

Article

Effect of Conventional and Microwave Heating on Protein and Odor Profile in Soymilk Powder

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Abstract: Soymilk contained serine protease enzymes with inhibitory activity against trypsin, causing a negative effect on nutritional absorption. This project aimed to investigate the effects of conventional heating (100 °C/20 min) and microwave heating (360–900 W/1–6 min) on trypsin inhibitor, protein digestibility, and odor profiles. Soymilk contained 46–47% protein, regardless of heating conditions. Using scanning electron microscopy, it can be seen that the conventionally heated sample had a smooth surface and a porous structure, whereas microwave-heated samples contained a protein matrix in clusters with rough surfaces. The molecular weight of proteins in SDS-PAGE was reduced to 19 kDa and <16 kDa after conventional and microwave heating, respectively, resulting in the reduction of trypsin inhibitor from 36.66 to 0.91 and 0.56–0.66 mg/g, respectively. Protein digestibility was significantly improved when either the conventional heating at 100 °C for 20 min or the microwave heating at 900 W for 6 min was applied. From e-nose analysis, the beany flavor was reduced after the microwave heating for 3–6 min, and the highest intensity of the sweet odor compounds were observed after the microwave heating at 600 W for 1 min. Due to the short processing time, microwave heating was then recommended for inhibition of trypsin inhibitors and improving protein digestibility.

Keywords: plant-based milk; soy protein; protein digestibility; trypsin inhibitor; odor; E-nose; green technologies



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1. Introduction

Soybean is one of the most popular legumes due to its high content of macronutrients such as proteins, carbohydrates, and lipids. Its composition may vary depending on the varieties and the growing conditions [1]. Moreover, soymilk is becoming popular worldwide due to its nutritional benefits, lactose-free property, and the increasing demand for plant-based protein. The United States Department of Agriculture (USDA) has reported that soymilk is rich in protein, calcium, potassium, fatty acids, and vitamins (e.g., vitamin A and D). However, the plant-based proteins showed lower digestibility than the animal-based proteins [2]. From previous studies, the in-vitro digestibility of soy protein varied from 66.1% [3] to 76% [4], while the digestibility of milk proteins: casein, whey, and milk protein meal (295 mmol N) were 74.4% [3], 75.5% [4], and 95% [5], respectively.

Soybean consists of a variety of anti-nutritional bioactive compounds that inhibit the digestion and assimilation of the foods nutrients, such as trypsin inhibitor (TI), antivitamin, phytate, and hemagglutinin. TI is a class of serine protease enzymes that can lessen the biological activity of chymotrypsin and trypsin, a proteolytic enzyme that is important for the digestion of proteins in living organisms. Trypsin is produced in its inactive form (called trypsinogen) in the pancreas, which is then activated during digestion as it enters the small intestine [6]. Consumption of TI can cause pancreatic hypertrophy or hyperplasia, due to an increase in the acinar cells of the pancreas in the body. Then, it can interfere dietary protein digestibility, the absorption process, and pancreatic secretory activity. TI activity in soybean was up to 94.1 U/m [3,7]. To inactivate TI, several ways have been developed. Among

those ways, thermal treatments have become the most widely employed [8]. However, the treatments cause nutrition loss (such as DPPH free radical-scavenging activity [9], loss of vitamins and amino acid [10]), affect functional properties (such as color degradation and flavor from the cause of the Maillard reaction [10,11] and protein particle, brought on by an increase in 11S content after heating [12], and consume a lot of energy [13].

TI inactivation in soymilk was mostly carried out at 93–121 °C. The use of a low temperature would take more time, whereas the use of a high temperature would shorten the processing time. For example, heating at 93 °C for 60–70 min or at 121 °C for 5–10 min could inactivate the TI in soymilk by 90% [14]. The United States Food and Drug Administration (FDA) recommends manufacturers to reduce TI activity by at least 80%, for any residual activity to not interfere with the protease's ability for protein digestion or human health. Moreover, in animal feed, trypsin doses should be less than 4 mg/g in order to provide animals with beneficial nutrients [15]. However, FDA recommendations thermal treatment that achieved at least 80% inactivation should also lead to nutritional and sensory changes and functionality loss.

A microwave is in an electromagnetic field consisting of both a magnetic field and an electric field produced by positively or negatively charged particles [16]. The waves oscillate, with a frequency from 300 to 300,000 MHz, in both phase and planes perpendicular to the direction of wave propagation and propagate in free space at the speed of light [17]. It is a nonionizing radiation that causes molecular motion by the movement of ions and rotation of dipoles, resulting in fast volumetric heating [18]. The amount of heat generated in the product during the microwave treatment was related to dielectric properties of the product. The dielectric constant (ϵ') determined the ability of the material to absorb the microwave energy while the loss factor (ϵ'') determined the ability to convert the absorbed microwave energy into heat. These dielectric properties were dependent on frequency, temperature, density, and moisture content of material [19]. For soymilk (at 20–70 °C), its dielectric constant and loss factor were in the range of 60.3–73.0 and 8.0–18.1, respectively [20] which had high potential to absorb and reasonably convert the microwave energy into.

The volumetric heating is different from conventional heating methods that transfer heat gradually from the outside to the inside of an object via convection, conduction, and radiation [21]. Therefore, microwave heating has less energy consumption and can be adapted to improve the quality of products [22]. Due to their high energy efficiency, microwaves are considered to be one of the best green technologies that can be used for food processing. Microwave treatment can be used for sterilization, pasteurization, and extraction of foods with a short processing time (approximately 50% of conventional processing time) [23]. Additionally, the evolution of microwaves has proven to be energy efficient in terms of the synthesis of food products, including various chemical processes [24,25]. From the previous study [17], microwave heating increased the in vitro protein digestibility of pigeon pea flour from 54 to 72%, due to changes in protein secondary structures. There was a 5% loss in β -sheets but a 5% gain in random coils, resulting in an increase in protein digestibility [26]. Moreover, microwaves could also reduce the amount of TI in velvet bean (*Mucuna pruriens* (L.)) by 100%, lentils (*Lens culinaris*) by 93.29%, black soybean by 76.91%, peanut seeds by 61.5%, and rice bran by 30.94% etc. [27]. In soymilk, by microwave treatment at 90 °C for 30 min, TI was reduced by 47% [7]. Increasing temperature to 100 °C for 10 min, TI in soymilk was reduced by 84% [2].

As mentioned above, soy protein has many health benefits. To increase bioavailability, TI should be thermally inactivated. However, heating for a long time might affect flavor and nutrients of soymilk. Therefore, this study aimed to use microwave processing to improve protein digestibility in soymilk. Its effects on protein, trypsin inhibitors, and volatile compound in soymilk would be determined and compared with those from the conventional heating. It would be useful to set up the green processing for soymilk in order to improve its nutritional availability.

2. Materials and Methods

2.1. Materials

Soybeans Material

Soybeans were purchased from a local company (Raitip, Thanya Farm Co., Ltd., Bangyai, Nonthaburi, Thailand). It was composed of 43.8% protein, 21.9% lipids, 23.4% carbohydrates, 6.5% dietary fiber, and 8.1% moisture content.

2.2. Soymilk Powder Preparation

To prepare soymilk, soybeans were cleaned and soaked at 25 °C for 24 h in water [2]. After soaking, it was washed in water for 3 times, then mixed with water in a ratio of 1:9 (*w/w*) using a blender (Otto, BE-127A, Otto Kingglass Co., Ltd., Bangkok, Thailand) for 3 min. To separate the solid from the soymilk, the obtained slurry was filtered through a 4-layer cheesecloth. Fresh soymilk was divided into two parts. The first part (100 g) was put into a beaker (with 15-mm thickness of soy milk layer) for conventional heating at 100 °C for 20 min using an induction stove (ZEBRA, ZB-ICV1900, Satien Stainless Steel Public Co., Ltd., Bangkok, Thailand). The second part (100 g) was put into a beaker (with 15-mm thickness of soy milk layer) for microwave heating (LG, MP-9482SR, LG Electronics, Seoul, Republic of Korea) at 360 W, 600 W, and 900 W for 1, 3, and 6 min. The experimental conditions of the microwave treatment were set from a preliminary study to obtain temperature of soymilk in the range of pasteurization, sterilization and UHT that had potential to reduce trypsin inhibitor [14]. The microwave was operated in a batch operation at a frequency of 2450 MHz, with a magnetron to convert electrical energy into microwave energy. The output powers of microwave were manipulated by a controller that controlled the ON-OFF time of a relay to obtain the average output power at the setting condition. In addition, a rotating plate (2.4 rpm) was used to ensure even distribution of the microwave for the uniform heating.

After heating, all soymilk samples were dried using a freeze dryer (Heto, Lyolab 3000, Thermo Electron Co., Ltd., Waltham, MA, USA) at −54 °C for 27 h. After that, the dried samples were ground and sieved to obtain soymilk powder with a particle size of 150 µm, and then stored at −18 °C for physical and chemical analysis with 3 replicates in each treatment.

2.3. Determination of the Structural Surface of Soymilk Powder

Microstructure of soymilk powder from fresh soy milk (control), conventional heating, and microwave heating was determined by scanning electron microscopy (SEM; FEI, FEG Quanta 450, Field Electron and Ion Co., Ltd., Hillsboro, OR, USA) with gold coating (Quorum, SC7620 Sputter Coater, Quorum Technology Co., Ltd., Lewes, UK). SEM images were acquired at 1000× and 10,000× of magnification.

2.4. Determination of Protein Content in Soymilk Powder

The protein content of soymilk powder from control, conventional heating, and microwave heating was determined using Kjeldahl's method [28]. Samples (0.5 g) were put into a digestion flask equipped with the Digest Automat (Buchi, K-360, Buchi Co., Ltd., Flawil, Switzerland). The samples were digested by H₂SO₄ with CuSO₄·5H₂O and K₂SO₂ as the catalyst and then heated at 420 °C. The amount of ammonia was determined by titration using a 0.1 mol of HCl solution. The nitrogen content was then multiplied by a sample-specific protein factor (5.71 for soy products) to obtain the protein content. The experiment was carried out with four replications.

2.5. Determination of the Molecular Weight of Proteins in Soymilk Powder

The molecular weight profiles of the soluble soymilk proteins were examined using SDS-PAGE with 15% acrylamide gel. A mixture of 5 mg of control sample, 5 mg of conventionally heated sample, and 15 mg of 600 W and 900 W microwave-heated sample were dissolved in distilled water (0.5 mL) and then centrifuged at 10,000 rpm for 1 min.

The supernatant part was selected for analysis. Standard molecular weight markers (20 µg of each sample) were mixed with 10 µL of 5X sample loading buffer containing 0.4 M dithiothreitol (DTT) and boiled at 100 °C for 10 min before loading onto 15% gel. SDS-PAGE was conducted at 150 V for 70 min. After 60 min running, gel was stained in the staining solution (15 mL) for 30 min. Each sample was de-stained for 4 times (15 min per time). The experiment was carried out with two replications. The molecular weight of proteins was estimated.

2.6. Determination of Trypsin Inhibitors in Soymilk Powder

A total trypsin inhibitor assay was performed using the approach provided by Vanga et al. (2020) [2]. Freeze-dried soymilk powder (0.5 g) was mixed with 25 mL of 0.01 mol NaOH at ambient temperature (25 °C) for 3 h with steady shaking, leading to a pH of 8.5. The supernatant (1 mL) was collected and diluted with deionized water until the two solutions could inhibit 40–60% trypsin activity. The 2 mL of trypsin solution (8 mg in 200 mL of 0.001 mol HCl) was mixed with 1 mL of diluted soymilk supernatant in 15-mL tubes. These tubes were pre-heated to 37 °C in a water bath. Then, the 5 mL of preheated (to 37 °C) benzyl-DL-arginine-para-nitroanilide (BAPNA) solution (0.08 g BAPNA in 2 mL dimethyl sulfoxide, which was diluted to 200 mL using pre-heated tris-buffer (pH = 8.2)) was added to the tubes and vortexed. After incubation at 37 °C for 10 min, the reaction was stopped using 30% acetic acid (1 mL). After that, the samples were centrifuged at 2500 rpm for 10 min. The absorbance of the supernatant was determined using a spectrophotometer at 410 nm (Shimadzu, UV-1900, Kyoto, Japan). The DI water and trypsin from bovine pancreas (essentially salt-free, lyophilized powder, ≥9000 BAEE units/mg protein, Sigma-Aldrich (Merck SA, Darmstadt, Germany), QC, Canada) were used as blank and standard trypsin, respectively [8]. Trypsin inhibitor (TI) content was calculated using Equation (1) [29].

$$TI \text{ (mg/g of sample)} = \frac{\text{Difference in absorbance}}{0.019 \times \text{sample weight (g)}} \times \frac{\text{Dilution factor}}{1000 \times \text{sample size (mL)}} \quad (1)$$

2.7. Determination of In Vitro Protein Digestibility in Soymilk Powder

In vitro protein digestibility (IVPD) of soymilk powder from control, conventional heating, and microwave heating was determined as described in the previous research using pepsin (#P-7012, Sigma-Aldrich, Oakville, ON, Canada) and pancreatin (#P-1625, Sigma-Aldrich, Oakville, ON) [2,30]. Soymilk powder (0.5 g) was mixed with 20 mL phosphate buffer (0.01 M, pH 7.0) vortex and incubated at ambient temperature (25 °C) for 30 min. Then, the mixture (2 mL) was collected to determine the initial protein content. As digestion progressed to the second step, the pH of the remainder was adjusted to 1.5 using 1 M HCl. Then, 100 µL pepsin solution (10 mg pepsin/mL in 0.01 M HCl) was added. Following a 30-min incubation at 37 °C, each sample was mixed with 1.0 M NaOH solution (100 L) to terminate the second stage digestion. Then, pH was adjusted to 7.8, before adding 300 µL pancreatin solution (10 mg/mL in sodium phosphate buffer, pH 7.0) to begin the third-stage digestion, which was incubated at 40 °C for 1 h. To terminate the overall digestive stages, 100 µL of a 150 mM Na₂CO₃ solution was added. The Smart BCA Protein Assay Kit (iNtRON Biotechnology, Inc., Gyeonggi-do, Republic of Korea) was used to calculate the protein content in soymilk samples before and after digestion. The in vitro digestibility of soymilk proteins was calculated using Equation (2).

$$IVPD(\%) = \frac{P_0 - P_1}{P_0} \times 100 \quad (2)$$

where P_0 is the initial protein content (µg/mL). P_1 is the final undigested protein content (µg/mL).

2.8. Determination of Volatile Flavor Profiles in Soymilk Powder

The freeze-dried soymilk samples (0.2 g) were placed into a 20-mL headspace vial. Then deionized water (2 mL) was added and vortexed for 10 s before immediately sealing with PTFE/silicone septa cap. The sample vials were placed in the auto-sampler tray to analyze volatile compounds using an electronic nose (E-nose) with metal oxide sensors, following the principle of gas chromatography (Alpha MOS, GC E-nose Heracles, Toulouse, France). The sample was initially incubated at 50 °C for 20 min to allow the volatile compounds to reach equilibrium in the gas phase of the headspace vial. Set up conditions were as follows: agitation speed at 500 rpm (5 s working and 2 s pause), injection volume at 5000 µL, injection speed at 200 µ/s, injector temperature at 200 °C, inject time at 30 s, trapping temperature at 50 °C, trapping duration at 37 s, detector temperature at 260 °C, and syringe temperature at 60 °C. The sample in gaseous phase was detected with two post-column (MXT-5 and MXT-1701) hydrogen flame ionization detectors. Data of volatile compounds were recorded and processed using the Alpha Soft Version 2021 program (Alpha M.O.S., Toulouse, France).

2.9. Statistical Analysis

All experiments were conducted in triplicate with data presented as mean ± standard deviation. An analysis of variance (ANOVA) was carried out to determine the variance of the data. Duncan's New Multiple Range Test was employed to determine a significant difference among the processing conditions of soymilk samples at the $p \leq 0.05$ level. IBM SPSS Statistics 28.0 software (Thaisoftup Co., Ltd., Bangkok, Thailand). In the experiment on flavor profiles, E-nose data were exported. Principal component analysis (PCA) using correlation and cluster analysis at the 95% confidence level were performed using the XLSTAT statistical software (XLSTAT-Sensory 2022 by Lumivero) for Microsoft Excel.

3. Results and Discussion

3.1. Effect of Heating Conditions on the Morphology of Soymilk Powder

The morphology of the soymilk powder with and without treatment were determined by scanning electron microscopy. The morphology of the control soymilk powder was shown in Figure 1A, which had no protein clusters but a smooth surface and little porosity. After being conventionally heated (Figure 1B), the surface of soymilk became more porous, possibly resulting in a high sulfhydryl content and contributing to a gel texture after cooling [12]. The yellow circle presented a cluster of soybean oil, which was not detected in the control sample but was observed in some heated samples. Reaction at the water-oil interface may provide a physical barrier and prevent droplet coalescence, causing a linked crystal network at the interface [31,32].

For microwave heating at 360 W for 1 min, the temperature was increased from 18 °C to 50 °C (Table 1). The characteristic of the porous structure (red arrow) was similar to the control, but its pores were slightly wider, due to rapid heating during microwave treatment generating high vapor pressure and porosity (Figure 1C). By increasing microwave heating time from 1 min to 3 min and 6 min, temperature was increased to a range of 87–89 °C, and protein aggregation occurred, appearing as clusters and rough surfaces (Figure 1D,E). This was in agreement with Zhang et al. [33], who found extending the heating time led to more holes or a larger cavity on the surface. Particle aggregation could then be clearly seen on the surface.

Similarly, increasing the microwave power could increase the degree of protein aggregation, clustering, and roughness of the surface due to the increased temperature in the range of 67–91 °C (Figure 1F–K). Due to the increased microwave power, the heating rate could be increased and then affected the hydrophilic groups locating on the protein surface [34]. Eventually, it affected the denaturation and aggregation of proteins [35]. In addition, the formation of a lipid film could be observed. Lipid had surface-contraction interfacial films assembled with protein and polysaccharide components. The high temperature helped to disperse water and lipid homogeneously, decrease particle size, and

promote a uniform distribution of protein [36]. Generally, particle sizes of soymilk protein could be classified into three types: small globules (<20 nm), small stable protein particles (20–40 nm), and large protein particles (>40 nm). The majority of protein in soymilk is normally in large particles (around 100 nm) [37].

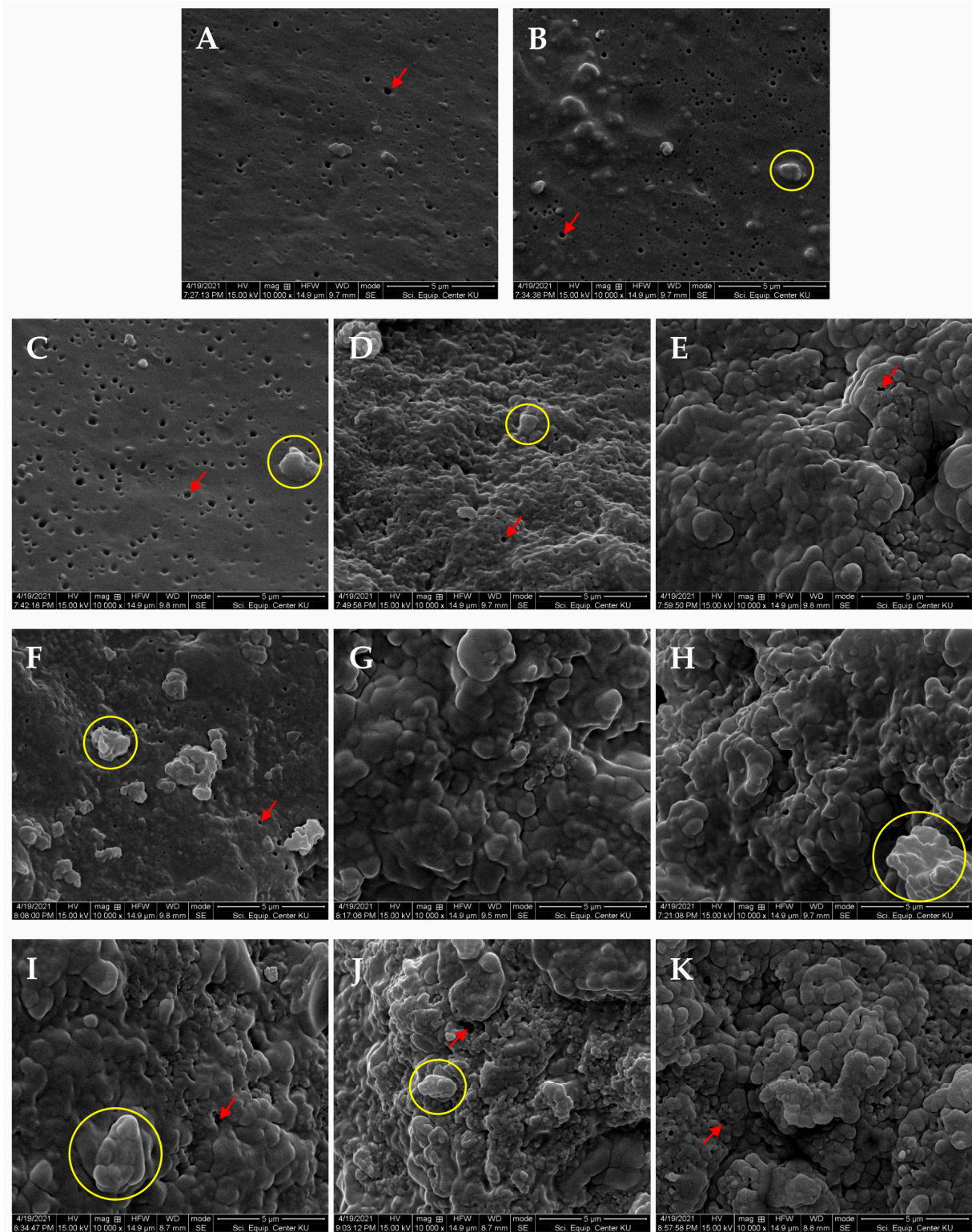


Figure 1. SEM of soymilk powder from control (A), conventional heating (B), microwave heating at 360 W for 1 min (C), 3 min (D), and 6 min (E), microwave heating at 600 W for 1 min (F), 3 min (G), and 6 min (H), microwave heating at 900 W for 1 min (I), 3 min (J), and 6 min (K).

Table 1. Temperature of sample during treatment and protein content in soymilk powder.

Process Parameters	Heating Process	Holding Time (min)	Temperature (°C)			Energy (kW·h)	Protein Content ^{ns} (%)
			Initial	Maximum	Final		
Control (raw soymilk)	-	-	19	19	19	0	46.71 ± 0.21
Conventional heating	100 °C 360 W	20	20	100	84	0.167	45.65 ± 0.36
		1	18	87	50	0.006	45.79 ± 0.63
		3	19	98	87	0.018	45.67 ± 0.99
		6	19	102	89	0.036	46.16 ± 0.49
Microwave heating	600 W	1	20	97	67	0.010	46.38 ± 1.64
		3	22	120	90	0.030	46.69 ± 1.98
		6	21	130	84	0.060	47.17 ± 1.86
	900 W	1	21	99	81	0.015	46.51 ± 0.41
		3	21	138	91	0.045	47.09 ± 1.76
		6	22	149	73	0.090	46.47 ± 0.23

^{ns} Values (mean ± standard deviation) in same column were not significantly different ($p > 0.05$).

3.2. Effect of Heating Conditions on Protein Content and Molecular Weight in Soymilk Powder

Protein content in all soymilk powder samples was in the range of 46–47% (Table 1), indicating that the heating condition did not affect the quantity of protein. In this study, the protein content of soymilk was consistent with that reported by Jinapong et al. [38], who reported the protein content of soymilk in a range of 48.79–50.31%. From the previous studies, protein content was 17.9–19.7% in almond milk powder [39], 21.43% in whey powder [40], and 25.50% in whole milk powder [41], which was less than that in soymilk. Therefore, soymilk has been used as a protein source for human foods for a long time.

Molecular weight of soluble protein in soymilk powder prepared by conventional and microwave heating was determined by SDS-PAGE gel electrophoresis (Figure 2). Comparing with the protein marker, the molecular weight of soluble protein in soymilk powder without pretreatment had major bands at 72, 52, 40, and 20 kDa, respectively. Soluble protein in the conventionally heated soymilk powder had the major bands at 72, 52, 38, and 19 kDa, respectively. By switching to microwave heating at 600 W and 900 W for 6 min, soluble proteins appeared at approximately <16 and 16 kDa, which were in a lower band than the control (raw soymilk). The control with temperature of 19 °C and the conventional heated samples had the band intensities of α' subunit (72 kDa), and α subunit (52 kDa) of β -conglycinin, acidic subunit of glycinin (38–40 kDa), trypsin inhibitor and basic unit (20 kDa) and glycinin acid polypeptide (19 kDa) [42,43].

Thermal treatments from both conventional heating and microwave heating increased maximum temperatures of soymilk to the range of 87–149 °C, resulting in structural rearrangements from the heat-related interactions between both hydrogen bonds involving in protein structure stabilization and those between amino acids and surrounding water molecules [42]. Due to the volumetric heating during microwave treatment associated with the dipolar rotation and the ionic mechanism, heating rate during the microwave treatment was faster; as well as, metamorphism was smaller than the conventional heating [7]. Consequently, trypsin inhibitor, the protein with molecular weight of 20.0–22.4 kDa [44,45] was not detected in the microwave-heated samples that reached the maximum temperatures at 130 °C and 149 °C during the microwave heating at 600 W and 900 W, respectively. In fact, protein patterns in the microwave-heated samples revealed electrophoretic bands corresponding to different subunits of B (acid and basic of glycinin) at 16 kDa and BX2 (oleosins) at <16 kDa [43,46]. In addition, microwave treatment also changed the secondary structure of proteins and decreased trimeric glycoprotein [42,47].

Increasing microwave power from 600 to 900 W did not further reduce the molecular weights of proteins in soymilk. Due to the absence of protein with a molecular weight of 20–22.4 kDa in all treated samples, both conventional heating and microwave heating could inhibit the trypsin inhibitor. Microwave heating at 600–900 W, on the other hand, reduced the processing time from 20 min in the conventional heating to 3–6 min that could reduce energy for heating by 53.89–82.04% (Table 1). The degree of reduction in protein molecular weight was dependent on the heating process condition and time [30].

Moreover, the reduced molecular weight of proteins may affect the quality and digestibility of soymilk proteins.

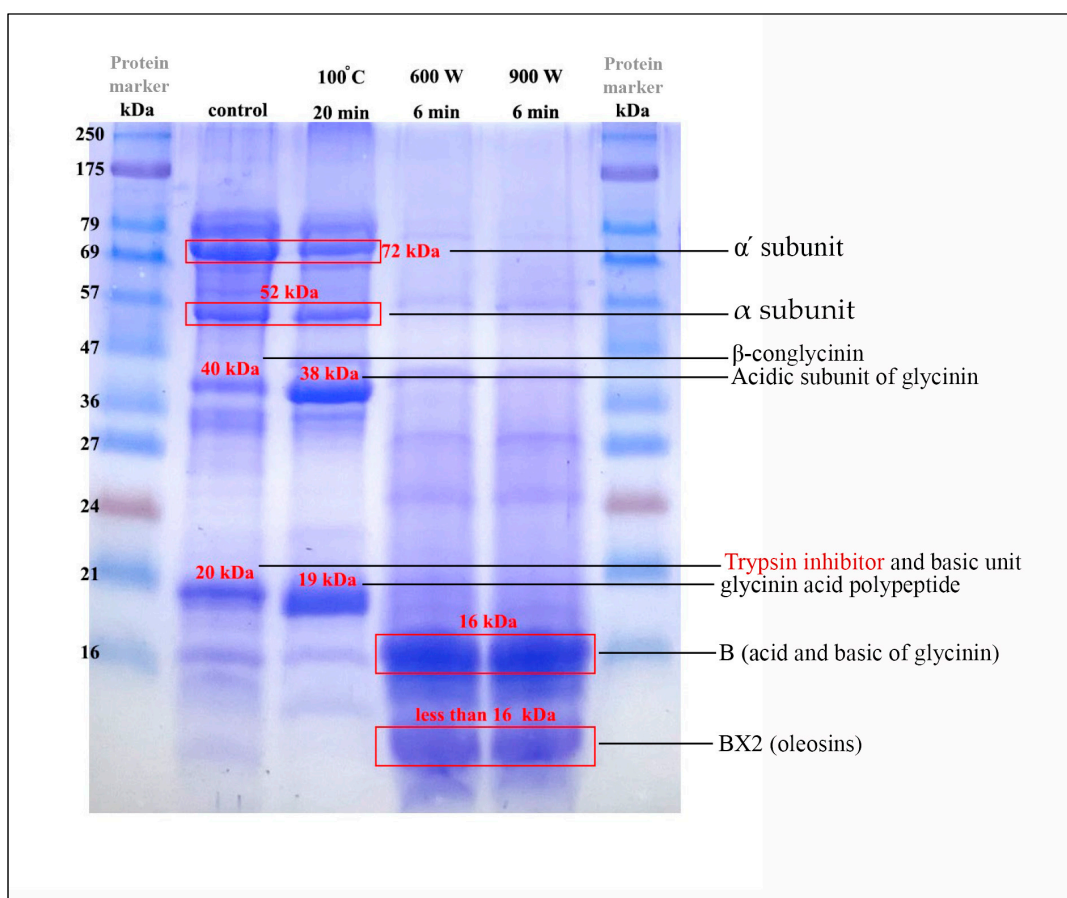


Figure 2. SDS-PAGE of proteins in control and treated soymilk powder.

3.3. Effect of Heating Conditions on Trypsin Inhibitor Activity in Soymilk Powder

The trypsin inhibitor content of soymilk without treatment (control) was 36.66 mg/g. After conventional heating, trypsin inhibitor content was significantly reduced to 0.91 mg/g. By switching to microwave heating, trypsin inhibitor content was decreased to a range of 0.56–0.66 mg/g, which was significantly lower than that of conventional heating in the shorten time (Table 2). According to FDA recommendation, TI activity should be reduced by at least 80%. In the case of soymilk, the initial trypsin inhibitor was 36.66 mg/g, thus the allowable concentration of trypsin inhibitor should be less than 7.33 mg/g. Therefore, both conventional heating and microwave heating conditions in this study could be used for reducing trypsin inhibitor. Although increasing microwave power might cause structural conformational changes in secondary protein structures during oscillating electric fields, which are usually related to digestion [47,48] and microwave heating was at a higher temperature than conventional heating during heating the samples, in this study, the increase in microwave power from 360 to 900 W did not cause a significant difference in trypsin inhibitor activity.

All treated soymilk from both conventional and microwave heating had a very small amount of trypsin inhibitor, compared with the control sample. This was coincided with the result from SDS-PAGE analysis, which found the disappearance of proteins with a molecular weight of 20.0–22.4 kDa after heating. Due to the volumetric heating, microwave processing needed only a few minutes to reach the temperature that could decrease the amount of trypsin inhibitor [49]. Therefore, the microwave treatment could be an alternative method to reduce trypsin inhibitor within short processing time. The conventional heating

required long holding times at a high temperature to obtain low residual trypsin inhibitor activity, possibly causing unintended losses of nutritious components such as cystine, arginine, and lysine, as well as changes in sensory quality, due to the development of the browning reaction and cooked flavor [7].

Table 2. Trypsin inhibitor and in vitro protein digestibility of soymilk powder.

No.	Process Parameters	Heating Process	Holding Time (min)	Trypsin Inhibitor (mg/g. db.)	In-Vitro Protein Digestibility (%)
1.	Control (raw soymilk)	-	-	36.66 ± 0.24 ^a	22.69 ± 0.56 ^c
2.	Conventional heating	100 °C	20	0.91 ± 0.00 ^b	40.31 ± 1.84 ^b
		360 W	6	0.56 ± 0.00 ^c	23.73 ± 1.26 ^c
3.	Microwave heating	600 W	6	0.66 ± 0.05 ^{bc}	25.94 ± 0.38 ^c
		900 W	6	0.65 ± 0.03 ^{bc}	55.67 ± 1.57 ^a

^{a–c} Values (mean ± standard deviation) in same column with different lowercase letters were significantly different ($p \leq 0.05$).

3.4. Effect of Heating Conditions on the In Vitro Protein Digestibility of Soymilk Powder

The in-vitro protein digestibility (IVPD) of soymilk powder from all treatments was determined. The control sample's protein digestibility was significantly lower than that of the heated samples. This was coincided with Zahir et al. [50], who reported that the protein digestibility of soybeans was improved after boiling, compared with uncooked soybeans. In this study, the highest degree of protein digestion (55.67%) was obtained from the microwave heating, followed by the conventional heating (40.31%). From previous study [51], IVPD of soymilk was varied in the range from 9.4% to 26.0%. In addition, the IVPD of the commercial soymilk was reported at 20.56% [52]. However, IVPD of soy protein isolate containing more than 80% protein was in the range from 66.1% [3] to 76% [4] that was much higher than IVPD in soymilk. It is possible that purity of protein in the soy protein isolate was much higher than that in the soymilk generally containing protein together with fat, starch and others compounds that could obstruct protein digestion.

In this study, the conventional heating at 100 °C for 20 min yielded significant improvements in IVPD, compared with the control, microwave 360 W and 600 W. This was since the heat transfer from outer to inner layers during the conventional heating provided the energy, causing high temperature to build up in sample slowly [21]. The high temperatures helped the structure change to the dense aggregation, leading to large molecular structures. However, heating for a long time caused protein denaturation, higher IVPD and a looser form [42,50].

For the case of microwave treatment in this study, the microwave power of 300–600 W for 6 min was not enough to significantly improve IVPD, although the molecular weight of proteins from microwave heating was much lower than the control. This was due to the fact that digestibility was not only related to molecular weight but was also dependent on other parameters such as structural property varying in porosity. In this study, the surface structure of the control (unheated) sample was smooth. After microwave heating at 300–600 W for 6 min, the surface structure was slightly different, compared with the control. However, the clear change in porosity was particularly observed after the microwave heating at 900 W for 6 min, which caused more porosity than microwave heating at 600 W and 360 W for the same processing time. As a result, microwave heat treatments increased IVPD of soymilk powder. Therefore, increasing microwave power to 900 W for 6 min maximized IVPD. Similar to Sun et al. [26], pigeon pea flours were treated using various treatments, including control, microwave, soaking, ultrasound, and grinding. Only microwave treatment significantly improved the degree of hydrolysis of the pea proteins to 71.6%, compared to the control's 54.4% ($p < 0.05$). The structure of the microwave-treated sample was changed and interacted with the protein matrix in clusters of the secondary

structures, including alpha-helix, beta sheet and turn, resulting in better digestibility of proteins [2,53].

3.5. Effect of Heating Conditions on Volatile Compound Profiles in Soymilk Powder

Distinctive traits of volatile compounds were marked as the basic characterization of the odor. By using suitable conditions for gas chromatography, volatile components of soymilk powder could be identified after exposure to the conventional heating and the microwave heating. The peak morphologies were achieved using an MXT-5 capillary column. Chromatograms of soymilk from different heating conditions showed 10 distinct peaks with the highest peak at retention time of 34.65 s from sensor 32.22-1, demonstrating ester compounds. Each volatile component and its retention time were identified using the AroChemBase database (Table 3).

Table 3. Possible compounds in soymilk powder and sensory description at each retention time.

No.	MXT-5		Relevance Index	Compound	Name	Formular	CAS	Sensory Descriptive
	Retention Time (min)	Sensor						
1.	15.67	15.62-1	86.44	Alcohols	Methanol	CH ₄ O	67-56-1	Alcoholic; Pungent; Strong
2.	17.49	17.42-1	91.96	Hydrocarbons	2-Methylbutane	C ₅ H ₁₂	78-78-4	Gasoline; Pleasant
3.	26.75	26.82-1	96.48	Other	1-Butanamine	C ₄ H ₁₁ N	109-73-9	Ammoniacal; Fishy
4.	34.65	32.22-1	86.37	Esters	Methyl butanoate	C ₅ H ₁₀ O ₂	623-42-7	Apple; Banana; Ester; Ethereal; Fruity; Green; Pineapple; Sweet
5.	40.01	41.67-1	98.76	Other	pyrrole	C ₄ H ₅ N	109-97-7	Chloroform; Coffee; cracker; Nutty; Sweet; Warm
6.	42.99	41.67-1	97.58	Aldehydes	(E)-3-Hexenal	C ₆ H ₁₀ O	69112-21-6	Apple; Fruity; Green
7.	48.07	52.61-1	95.95	Aldehydes	furfural	C ₅ H ₄ O ₂	98-01-1	Almond; Baked; benzaldehyde; Bread; Fragrant; Sweet; Woody
8.	49.07	52.61-1	94.54	Ketones	(E)-3-hexen-2-one	C ₆ H ₁₀ O	4376-23-2	-
9.	55.57	52.61-1	96.19	Aldehydes	(E,E)-2,4-hexadienal	C ₆ H ₈ O	142-83-6	Citrus; Floral; Green; Spicy; Sweet; Vegetable
10.	61.15	64.10-1	94.63	Ketones	1-octen-3-one	C ₈ H ₁₄ O	4312-99-6	Dirty; Dusty; Earthy; Herbaceous; Metallic; Mushroom; Musty

In this study, the main components identified in soymilk powder were esters or methyl butanoate, which provided a green odor. Soymilk is known for its unique beany flavor, also known as “grassy flavor” or “oxidized oil flavor” [54]. Temperature and time of heat treatment of soymilk played an important role on the green odor compounds, especially in the conventional heating and the microwave heating at 6 min that significantly decreased green odor ($p \leq 0.05$). In fact, exposure to the microwave caused the friction of molecules and cell ruptures, resulting in leakage of some compounds from cell [48]. Then the microwave treatment at 360 W for short time (1 min) showed higher green odor than the untreated samples. However, extending the microwave treatment for a longer time could generate more heat to evaporate some volatile compounds particularly the green odor. Therefore, the microwave treatment at 360 W for 3–6 min could reduce the green odor (Table 4). Similarly, an increase in microwave power possibly increased heating rate. As a result, the green odor compounds were reduced significantly after only 1 min of the microwave treatment at 600 W or 900 W.

With the applied heat from both conventional heating and microwave heating, other odor compounds including alcohols: methanol, aldehydes: (E)-3-hexenal, hydrocarbons: 2-Methylbutane, (E,E)-2,4-hexadienal, ketone: (E)-3-hexen-2-one, and others: 1-butanamine, pyrrole, and propyl cyclopentane were reduced, compared to the control condition (with the exception of the microwave treatment at 600 W for 1 min that showed an increase in the sweet odor compounds (pyrrole) significantly ($p \leq 0.05$)).

Table 4. Peak area at each retention time in control and treated soymilk powder.

Compounds	Peak Area										
	Control (Raw Soymilk)	Conventional Heating 100 °C 20 min	Microwave Heating 360 W 1 min	Microwave Heating 360 W 3 min	Microwave Heating 360 W 6 min	Microwave Heating 600 W 1 min	Microwave Heating 600 W 3 min	Microwave Heating 600 W 6 min	Microwave Heating 900 W 1 min	Microwave Heating 900 W 3 min	Microwave Heating 900 W 6 min
Alcohols	345.45 ± 16.10 ^a	319.02 ± 56.26 ^{ab}	337.20 ± 4.23 ^{ab}	144.08 ± 1.46 ^d	131.04 ± 6.82 ^d	277.89 ± 25.93 ^b	163.86 ± 8.58 ^{cd}	209.44 ± 50.76 ^c	112.00 ± 7.45 ^{de}	59.20 ± 3.13 ^e	272.00 ± 47.89 ^b
Hydrocarbons	2367.53 ± 107.17 ^a	150.71 ± 2.42 ^h	2223.65 ± 36.11 ^b	536.63 ± 24.56 ^{de}	366.99 ± 1.11 ^f	1320.27 ± 43.14 ^c	618.80 ± 29.51 ^d	244.44 ± 5.35 ^g	252.23 ± 29.03 ^g	454.71 ± 26.49 ^e	179.72 ± 5.01 ^{gh}
Other	1299.22 ± 81.40 ^b	76.56 ± 5.12 ⁱ	1843.48 ± 54.72 ^a	357.73 ± 17.95 ^{de}	300.10 ± 0.21 ^{ef}	782.53 ± 16.94 ^c	427.88 ± 18.99 ^d	193.83 ± 6.43 ^{gh}	228.74 ± 8.61 ^{fg}	327.06 ± 11.92 ^e	135.23 ± 7.67 ^{hi}
Esters	18142.42 ± 612.43 ^b	472.38 ± 147.16 ^h	23,722.11 ± 362.01 ^a	4746.38 ± 72.68 ^e	4379.44 ± 24.73 ^e	9059.87 ± 180.98 ^c	6048.86 ± 90.21 ^d	2216.05 ± 111.82 ^g	3217.16 ± 109.25 ^f	4408.08 ± 171.15 ^e	1783.05 ± 55.25 ^g
Others	898.09 ± 70.71 ^d	ND ⁱ	501.98 ± 2.97 ^d	641.68 ± 12.78 ^c	287.79 ± 9.72 ^e	2144.47 ± 9.21 ^a	1010.54 ± 21.11 ^b	133.44 ± 3.10 ^g	132.88 ± 13.47 ^g	160.88 ± 13.42 ^f	76.34 ± 2.28 ^h
Aldehydes	1809.76 ± 45.19 ^d	123.49 ± 32.81 ^b	396.67 ± 25.63 ^c	283.90 ± 7.55 ^e	261.08 ± 16.59 ^e	156.84 ± 10.49 ^f	290.27 ± 5.78 ^d	392.38 ± 2.09 ^b	462.25 ± 8.62 ^a	300.57 ± 7.37 ^d	168.86 ± 2.94 ^f
Aldehydes	1354.85 ± 38.08 ^a	189.89 ± 27.45 ^{ef}	597.70 ± 13.83 ^b	319.72 ± 3.08 ^d	309.06 ± 11.73 ^d	561.64 ± 18.66 ^b	387.60 ± 12.01 ^c	301.20 ± 25.42 ^{ef}	221.11 ± 15.61 ^{ef}	235.45 ± 22.09 ^e	182.31 ± 5.81 ^f
Ketones	1036.15 ± 56.92 ^a	472.38 ± 147.16 ^g	447.14 ± 62.37 ^{ef}	547.74 ± 9.89 ^{cd}	475.65 ± 8.32 ^{de}	560.13 ± 5.19 ^c	550.80 ± 31.81 ^{cd}	193.83 ± 6.43 ^{ef}	473.05 ± 27.79 ^{de}	639.61 ± 30.58 ^b	387.13 ± 3.61 ^{fg}
Aldehydes	612.80 ± 80.74 ^a	174.58 ± 21.35 ^b	105.17 ± 9.03 ^c	83.12 ± 2.13 ^c	126.96 ± 10.80 ^{bc}	83.21 ± 4.69 ^c	86.91 ± 2.93 ^c	104.15 ± 5.78 ^c	73.19 ± 4.36 ^c	81.18 ± 0.35 ^c	109.75 ± 5.05 ^c
Ketones	663.58 ± 124.90 ^a	236.93 ± 27.96 ^b	111.43 ± 12.06 ^c	128.88 ± 1.97 ^c	182.22 ± 17.54 ^{bc}	118.49 ± 4.31 ^c	113.99 ± 8.59 ^c	146.62 ± 6.22 ^c	117.02 ± 0.98 ^c	115.42 ± 12.57 ^c	129.08 ± 4.93 ^c

^{a–i} Values (mean ± standard deviation) in same rows with different lowercase letters were significantly different ($p < 0.05$). ND: not detected.

Based on the information of volatile compounds from E-nose analysis, the principal component analysis (PCA) plots for the unheated soymilk powder (control), conventional heating and microwave heating were demonstrated in Figure 3. The combined contribution of two main principal components (PC1 and PC2) reached 82.21% (64.765% from PC1 and 17.441% from PC2), showing that the PCA could explain variation in volatile compounds of both untreated and treated soymilk. Based on volatile compound profiles, all soymilk samples could be clearly clustered into five groups. The first group consisted of only a control sample or untreated soymilk powder wherein no thermal effect occurred. The second and the third groups consisted of the microwave heating for 1 min at 360 W and 600 W, respectively. Due to their unique odor compounds, the microwave heating at 360 W for 1 min showed the dominant green odor while the microwave heating at 600 W for 1 min showed the dominant sweet odor. The fourth group was related to the samples from the medium heat treatment either lower microwave power for longer time or higher power for shorter time, consisting of the microwave at 360 W for 3–6 min, 600 W for 3 min and 900 W for 1–3 min. Lastly, the fifth group was composed of those samples from intensive heating: conventional heating for 20 min, microwave heating at 600 W and 900 W for 6 min. The intensive heating enhanced the loss of volatile compounds, resulting in low intensity of green odor and other volatile compounds [55].

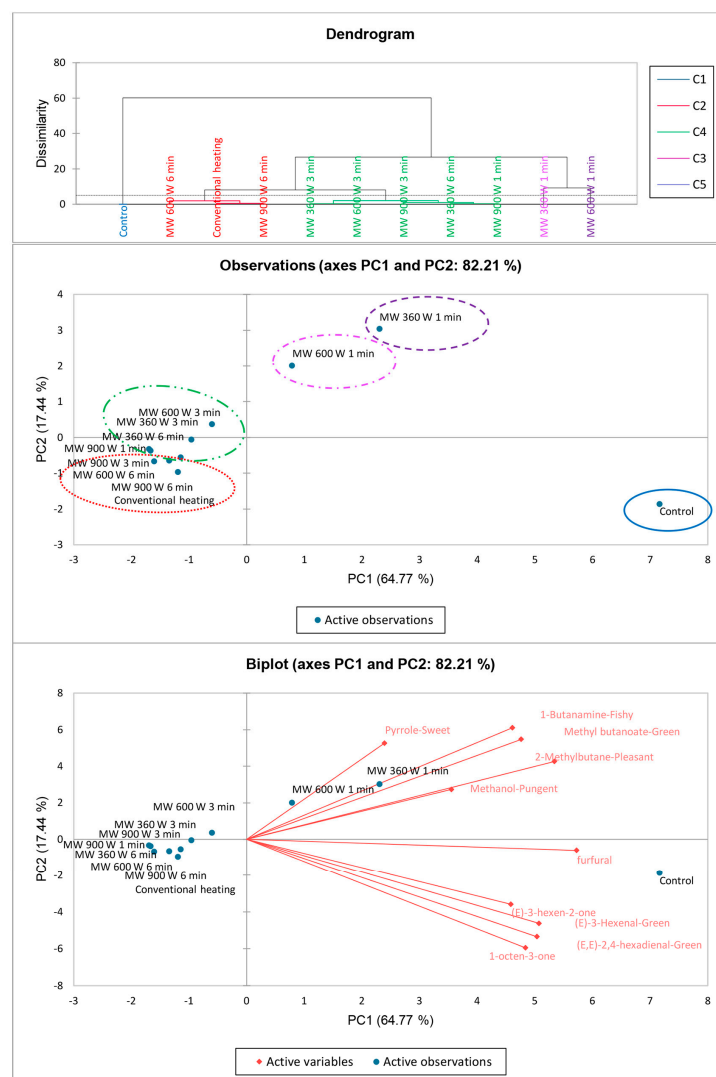


Figure 3. PCA plot of control and treated soymilk powder based on volatile compounds performed by E-nose.

4. Conclusions

To reduce trypsin inhibitor in soymilk from 36.66 to 0.65 mg/g (more than 80% reduction which was recommended by FDA) and improve protein digestibility from 22.69% to 55.67%, the microwave with frequency of 2450 MHz operated in a batch operation at 900 W for 6 min (to reach the maximum temperature at 149 °C) should be recommended. It provided a similar effect on protein characteristics to the conventional heating setting at 100 °C for 20 min without having a significant effect on protein content (45.65–47.17%). In addition, the microwave heating could be used for enhancing sweet odor compounds and reducing green odor compounds. Together with its short processing time (6 min) compared with the conventional heating (20 min), the microwave heating at 900 W could reduce the energy by 53.89%. Therefore, the microwave heating, one of many green technologies, could be an alternative heating method to inactivate the trypsin inhibitor and improve the nutritional availability and digestibility of proteins in soymilk products without compromising in odor quality. This knowledge could also be extended to apply to other foods and feeds that have the problem of active trypsin inhibitors in order to sustain nutritional availability for humans and animals. However, the transition from laboratory scale (a batch operation) to industrial implementation (a continuous operation) demands justification and optimization of the process parameters such as thickness of the processed layer of material, speed of conveyor movement, etc.

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