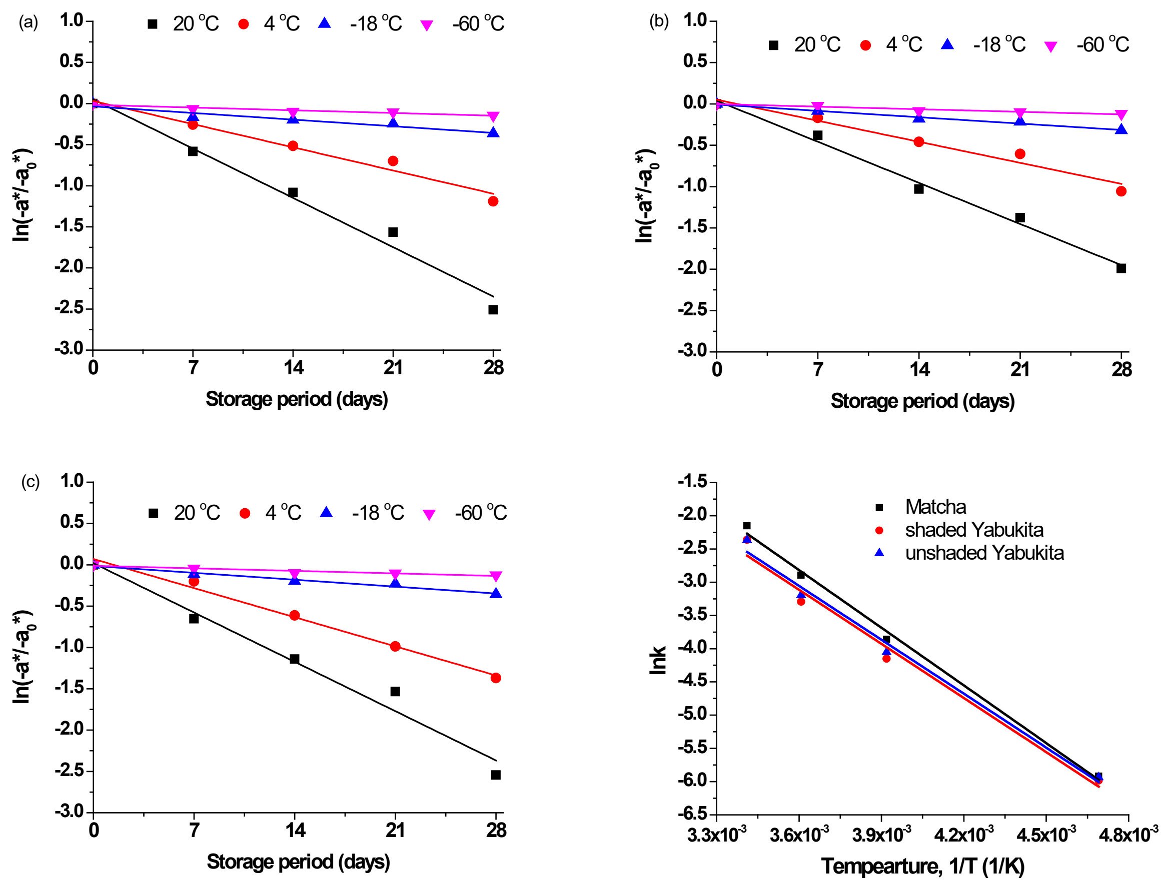



Fig. 4. First-order degradation of visual green color in (a) Matcha paste (b) shaded Yabukita paste (c) unshaded Yabukita paste, and (d) Arrhenius plot for degradation of color in different green tea paste during storage

The temperature-dependent degradation rate constant was determined by the Arrhenius plot as shown in Fig. 5d. Variation of degradation rate constants of ascorbic acid with storage temperature obeyed the Arrhenius relationship. The activation energy during storage at 20, 4, -18, and -60 °C for four weeks was estimated by using equation 3, as shown in Table 3. The activation energy for all types of green tea pastes did not vary significantly. We found the activation energy in the range of 18.47±1.58 to 17.20±3.01 kJ/mol, which was much lower than the values reported earlier that lay in the range of 42–105 kJ/mol, for ascorbic acid degradation during storage of different citrus juices over the temperature range of 0 – 45 °C (Polydera, Stoforos, & Taoukis, 2003; Van Bree et al., 2012).

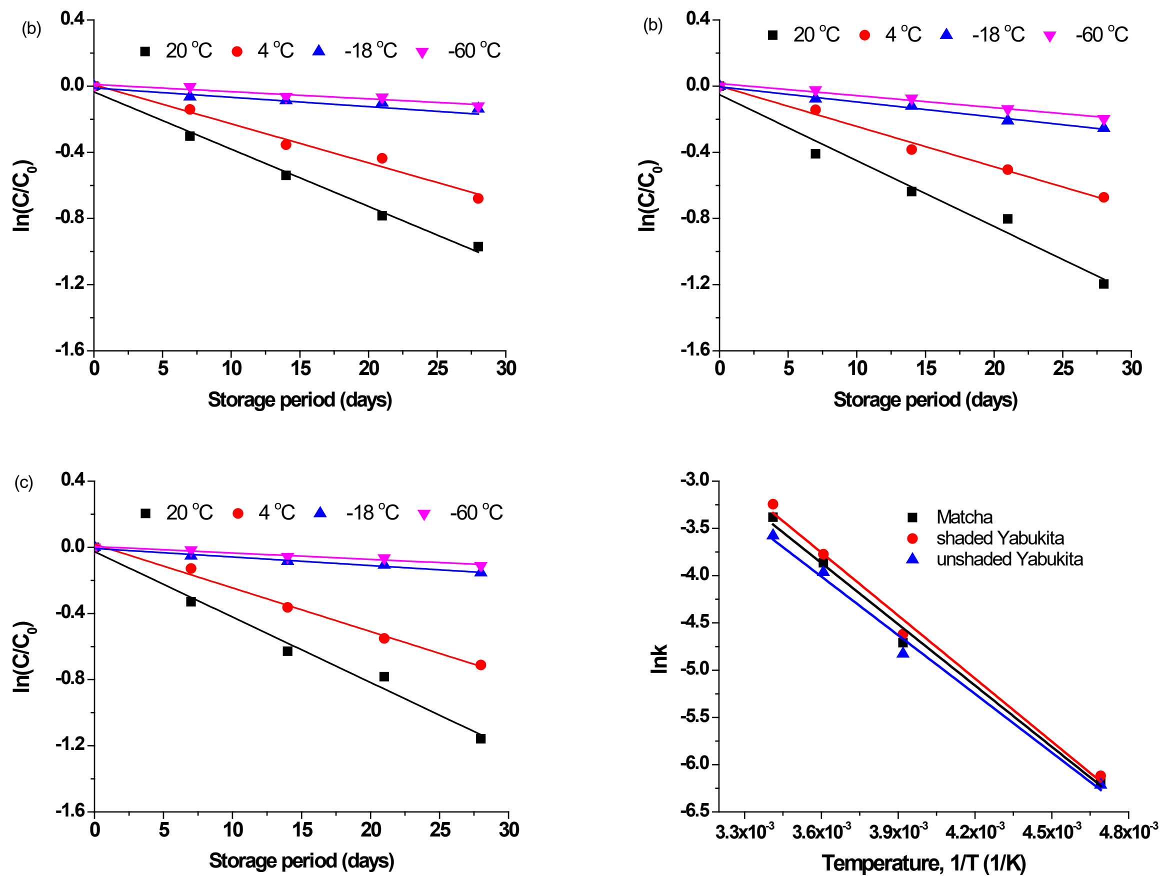



**Fig. 5.** First-order degradation of ascorbic acid in (a) Matcha paste (b) shaded Yabukita paste (c) unshaded Yabukita paste during storage, and (d) Arrhenius plot for degradation of ascorbic acid in different green tea paste during storage

### *3.5. Total viable count*

TVC is the numerical estimation of the concentration of microorganisms in a sample, including bacteria, yeast, and mold spores. In the present study, the TVCs of the green tea pastes were analyzed and the data are presented in Fig. 6a-d. The result shows a constant microbial load ranging from $1\times10^2$ to $1\times10^3$ cfu/g, when the green tea pastes were stored at a cold temperature such as -18 °C and -60 °C. Our findings agreed with Geiges (Geiges, 1996), who reported that the number of microorganisms in frozen food remains virtually constant during storage at -30 °C or below and there is no growth of microorganisms at -18 °C. The highest TVC was recorded at 20 °C in all types of green tea pastes, and it crossed the permissible limit of $1\times10^4$ cfu/g within one week. According to Codex Alimentarius (1997) and Gulf Standards (2000), the permissible limit of the total microbial load is in the ranges of $1\times10^4$ to $1\times10^5$ cfu/g. A higher recommended TVC of $\leq1\times10^7$ cfu/g for tea (*Camellia sinensis*) was reported by the European Tea Committee (ETC) and European Herbal Infusions Association (EHIA), 2018). Among the green tea pastes, shaded Yabukita contained a higher microbial load of $8.3\times10^5$ to $1.27\times10^8$ cfu/g between 1st week and 4th week at ambient conditions (20 °C). This may be attributed to the effects of smaller particle size, and higher water activity (aw = 0.94), both of which may accelerate the microbial growth. Another possible reason for the higher colony count in the shaded green tea paste is that it contained lower levels of phenolic compounds compared to the unshaded green tea paste. Several researchers have reported that green tea polyphenols (mainly catechin) have potential antimicrobial activity, and are found in higher amounts in unshaded green tea (Sano et al., 2018; Taguri, Tanaka, & Kouno, 2004; Taylor, Hamilton-Miller, & Stapleton, 2005). Among the green tea pastes, dry milled Matcha showed lower microbial counts than the MWM green tea paste. The heat produced during dry milling could kill microorganisms, resulting in lower TVCs. The MWM Hojicha paste showed a total microbial count in the range of $2.3\times10^5$ to $5.1\times10^8$ cfu/g at 20 °C.

All green tea pastes contained less than the permissible number of TVCs ($\leq 1\times 10^5$) until the third week when stored at 4 °C.

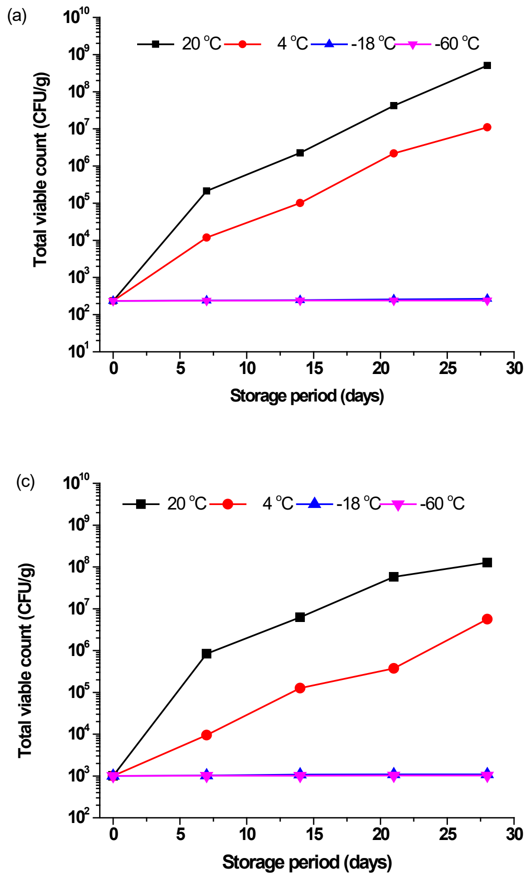



**Fig. 6.** Total viable counts in (a) Matcha paste, (b) shaded Yabukita paste, (c) unshaded Yabukita paste and (d) Hojicha paste during storage

## 4. Conclusion

A comparative study between dry milled Matcha paste and MWM green tea paste showed that MWM had better physical and functional properties than dry milled Matcha. In terms of physical characteristics, MWM green tea paste showed excellent solubility in cold water, more smooth (smaller particles) and better visual green color. Regarding functionality, MWM green tea paste exhibited higher ascorbic acid, total phenolic content and antioxidant activity than Matcha paste. Among the all green tea pastes, shaded Yabukita one demonstrated the better physical and nutritional quality than the other green tea pastes. On the other hand, storage tests revealed that all MWM green tea pastes were degraded slightly faster than the Matcha paste at 20 °C. A kinetic study indicated that the degradation rate of ascorbic acid and color did not differ significantly during cold storage. The TVCs of all the green tea pastes were done, and results show that all green tea pastes reached impermissible levels of TVC within one week at 20 °C. Whereas storage under refrigeration (4 °C) kept the viable count within the permissible limit for 21 days and no microbial growth was observed under frozen storage (-18 °C and -60 °C). The MWM green tea paste could be used as a food additive or in the development of value-added products, enriched with more functional compounds than conventional green tea paste.

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**Table caption**

**Table 1.** Physical properties of matcha and different micro wet milled green tea pastes.

**Table 2.** Antioxidant properties of matcha and different micro wet-milled green tea pastes.

**Table 3.** Rate constants and activation energy values for color and ascorbic acid in green tea pastes, upon storage under different conditions.

**Figure caption**

**Fig. 1.** Micro wet milling system: (a) schematic diagram of MWM system, (b) Upper milling stone (c) Lower milling stone.

**Fig. 2.** Effect of milling on particle morphology (SEM image) of green tea paste (a) Matcha green tea pasted (b) micro wet milled green tea paste; Effect of MWM operational parameters on particle sizes: (c) water to green tea ratio and feeding rate; (d) rotational speed and feeding rate.

**Fig. 3.** Comparison on the (a) particle size distribution and (b) particle size of the different green tea pastes.

**Fig. 4.** First-order degradation kinetics of visual green color in (a) Matcha paste (b) shaded Yabukita paste (c) unshaded Yabukita paste, and (d) Arrhenius plot for degradation of color in different green tea paste during storage.

**Fig. 5.** First-order degradation kinetics of ascorbic acid in (a) Matcha paste (b) shaded Yabukita paste (c) unshaded Yabukita paste during storage, and (d) Arrhenius plot for degradation of ascorbic acid in different green tea paste during storage.

**Fig. 6.** Total viable counts in (a) Matcha paste, (b) shaded Yabukita paste, (c) unshaded Yabukita paste and (d) Hojicha paste during storage.

**Author contributions:**

Md Zohurul Islam: Formal analysis, Investigation, Data Curation, Writing - Original Draft

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Mito Kokawa: Validation, Writing - Review & Editing, Supervision

Shinya Fujii: Sample collections, Review & Editing