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Quality Assessment of Selected Pumpkins Based on their Dry Matter and Total Soluble Solids

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Abstract

THE STUDY was conducted to determine the role of dry matter and total soluble solids (TSS) as quality indicators in the assessment of pumpkins (Thong Lanna (TL) 2, TL7, TL11, TL17, and commercial cultivar (CC)). Pumpkins were categorized into two groups, sticky (TL11, TL17, and CC) and less sticky (TL2 and TL7), based on their adhesiveness value, wherein the adhesiveness had a highly negative association with amylose content. On the other hand, the starch content showed a highly positive association to amylose content. Less sticky pumpkins that contained high dry matter and total soluble solids (TSS) exhibited a high antioxidant (total flavonoid contents and total carotenoids) and antioxidant capacity (2,2-diphenyl-2-picryl-hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). The other traits observed in less sticky pumpkins were lower fruit weight and pulp firmness, and higher sweeter pulps and starch content than sticky pumpkins. A pumpkin with a high dry matter and TSS showed a low adhesiveness value. Moreover, the shape and sizes of starch granules did not indicate the stickiness of cooked pulp. Therefore, dry matter and TSS could be used to rapidly determine antioxidant capacity in these pumpkins, which would be convenient, whereas amylose content is for the stickiness of cooked pulp.

Keywords: Amylose content, Cooking quality, quality indicator, SEM, Stickiness.

Introduction

Food plays an essential role due to its health benefits and human perception, and a nutritious vegetable is also needed for human nutrition. Pumpkin (*Cucurbita moschata* Duchesne ex. Poir) is an important crop and source of innumerable nutritive compounds, including carotenoids, flavonoids, vitamins, and high health benefits minerals (Ajuru and Nmom, 2017; Gbemenou et al., 2022). Likewise, as part of the strategy program to meet the consumer's demand to secure quantity and quality of food, the plant breeders employ inbreeding of different crops. The developed pumpkin inbreds are tested under various agro-climatic conditions to determine their growth and yield. Therefore, the characterization of the physico-chemical and the antioxidant properties of their fruit is essential as the former.

Selecting different inbred crops, such as pumpkins, would take much work. Thus, an easy selection process yet manageable to use is needed, which only requires a short time to identify how good the quality it is. Dry matter and total soluble solids (TSS) are linked to the quality of fruits and

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vegetables. They are simple techniques and often employed as an indicator in the selection process. Likewise, the association of physical and chemical attributes with dry matter and TSS is used in the horticultural sector (Palaityte et al., 2017; Valverde-Miranda et al., 2021), thus, it shortens the selection procedures. TSS is a simple chemical analysis used as a harvest index, and dry matter served the same as the former, including for maturity and quality (Scalisi and O'Connell, 2021).

Previous studies reported that a pumpkin with a high dry matter exhibited a high vitamin C content, sugars, total phenolics, and flavonoids (Javaherdashti et al., 2012; Medelyaeva et al., 2021). Another study reported that dry matter and the TSS of cucumber showed a positive relationship (Valverde-Miranda et al., 2021). Still, dry matter and flesh firmness in apple fruit showed no association (Palmer et al., 2010). Moreover, dry matter and soluble solids content were reported to have a strong relationship (Travers, 2013; Scalisi and O'Connell, 2021). These studies prove that dry matter and TSS are good quality indicators in the selection process. However, the study on dry matter and TSS as quality indicators that affect the cooking quality (stickiness of cooked pumpkin) and antioxidant activity of newly inbred pumpkins is limited. Therefore, the objective of this work was to investigate the role of dry matter and total soluble solids (TSS) as quality indicators in the assessment in five pumpkin genotypes (Thong Lanna (TL2), TL7, TL11, TL17, and commercial cultivar (CC)) as part of the selection criteria.

Materials and Methods

Plant material and preparation

Organically grown different pumpkin (*Cucurbita moschata* Duchesne ex. Poir*)* inbreds (Thong Lanna (TL2), TL7, TL11, TL17) and F_1 hybrid (commercial cultivar (CC) with the same fruit maturity (45 days after flowering) were used in the study .The organically produced pumpkin fruits came from Rajamangala University of Technology Lanna, Lampang, Thailand .Each pumpkin fruit was washed, dried with a clean cloth, and weighed individually .Peeled and chopped raw fruit samples were applied with liquid nitrogen (N_2) , and steamed samples were stored at -20 ˚C for further analysis. The Thong Lanna pumpkin inbreds were selected because they were registered under the Plant Varieties Act of Thailand.

Fruit weight and dry matter

A digital weighing scale)Model P4102, OHAUS Corporation, USA ((was used to determine the individual fruit weight of all pumpkins .Raw and steamed samples (10 g each) were dried at 70 ˚C for 48 h using an oven (Memmert, Models 30-1060) following AOAC (2000).

Pulp color

Based on the Commission International del' Eclariage) CIE (color system) $L*$, $a*$, b *, (the pulp color of all pumpkins was recorded using a colorimeter) Model CR-400, Minolta, Japan .(The pulp color change of the pumpkins was determined using the equation below.

$$\Delta E = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

Pulp firmness and adhesiveness

Four pieces of equal thickness (2 cm) of sliced raw fruit were used. The analysis conditions for the raw samples were 5 mm/sec pre-test speed, test speed 1 mm/sec, and 1 mm/sec post-test speed using a 5 mm probe (cylinder) diameter. Five measurements of each piece of the raw sample were analyzed for firmness and adhesiveness, and the obtained values were averaged.

A steaming procedure for steamed samples was done previously to obtain cooking duration. Before steaming, a stainless cork borer was used to attain uniform 1 cm^3 pulp (length × width × diameter). Then, samples were steamed for 5 to 10 min in boiling water, and the steamed samples were taken out every 2 min and pressed between two glass petri dishes. Easily pressed samples were sufficiently cooked, and the steaming preparation lasted 10 min. Finally, the steamed sample was cooled for 5 minutes at room temperature (28 ± 2˚C).

The 20 pieces of steamed pulps for each experimental batch were used to analyze the firmness and adhesiveness. The test was done following the analysis conditions: 1 mm/sec pre-test speed, 1 mm/sec test speed, and 5 mm/sec post-test speed using a 35 mm probe (cylinder) diameter. The averaged values were recorded. The textural profile of raw and steamed samples was determined using a texture analyzer (TA.XT plus Texture Analyser, Stable Micro Systems, Serial No. 10047, United Kingdom). These details of analyzing the firmness and adhesiveness were followed based on the previous report (Rosales et al., 2023).

Total soluble solids (TSS)

A digital refractometer (Atago) recorded the TSS of raw and steamed samples. Shortly, TSS was analyzed by mixing two grams of sample and 2 mL distilled water to extract better the juice of the pumpkins.

Total sugar content, amylose content, and starch content

Sample extraction was done by adding 10 mL of 80 %ethanol to a 5 g fresh weight sample, and centrifugation at12000 rpm was done .The supernatant was collected to analyze total sugar, total flavonoid content, and antioxidant capacity using 2,2-diphenyl-2-picryl-hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays of the pumpkins .For the reducing sugar, extraction was done by adding 10 mL distilled water to a 10 g FW sample, then homogenizing and centrifuging at 12000 rpm afterward .

All the samples and standards were observed using a spectrophotometer)Hanon i5, Jinan Hanon Instruments Co., Ltd., China (to record their absorbance .All the chemicals used in the experiment were analytical grade.

To determine total sugar content, 0.5 mL supernatant was mixed with 1 mL 5 %phenol, and 5 mL 95 %sulfuric acid was done .The samples and standard)glucose (were incubated at 60 ˚C for 60 min .The absorbance was observed at 490 nm (Dubois et al., 1956).

As for reducing sugar content, 1 mL supernatant was mixed with 1 mL of 3,5-dinitro salicylic acid reagent, then boiled for 10 min and cooled in ice before reading the absorbance of the samples and (glucose) standard at 540 nm (Miller, 1959).

A powdered dry sample (0.1 g) was mixed with 1 mL of 95% ethanol, 9 mL 1 N sodium hydroxide, and 90 mL distilled was added, and filtered afterward to analyze amylose content .Then, in a 50-mL volumetric flask, 25 mL distilled water was added, followed by 1 mL acetic acid, 2 mL iodine solution, 10 mL extract sample, and distilled water to make up to 100 mL .The absorbance of the sample and amylose)standard (was observed at 620 nm using a spectrophotometer (Juliano, 1971) .

Starch content was determined by making a minor modification using the anthrone reagent method .One-gram FW sample with 5 mL distilled water was homogenized and centrifuged after adding 25 mL hot) 73 ˚C (80% ethanol .Then, decanting the alcoholic solution was done .The extraction procedure was repeated by adding 30 mL of 80 %ethanol .

After removing the ethanol in the residue by evaporation in boiling water, 5 mL distilled water and 3.25 mL 52 %perchloric acid was added to the residue and stirred for 5 min continuously, then occasionally for the next 15 min .Afterward, 20 mL of distilled water was added before centrifugation . Next, the extract of the sample was transferred to the 100-mL volumetric flask .The procedure was repeated by adding the same amount of distilled water and 52 %perchloric acid, then stirring it occasionally for the next 30 min .Finally, the extract was transferred to the volumetric flask, distilled water was added to make up to 100 mL, and filtration was done .One mL extract sample was mixed with freshly made anthrone reagent)10 mL (and incubated in a boiling water bath for 12 min . After cooling down, the absorbance of the sample and glucose)standard (was observed at 630 nm using a spectrophotometer (Clegg, 1956).

Starch isolation and scanning electron microscopy (SEM)

Starch isolation was done using the method of Sing et al. (2007) with minor modifications. The juice of pumpkins from fruit pieces was extracted using a homogenizer. A muslin cloth was used to extract the juice, and the deposit left on the cloth was washed 3-4 times with distilled water. A glass beaker collected the filtrate, and the residue left on the muslin cloth was discarded. The filtrate was centrifuged three times at 3800 *xg* for 20 min each. At every centrifugation, thesupernatant was discarded and reslurried using distilled water. After the centrifugation, the filtrate was kept undisturbed overnight. The solid layer of starch settled down and was collected and dried in an air oven at 40 ˚C for 24 h.

Scanning electron micrographs were obtained using a scanning electron microscope (Jeol JSMM6610LV). The pumpkin powder was sprinkled on a carbon tape stub and coated with gold. During micrography, an accelerating potential of 10kV was used.

Total flavonoid contents

All the samples were homogenized separately before the analysis and the supernatant was collected. After mixing the supernatant) 0.5 mL (with 1 mL 10 %aluminum chloride, 1 mL 1 M sodium acetate, and 2.8 mL distilled water, it was kept at room temperature for 40 min .Standard ()quercetin (and the samples observed its absorbance at 415 nm (Chang et al., 2002). Quercetin was expressed as mg quercetin equivalents QE/g of fresh sample.

Total carotenoids

All the samples were homogenized separately before the analysis and the supernatant was collected. Briefly, 40 mL of acetone was mixed with 1 g of FW sample for 4 min, and it was filtered using Whatman No. 1 with a Buchner funnel under vacuum .Further, 40 mL of acetone was added to attain a colorless sample .Finally, the absorbance was observed at 450 nm using a spectrophotometer (Bonina-Noseworthy et al., 2016).

Antioxidant capacity

DPPH radical scavenging assay**.** Brand-Williams et al. (1995) followed the method with minor modifications .All the samples were homogenized separately before the analysis and the supernatant was collected. The 0.5 mL supernatant was mixed with 2.85 mL freshly made DPPH working solution and incubated for 30 min in dark conditions, and absorbance (abs) was observed at 515 nm. The DPPH radical scavenging activity was calculated using the equation below .

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control} \times 100$$

Ferric reducing antioxidant power (FRAP) Assay. Minor modifications were made to the method of Benzie and Strain (1996) to determine the antioxidant capacity of the samples .All the samples were homogenized separately before the analysis and the supernatant was collected. The 0.5 mL supernatant was mixed with 2.85 mL freshly made FRAP working solution and incubated for 30 min in dark condition .The absorbance of the samples and standard)Trolox) was observed at 593 nm.

Statistical analysis

Pumpkin fruits (four fruits per replicate) and four replications were arranged in a completely randomized design and statistically analyzed using the SPSS program)SPSS for Windows Version 17.0, Released 2008, SPSS Inc., Chicago, Illinois, USA(. The fruits were about the same weight and harvested with the same fruit maturity. Mean standard errors)SE (were used to present the data . Means were compared by Tukey's Honestly Significance Difference)HSD (test. A Pearson correlation was done to determine the relationship of the attributes using the SPSS program. A stepwise multiple regression was used using SPSS to determine the predictors that affect the dependent variable before performing the path analysis.

Results

Fruit weight and dry matter

A significant difference in the fruit weight was observed. TL11 and TL17 had the highest fruit weight, whereas TL2, TL7, and CC were not different (Fig. 1A). Conversely, TL2 and TL7 had the lowest fruit weight but were not significantly different from CC. The dry matter of TL2 was significantly higher than the other pumpkins in raw and steamed forms (Fig. 1B).

Pulp firmness and adhesiveness

The pulp firmness of the raw and steamed pumpkins differed significantly (Fig. 1C). CC showed the highest pulp firmness in the raw pumpkin, while TL7 had the least firmness. However, after steaming, TL7 was firmer than the CC but not significantly different from TL11 and TL17. On the other hand, TL2 had the lowest pulp firmness, not different from TL11 and the CC. The adhesiveness of TL11 and CC did not significantly differ from each other, and they were higher than TL2 and TL7. However, all these pumpkins were outranked by TL17 (Fig. 1D).

Pulp color and color change

In the raw pumpkin, the $L*$ value of TL7 was higher than the CC and other pumpkins (Fig. 2A). TL2 showed the lowest $L*$ value, while TL17 was not different from CC. However, TL2 had the highest $a*$ value, whereas TL7, TL11, and TL17 were the lowest (Fig. 2B). The latter pumpkins were lower than the CC. The $b*$ value of the pumpkins was significantly different. TL2 exhibited the highest $b*$ value, higher than the CC, while the lowest was TL11 (Fig. 1E). The CC was not significantly different from TL7. In contrast, TL17 was lower than CC.

The chroma of TL2 exhibited the highest, whereas TL11 had the lowest. The former and the latter were significantly higher and lower than the other pumpkins, respectively (Fig. 2D). After steaming, the chroma of TL7 and CC were significantly higher than TL17. However, they showed a similarity with TL2 and TL11. However, the hue angle of the pumpkins showed a significant variation in raw and steamed pulps. TL7, TL11, and TL17 hues were higher than CC and TL2. However, TL11 and TL17 had higher hue angles after steaming than the other pumpkins (Fig. 2E).

TL7 depicted the highest color change compared to the CC and other pumpkins (Fig. 2F). But CC was not different in the color change to other pumpkins.

TSS, total sugars, amylose, and starch contents

A significant variation of the TSS among the pumpkins was observed. TL2 and CC were significantly higher than the other pumpkins (Fig. 3A). After steaming, the TSS of TL7, TL11, and TL17 increased but was not significantly lower than that of TL2 and CC. Fig. 3B depicted the significant differences in the total sugar content of the pumpkins before and after steaming. The raw TL11 was higher than the CC, whereas TL17 had the least total sugar content. The total sugar content of all pumpkins after steaming were increased. Steaming contributed to the increase of the total sugar in TL2, the highest among the pumpkins.

There was a variation in the reducing sugar of the raw pumpkins, TL17 being the highest, while CC was the lowest (Fig. 3C). The other pumpkins (except TL17) were significantly higher than the CC. The reducing sugar content of all pumpkins was reduced after steaming, and the highest reduction was observed in TL11, while CC had the least decline.

The amylose content of the raw pumpkins had significant variation (Fig. 3D). TL7 was significantly higher than the CC, TL11, and TL17.

Conversely, TL17 obtained the lowest amylose content, markedly lower than the CC. In addition, the starch of the pumpkins was significantly different. More than 20% starch content was observed in TL7, TL2, and CC, which were considerably higher than TL11 and TL17 (Fig 3D).

Starch morphology using SEM

Mixtures of spherical, oval, polyhedral, and dome-shaped granules were observed in all pumpkins. These shapes had been also observed in New Zealand Kamo Kamo starch (Singh et al., 2007). More number of spherical-shaped granules (small and big sizes) were observed in all pumpkins. There were few numbers of dome-shaped granules observed in all pumpkins.

Oval-shaped starch granules were observed in TL2 (Fig. 4A) and TL7 (Fig. 4B). TL11 (Fig. 4C), TL17 (Fig. 4D), and CC (Fig. 4E) have no oval-shaped starch granules. Size distribution variation was observed in the SEM wherein CC followed by TL11, and TL7 had smaller granule sizes. Bigger granule sizes were observed in TL17, followed by TL2.

Antioxidants

Fig. 5A depicted that the total flavonoid contents of raw and steamed pumpkins were significantly different. CC had the highest content, and TL2 followed it. The lowest content was observed in TL7, more than three times lower than the CC and TL2. In comparison, TL11 was two times lower than the latter pumpkins. Steaming was observed to be beneficial to TL7 because the content was markedly increased, whereas other pumpkins showed otherwise. The most reduction in the content was observed in CC, followed by TL2, TL17, and TL11. Regarding the differences among pumpkins, TL2 was the highest, followed by CC. Moreover, TL2 contained two times higher flavonoids compared to TL11. TL11 had the lowest content and was lower than TL7 and TL17.

On the other hand, a significant variation in the total carotenoids among pumpkins in raw and steamed was observed. TL2 was significantly higher than the CC and other pumpkins (Fig. 5B). TL11 and TL17 were recorded as the lowest among the pumpkins and were significantly lower than the CC. The content of the total carotenoids in a raw pumpkin was changed after steaming, which increased most of them. For example, TL11 and TL17 had a 2-fold increase after steaming compared to the raw.

Antioxidant capacity

A significant difference among the pumpkins in their antioxidant capacity was observed. The highest DPPH was observed in TL2 and CC in raw pumpkin, followed by TL11 and TL17 (Fig. 5C). The least DPPH was observed in TL7. The DPPH values across pumpkins after steaming were doubled, and CC was the highest. TL2 just followed this pumpkin. The differences in the DPPH values of other pumpkins were similar to those in raw form.

On the other hand, TL11 exhibited the highest FRAP value, higher than the CC (Fig. 5D). The other pumpkins, specifically TL2 and TL17, were comparable with CC. TL7 was significantly the lowest in FRAP value among the pumpkins. However, steaming caused an increase, which tripled their FRAP values. Although there was an increase, TL2 was significantly higher than the other pumpkins. CC was higher than TL7 and TL17. Steamed TL7 and TL17 were almost two-fold lower than TL2.

Relationship of dry matter and TSS to adhesiveness and antioxidant properties

A very high and positive correlation was observed between TSS and dry matter in raw-to-raw forms (Table 1a). Moreover, a high and positive relationship was observed between dry matter and antioxidants

(total carotenoids and total flavonoid contents) (Table 1a). However, dry matter and DPPH showed a moderate positive correlation to each other. On the other hand, TSS had no connection with the adhesiveness and FRAP in raw-to-raw correlation (Table 1a). Likewise, TSS exhibited a significant and positive correlation of the quality indicators to total flavonoid contents, total carotenoids, and DPPH. The relationship of TSS with the antioxidants may be due to the influence of dry matter on the pumpkins. Interestingly, amylose and adhesiveness showed a very high and negative correlation.

A high and positive relationship was observed between dry matter and all attributes, such as dry matter, total carotenoids, total flavonoid contents, DPPH, and FRAP for raw-to-steamed attributes (Table 1c). Moreover, raw dry matter showed a very high and positive correlation to the TSS of steamed pumpkins. For the relationship of raw TSS to steamed attributes, there was a very high and positive correlation to TSS, total flavonoid contents, total carotenoids, DPPH, and FRAP (Table 1c).

With regards to path analysis, a^* and total sugar characteristics showed a very high significant direct effect on dry matter with 0.72 and 0.45, respectively (Table 2a). Both a^* and total sugar displayed a very significant total effect with values of 0.82 and 0.61. Whereas total sugar and total flavonoid contents showed a very high significant direct effect with 0.50 and 0.42, respectively, by dry matter (raw form). The pulp firmness, total sugar, and total flavonoid contents exhibited a very significant total effect with -0.76, 0.71, and 0.73 values, respectively. Likewise, a significant indirect effect of pulp firmness was observed.

TSS and total flavonoid contents had a significant direct effect on TSS (steamed form) (Table 2c). TSS and total flavonoid contents had a significant indirect effect to each other was observed. Moreover, these attributes showed a very high significant total effect on TSS (raw).

Discussion

Pumpkin fruits are a good source of human nutrition, and the stickiness of cooked pulp is usually the preference of the consumers, and it is used as a basis for quality. Therefore, a sticky attribute is a significant concern in a breeding program of pumpkins. In addition, providing scientific information on the physico-chemical and antioxidant properties of the pumpkins for the consumers will also significantly impact their decision-making in the purchase.

The texture is essential in quality improvement (Fellows, 2017), influencing the consumers' acceptance and perception of the product. This study divides the pumpkins into two groups based on their adhesiveness or stickiness and amylose content. Therefore, the differences related to these characteristics could contribute to a manageable selection for consumption.

Generally, the adhesiveness or stickiness of cooked crops containing high starch content (rice, beans, and potato) is markedly related to amylose content (Juliano, 1985; Talja et al., 2008; Miranda et al., 2019). As per previous studies, the high adhesiveness is due to very low amylose content (Li and Gilbert, 2018; Tao et al., 2019), just like in pumpkins. Although it is evident that rice is different from pumpkin, the former consists of very high amylose content for non-sticky rice, and sticky rice has a very low amylose content (Juliano, 1985; Verma and Srivastav, 2017; Qiu et al., 2021). The range of amylose content in rice is very low (2-9%) and low (9-20%), showing a sticky and tender texture after cooking, respectively (Juliano, 1985), which can be adapted to categorize the pumpkins.

On the other hand, the other commodities, such as potatoes having higher amylose content, ranged from 11.9 to 20.1% (Talja et al., 2008) compared to the pumpkins in this study containing 2.29 to 12.41% amylose content. Therefore, the cooking quality of the pumpkins in this study could not be compared with potatoes. Thus, in this study, the pumpkins were ranked based on their amylose into two classes (very low and low amylose content). In addition, their cooking texture indicated by adhesiveness value could be classified into two categories: sticky and tender (less sticky).

Amylose and adhesiveness exhibited a negative association (Table 1a), indicating that high adhesiveness contains low amylose, in agreement with previous studies reporting that the high adhesiveness of pumpkin is due to very low amylose content (Li and Gilbert, 2018; Tao et al., 2019). Therefore, the pumpkins with lower amylose content and high adhesiveness values were labeled TL17, TL11, and CC, whereas TL2 and TL7 were less sticky.

Aside from considering the stickiness of the pumpkins in the selection process, the quality criteria such as dry matter and TSS could also be used. Consumers use TSS as an essential quality criterion; a higher TSS would mean a better taste (Rahman et al., 2021). Likewise, dry matter is an important quality indicator for yield and starch (Palaityte et al., 2017; Grisales et al., 2015). A high dry matter and TSS showed a positive association with each other (Tables 1a and 1b), consistent with reports by Valverde-Miranda et al. (2021), Scalisi and O'Connell (2021), and Travers (2013).

Based on the path analysis, dry matter (raw) displayed that those pumpkins with content of this had high $a*$ value and amount of total sugar. A high raw dry matter and $a*$ value were observed in TL2 wherein it had a high amount of sugar, although it ranked second after TL11. Likewise, the raw dry matter could predict the steamed attributes of pumpkins. It was observed that a high dry matter in raw pumpkin would have soft pulp (low firmness value), but high contents of total sugar (Figure 3b) and total flavonoids (Figure 5a), which was observed in steamed TL2. This means that consumers may relish a steamed pumpkin sweet and a high number of antioxidants. Moreover, a high dry matter content can be used as a quality indicator for cooked pumpkin. Another quality indicator that can be used is the TSS, it was observed that having high raw TSS showed a high amount of TSS and total flavonoids in steamed pumpkins. These attributes were observed in TL2. Based on the results, raw dry matter and TSS could be used for crop improvement of pumpkins as quality indicators for cooked pumpkins. This was used to determine the parameters that show direct and indirect effects on the independent variables, which are helpful for the plant breeders.

Taste plays a vital role in the consumers' decision-making, and if their preference is a sweeter pulp, and maximizes the health benefits like a good source of antioxidants. Therefore, especially the perception of the consumer on the product is as healthy as possible without forgoing the taste quality; in this situation, TL2 significantly contained higher total sugar, and the consumers may relish the sweet pulp of this pumpkin with high antioxidant properties. However, if consumers prefer a not-too-sweet and sticky pulp, the TL17 possesses the lowest total sugar but low antioxidant properties. The other choices are stickier pulps of TL11 and CC, but the antioxidant properties were significantly lower than TL2.

On the other hand, in the raw-to-raw correlation analysis, pumpkins with a very high dry matter showed a high TSS, and a moderate to high and positive correlation to DPPH, total sugar, total carotenoids, and total flavonoid contents (Table 1a). A pumpkin that exhibited a high dry matter was observed in TL2 (less sticky pumpkin), which is composed of high contents of those attributes. CC was also high in dry matter, second rank after TL2, and displayed high amounts of total flavonoid and DPPH value. Those sticky pumpkins (TLL11, and TL17) and less sticky pumpkin (TL7) had lower total flavonoid contents and DPPH values than TL2 and CC. But the total carotenoids were higher in TL7 than CC.

Flavonoids are a good source of antioxidants due to their capacity to donate hydrogen atoms to free radicals, hence they provide a significant antioxidant component (Mokhtar et al., 2021). There was a significant variation in total flavonoid contents among the pumpkins (Figure 5A). Our results concurred with the previous report wherein there was also a variation of the flavonoids observed in *Cucurbita* species (Hagos et al., 2023). CC displayed the highest content of total flavonoids followed by TL2, and TL17, while TL7 showed the least. The total flavonoid contents of TL7 and TL11 might need a longer fruit maturity wherein according to a previous study a mature pumpkin had a higher amount than a young fruit (Mokhtar et al., 2021). A further study on the different fruit maturity using the same pumpkin as in the study is recommended. Moreover, TL2 was 2 to 4 folds higher than TL7 and TL11, and this pumpkin may be promoted for commercialization because of its high total flavonoid contents. On the other hand, for antioxidants and their capacity, this study also concurred with the previous study wherein a pumpkin with a high dry matter contained high flavonoids and antioxidant capacity (Javaherdashti et al., 2012). These attributes and their positive relationship were characterized in TL2 and CC; thus, TL2 could be used for further commercialization to make it available the same as the CC. Moreover, a pumpkin with high TSS exhibited a high antioxidant capacity, the same observation as Moreno-Resendez et al. (2016). Therefore, based on our results and previous reports, quality indicators such as dry matter and TSS could be used for mass production and baseline scientific information for the crop improvement of pumpkins in support of the availability of nutritious food.

The content of total carotenoids is responsible for the color of the pumpkins. Less sticky pumpkin, TL2 had higher total carotenoid content. Similarly, regular kernel corn (yellow) contained higher total carotenoids than waxy corn (Hu and Xu, 2011). However, a raw pumpkin with a high dry matter and TSS exhibited a high total carotenoid content, as recorded in TL2, contrary to a previous study wherein a pumpkin with a high dry matter content showed a low carotene content (Kuliakina et al., 2020). This difference may be caused by cultivar, maturity, and cultivation practice. As the fruit matures, the carotenoid contents were increased (Sharma and Ramana Rao, 2013). Variations in carotenoids were also observed in pumpkin species, as previously reported (Norshazila et al., 2014; Itle and Kalbelka, 2009), which concurred with the study that there were differences. Likewise, the cultivation practices, such as fertilizer management and plant spacing were affected the carotenoids (Mutua et al., 2021; Wadas et al., 2012). On the other hand, carotenoids are varied from orange to red and these compounds have positive correlation with the color values. The $a*$ and $b*$ values showed positive association with the total carotenoids (Table 1a). Moreover, the chroma of TL2 had the highest among the raw pumpkins, which means that it has vivid yellow-orange pulp. The color becomes vivid as the chromaticity increases (Itle and Kabelka, 2009). Thus, the other pumpkins had lesser chromaticity, hence, it would have a dull yellow-orange because of their chroma values (Figure 2D). These observations concurred with the previous study (Itle and Kabelka, 2009) that high chroma values have vivid colors.

It was reported that the firmness (hardness) of the fruit positively correlated with the starch content (Stevenson, 2003). However, our results showed otherwise, indicating that there was no relationship. Corrigan et al. (2001) found that low and high-starch winter squash cultivars had the same hardness or firmness. Those pumpkins with low firmness had higher starch content, which was observed in TL2 and TL7. The CC also had high starch content, and at the same time, the pulp firmness was high. But TL2 also contained a high amount of starch, but it had the lowest firmness. This indicates that starch content may not have a direct effect on the pulp firmness and a further investigation is recommended to identify the responsible contributory to the pulp firmness such as pectin.

Moreover, it was observed that the size of the starch granule might not involve the firmness of the pulp. Although CC had the firmest pulp, it contained fewer bigger starch granule sizes (Fig. 4E) as compared to TL17 (less firm pulp), which had a bigger granule size (Fig. 4D). On the other hand, the pulp firmness after steaming, TL7, TL11, and TL17 was more remarkable than TL2. However, an additional image analysis and size distribution are recommended for future investigations.

The starch content was not involved with adhesiveness in this study. However, there was a negative connection between adhesiveness and starch content, as a high adhesiveness value contained a low starch content. This was observed in sticky pumpkins like in TL11 and TL17. However, CC is a sticky pumpkin, but the starch content was high, comparable with a less sticky pumpkin (TL2). This suggests that starch content could not be a good indicator of the stickiness of cooked pumpkins.

The adhesiveness of the pumpkins had no relationship with the size and shape of the starch granule. This was also documented in rice that the granule size of starches and waxy and non-waxy rice cultivars varied (No et al., 2019; Wani et al., 2012).

The fruit weight of less sticky pumpkins was lower than the sticky pumpkins, especially the TL11 and TL17. Additionally, a pumpkin with low fruit weight would not exhibit a low dry matter content or vice versa. It was observed that raw TL2 had the lowest fruit weight, but it contained the highest dry matter content, whereas TL17 had the highest fruit weight, but its dry matter content had the lowest. These observations were consistent with the study of Kuliakina et al. (2020). This indicates that the dry matter content and fruit weight of the pumpkins are cultivar dependent.

Raw attributes of pumpkins displayed a positive correlation with the steamed attributes (Table 1b). Raw dry matter exhibited positive association with the steamed pumpkin attributes, such as TSS, total carotenoids, FRAP, total sugar, dry matter, total flavonoid contents, and DPPH. This means that in the selection process of pumpkin for consumption would be manageable.

The total carotenoids were increased after steaming because of the softening and destruction of the cell structures that freed more carotenoids. In addition, the loosening of carotene-binding fibers (Carvalho et al., 2014) increased the total content and concurred with other reports (Matova et al., 2021). However, this was not a positive result for the TL7, where the content was decreased.

Reasons such as firmness, steaming time, and heat sensitivity might contribute to the decline of carotenoid contents. TL7 had the highest firmness among pumpkins after steaming; hence, it may need a longer cooking time to be enough to free more carotenoids. Another may be due to the sensitivity of carotenoid derivatives to heat, leading to degradation. The total carotenoid content declined due to high temperature, leading to the degradation of β-carotene by isomerization and epoxidation (Moreira et al., 2020). Moreover, carotenoids are sensitive to light, heat, and oxygen associated with isomerization and epoxidation products (Song et al., 2017). In effect, longer cooking times and high temperatures should be avoided. A shorter cooking time may be needed to prevent carotenoid loss in TL7. The variation of the total carotenoids among pumpkins was significant, wherein TL2 having a golden-brown pulp, had the highest content compared to the other pumpkins. TL11 was significantly the lowest in total carotenoids, with light yellow-orange pulp. It was observed that all the steamed pulps reduced their L^* value, possibly due to the degradation of carotenoids and brown color caused by the Maillard reaction, wherein reducing sugar is one factor in the process. In this respect, in the study the reducing sugar was reduced after steaming (Figure 3C); therefore, Maillard's reaction would occur. Releasing total flavonoid contents and carotenoids in cooked pulps increased antioxidant capacity. The DPPH values of all pumpkins were doubled, but there was a 2 to 5-fold increase in FRAP values after cooking.

On the other hand, only steamed TL7 increased in total flavonoid contents after cooking, whereas the other pumpkins decreased. The reduction of the content from most of the pumpkins may relate to the heat sensitivity of the specific flavonoid—more than 10% degradation of rutin at 70 ˚C after 2 h of treatment (Ioannou et al., 2020). However, high degradation occurred at 90-110 ˚C and completed degradation at 130 ˚C (Ioannou et al., 2020). Therefore, the beneficial cooking temperature would be 70 ˚C. The increase in TL7 might have bounded flavonoids converted into free form and contain a specific flavonoid class that can withstand heat. Another class is the flavone, which has a melting point of 94-97 ˚C (National Center for Biotechnology Information, 2021), which may contribute to high total flavonoid content in TL2 because the flavone might be higher than the other pumpkins. It can be observed that each flavonoid class is affected by complex factors such as melting point and time exposure to heat.

Conclusions

1. Knowledge of a quality product proves to influence the food choices of consumers. Our study provides more information that amylose content and adhesiveness can be employed to classify the tested pumpkins into two groups, very low amylose content and sticky (TL11, TL17, and CC) and low amylose and less sticky pumpkins (TL2 and TL7). A pumpkin with high dry matter and TSS could be promoted to the producers and consumers as an additional source of nutritious vegetables due to its excellent quality (raw and cooked). Based on the

results of the correlation and path analyses of raw pumpkin to steamed pumpkin, raw pumpkins with a high dry matter content and TSS showed a higher content of total carotenoids, total flavonoid contents, and antioxidant capacity (DPPH and FRAP assays). These attributes were observed in TL2, a less sticky pumpkin. From a health perspective, TL2, a vivid yellow-orange fleshed pumpkin, which turned into a golden-brown colored pulp after cooking, was sweeter and contained higher antioxidants (total carotenoids and flavonoids content) and high antioxidant capacity. At the same time, sticky pumpkins had a lower content of these attributes. TL2 may be used as a parent line for advanced crop improvement of pumpkins.

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Conflicts of interest

The authors declared no competing interests.

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TABLE 1a. Pearson correlation of raw attributes (vertical) to raw attributes (horizontal)

	DM	TSS	FW	PF	ADH	AMY	STR	*L**	*a**	*b**	TS	TC	TFC	DPPH	FRAP
DM	-	0.80**	−0.43	−0.13	−0.52*	0.45	0.47*	−0.74	0.82**	0.56**	0.62**	0.64**	0.68**	0.59**	0.01
TSS		-	−0.38	0.13	−0.25	0.28	0.77**	−0.80**	0.86**	0.66**	0.42	0.57**	0.89**	0.77**	0.01
FW			-	0.65**	0.79**	−0.86**	−0.69**	0.03	−0.50*	−0.58**	−0.31	−0.84**	−0.08	0.15	0.53*
PF				-	0.78**	−0.88**	−0.32	−0.22	−0.26	−0.48*	0.09	−0.70**	0.40	0.58**	0.71**
ADH					-	−0.92**	−0.57**	−0.01	−0.48*	−0.56**	−0.40	−0.85**	0.04	0.24	0.43
AMY						-	0.70**	0.02	0.54*	0.73**	0.18	0.94**	−0.02	−0.29	−0.70**
STR							-	−0.47*	0.80**	0.91**	0.15	0.88**	0.63**	0.32	−0.51*
*L**								-	−0.81**	−0.46*	−0.21	−0.30	−0.87**	−0.88**	−0.21
*a**									-	0.80**	0.23	0.77**	0.75**	0.59**	−0.22
*b**										-	−0.04	0.88**	0.53*	0.23	−0.62**
TS											-	0.24	0.22	0.27	0.39
TC												-	0.31	0.03	0−.60**
TFC													-	0.92**	0.16
DPPH														-	0.48*
FRAP															-

** - significant at 1% level; * - significant at 5% level;

DM – dry matter; TSS – total soluble solids; FW – fruit weight; PF – pulp firmness; ADH – adhesiveness; STR – starch; TS – total sugar; TC – total carotenoids; TFC – total flavonoid contents; DPPH – 2,2-diphenyl-2-picryl-hydrazyl; FRAP – ferric reducing antioxidant power.

TABLE 1b. Pearson correlation of raw pumpkins (vertical) to steamed attributes (horizontal)

	DM	TSS	PF	ADH	TS	TC	TFC	DPPH	FRAP
DM	0.72**	0.80**	−0.76**	0.10	0.71**	0.79**	0.73**	0.67**	0.77**
TSS	0.46*	0.90**	−0.68**	0.17	0.42	0.84**	0.87**	0.89**	0.84**
PF	−0.47*	0.17	−0.28	0.21	−0.25	−0.31	−0.13	0.49*	0.00
TS	0.44*	0.40	−0.35	−0.14	0.93**	0.19	0.12	0.35	0.37
TC	0.58**	0.49*	−0.21	−0.17	0.47*	0.84**	0.74**	0.26	0.53*
TFC	0.27	0.92**	−0.76**	0.19	0.14	0.74**	0.84**	0.97**	0.77**
DPPH	0.30	0.82**	−0.75**	0.28	0.15	0.53*	0.61**	0.91**	0.74**
FRAP	−0.03	0.09	−0.25	0.15	0.17	−0.32	−0.33	0.27	0.12

** - significant at 1% level; * - significant at 5% level

DM – dry matter; TSS – total soluble solids; FW – fruit weight; PF – pulp firmness; ADH – adhesiveness; TS – total sugar; TC – total carotenoids; TFC – total flavonoid contents; DPPH – 2,2-diphenyl-2-picryl-hydrazyl; FRAP – ferric reducing antioxidant power.

TABLE 2a. The direct (bold-faced), indirect and total effects of dry matter (raw) to the raw attributes

DM	*a**	TS
*a**	**0.72****	0.16
TS	0.10	**0.45****
Total effect	0.82**	0.61**

TABLE 2b. The direct (bold-faced), indirect and total effects of dry matter (raw) to the steamed attributes

DM	PF	TS	TFC
PF	**-0.36****	0.12	0.21**
TS	-0.16	**0.50****	0.11
TFC	-0.24**	0.09	**0.42****
Total effect	-0.76**	0.71**	0.73**

TABLE 2c. The direct (bold-faced), indirect and total effects of total soluble solids (raw) to the steamed attributes

TSS	TSS	TFC
TSS	**0.60****	0.50**
TFC	0.31**	**0.37****
Total effect	0.91**	0.87**

** - significant at 1% level
DM – dry matter; TS – total sugar; PF – pulp firmness; TFC – total flavonoid contents; TSS – total soluble solids
Residual for dry matter to the raw attributes – 86.86%
Residual for dry matter to the steamed attributes – 93.89%
Residual for total soluble solids to the steamed attributes – 86.11%

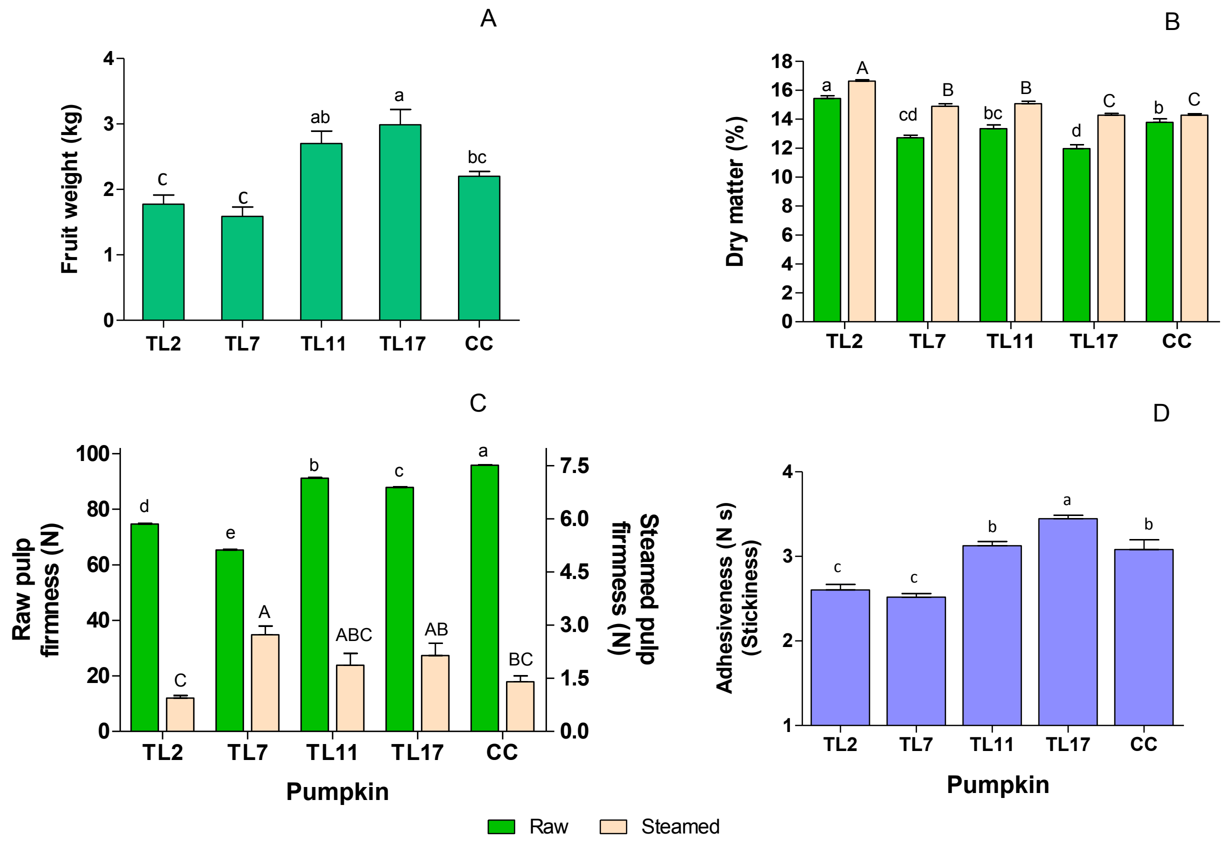


Fig. 1. The fruit weight (A) of raw pumpkins, dry matter (B), and pulp firmness (C) of pumpkins before and after steaming, and the adhesiveness in raw pumpkins (D). TL – Thong Lanna; CC – Commercial Cultivar

Bars are means ± standard error (SE); Means followed by different lowercase letters ([a–e]) for raw pumpkins and uppercase letters ([A–C]) for steamed pumpkins in a graph are significantly different at $P \leq 0.05$ by using Tukey's Honestly Significant Difference (HSD) test.

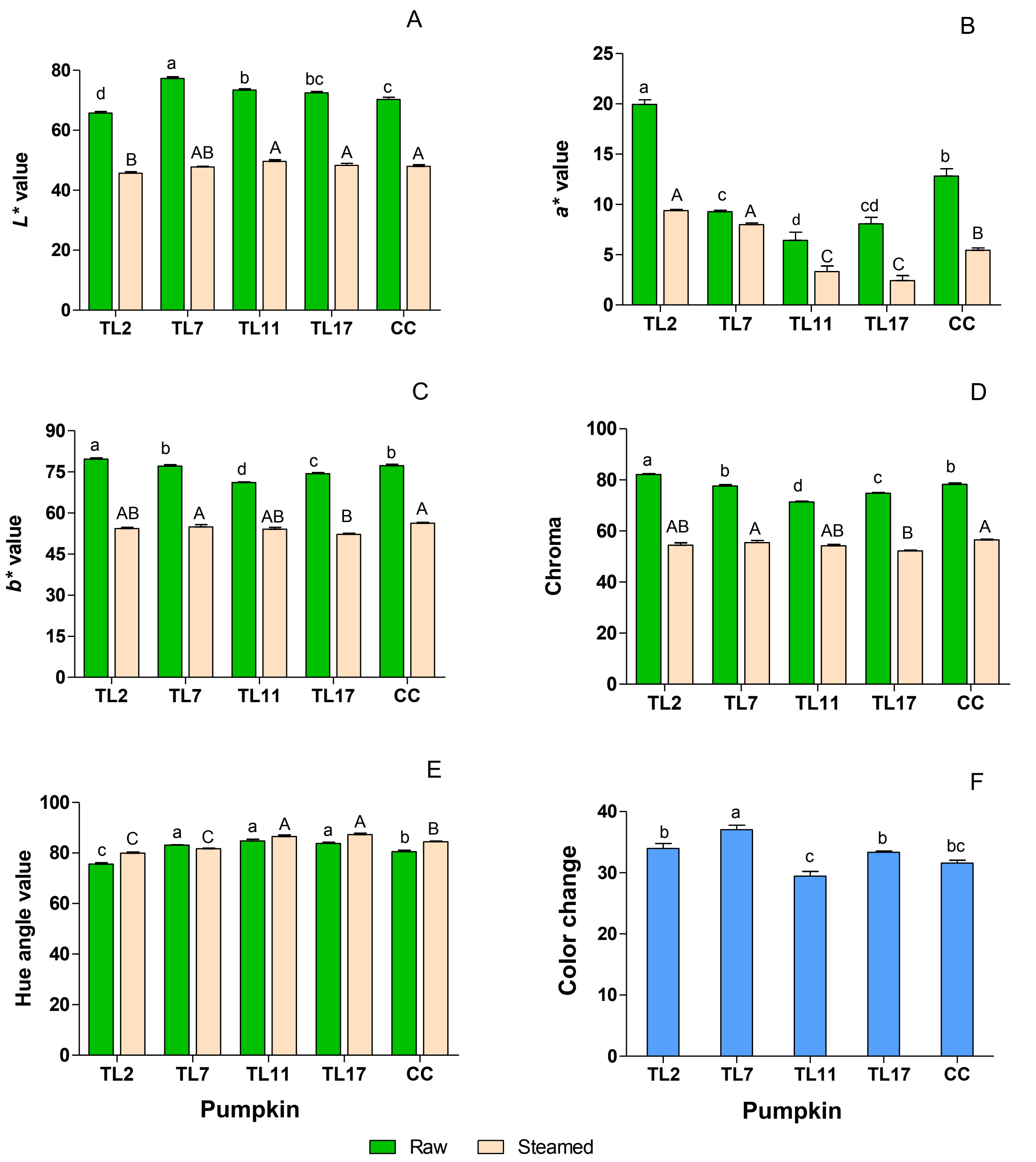


Fig. 2. The *L, *a**, *b**, chroma and hue of pumpkins before and after steaming, and color change of pumpkins after steaming. TL – Thong Lanna; CC – Commercial Cultivar**

Bars are means ± standard error (SE); Means followed by different lowercase letters ($^{a–d}$) for raw pumpkins and uppercase letters ($^{A–C}$) for steamed pumpkins in a graph are significantly different at $P \leq 0.05$ by using Tukey's Honestly Significant Difference (HSD) test and *t*-test.

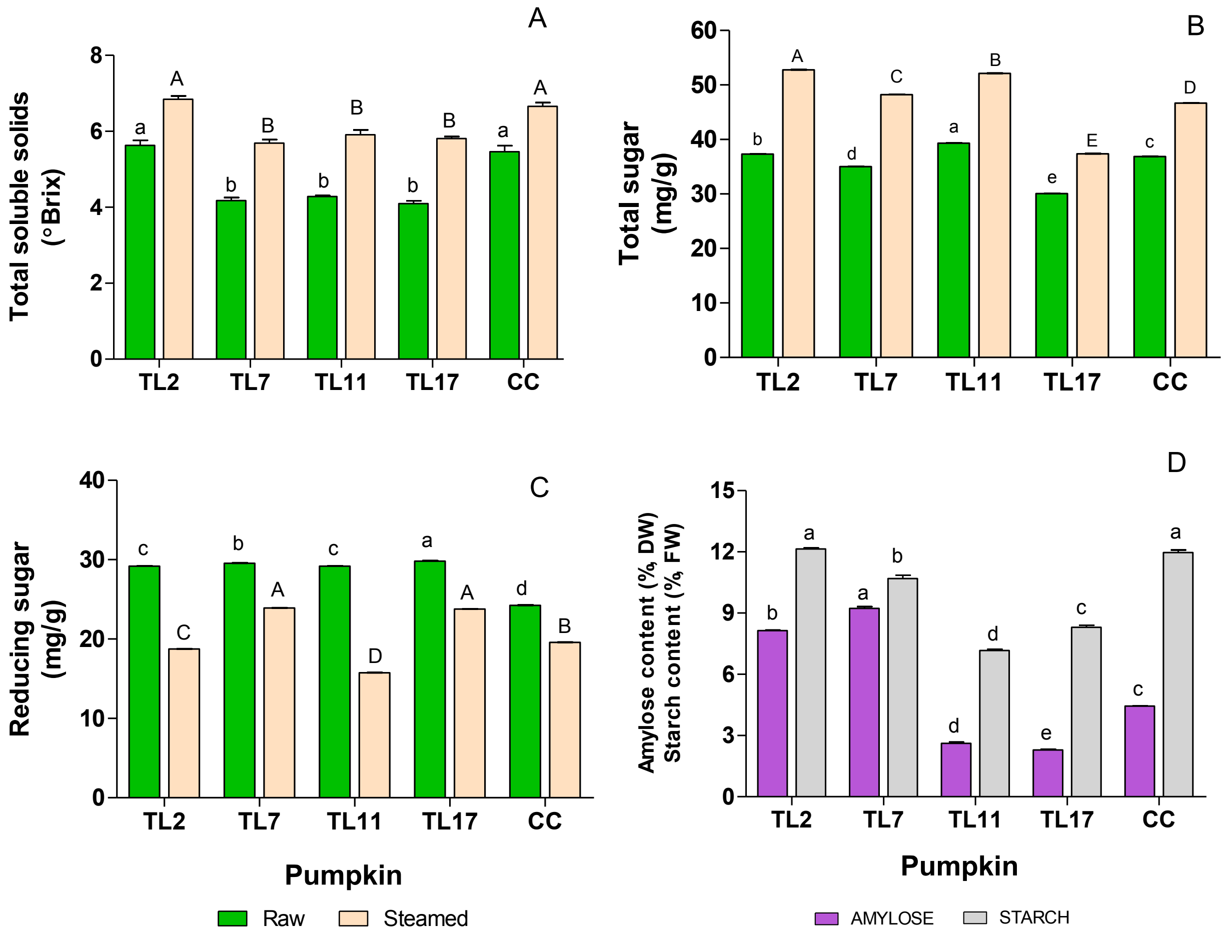


Fig. 3. The total soluble solids (A), total sugar (B), reducing sugar (C) of pumpkins before and after steaming, and amylose content and starch content of raw pumpkins (D). TL – Thong Lanna; CC – Commercial Cultivar

Bars are means ± standard error (SE); Means followed by different lowercase letters ($^{a–e}$) for raw pumpkins and uppercase letters ($^{A–D}$) for steamed pumpkins in a graph are significantly different at $P \leq 0.05$ by using Tukey's Honestly Significant Difference (HSD) test.

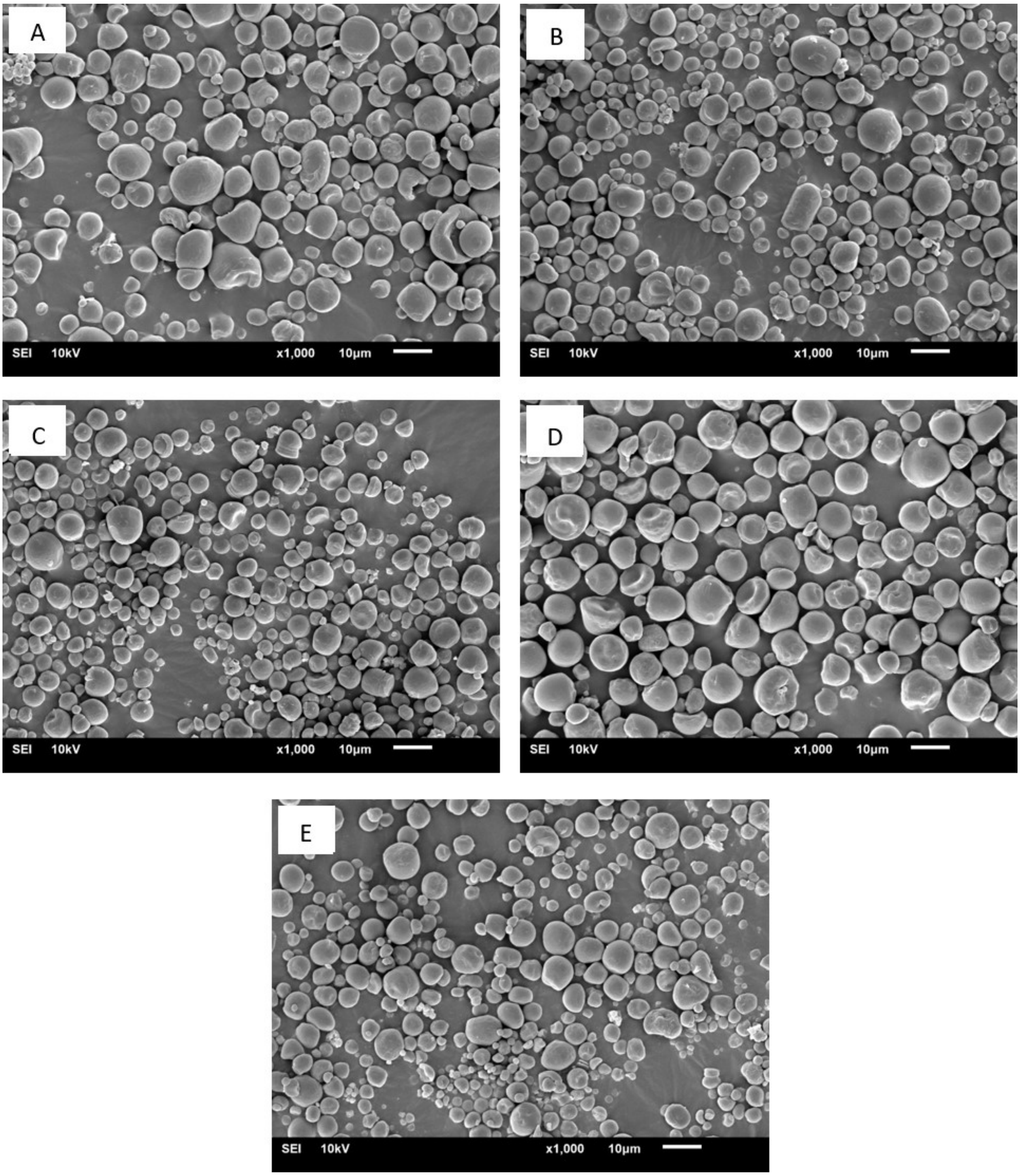


Fig. 4. Scanning electron micrographs (SEM) of starch granules from five pumpkins TL2 (A), TL7 (B), TL11 (C), TL17 (D), and CC (E). TL – Thong Lanna; CC – Commercial Cultivar

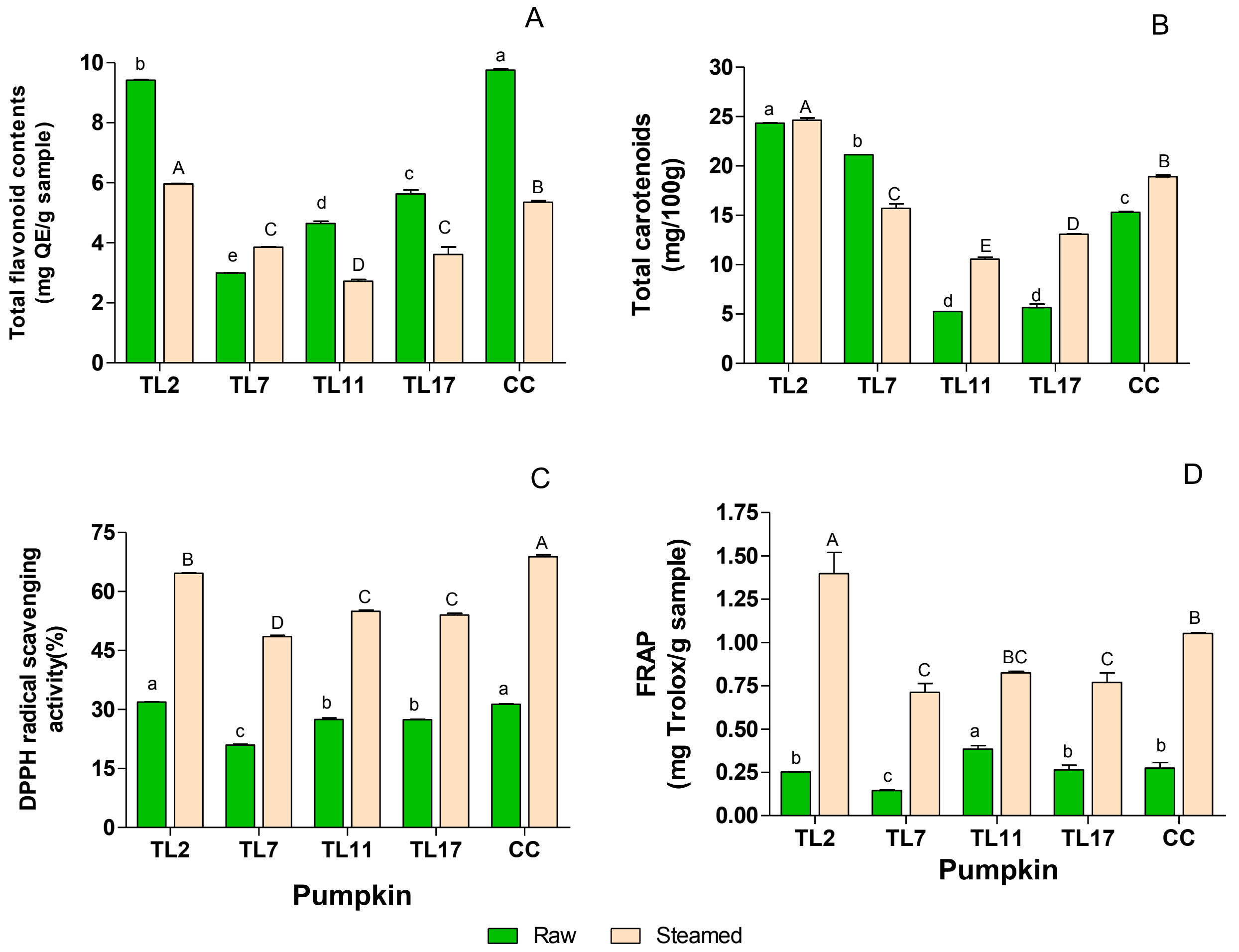
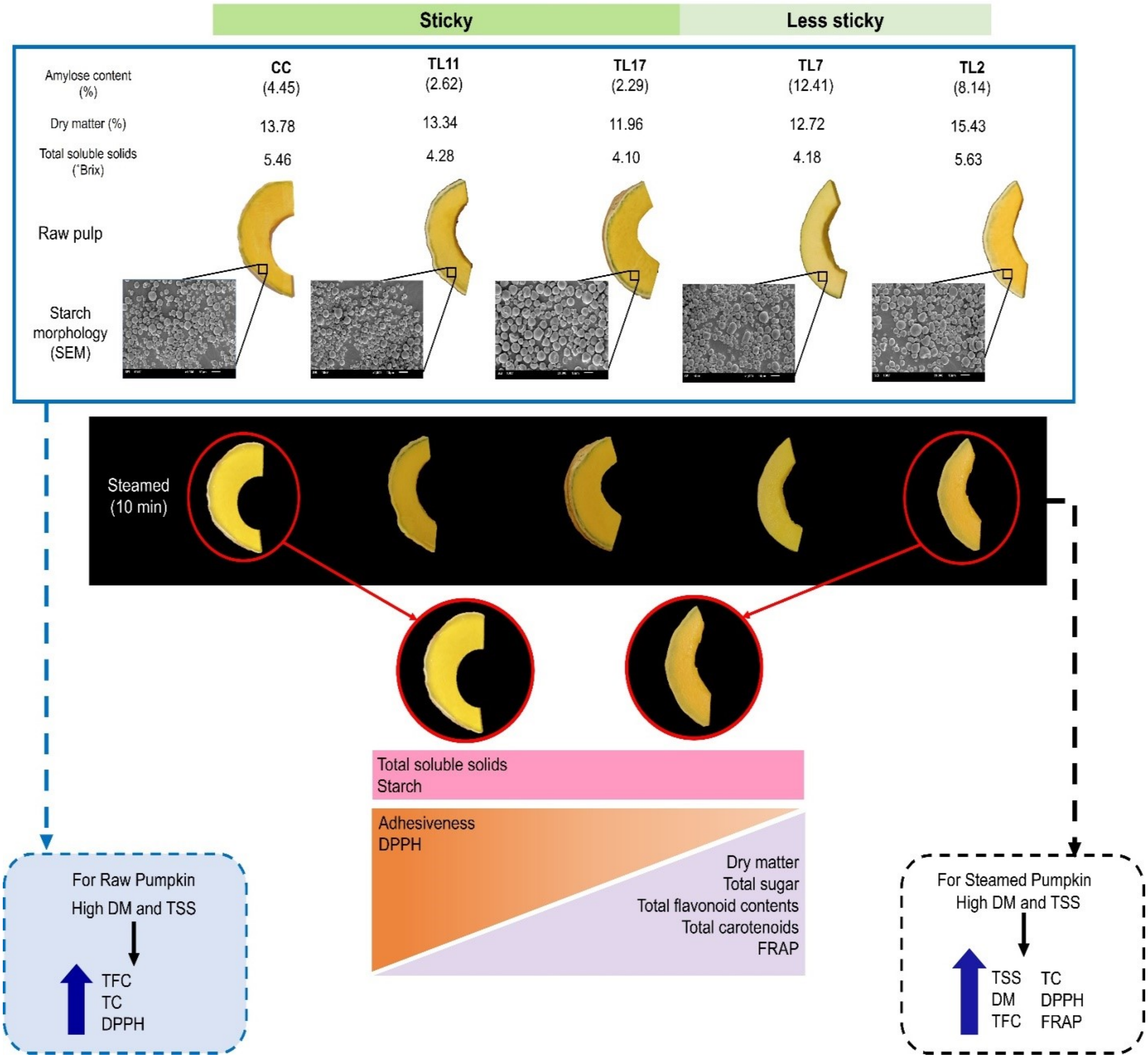


Fig. 5. The total flavonoid contents (A), total carotenoids (B), DPPH radical scavenging activity (C), and FRAP (D) value of pumpkins before and after steaming. TL – Thong Lanna; CC – Commercial Cultivar
Bars are means ± standard error (SE); Means followed by different lowercase letters ($^{a–d}$) for raw pumpkins and uppercase letters ($^{A–E}$) for steamed pumpkins in a graph are significantly different at $P \leq 0.05$ by using Tukey's Honestly Significant Difference (HSD) test.



Graphical abstract. The classification of raw pumpkins based on the amylose content with their starch morphology (SEM), and the color of pulps and the characteristics of pumpkins after steaming. Blue broken lines shows that high dry matter (DM) and total soluble solids (TSS) in raw pumpkin had higher total flavonoid contents (TFC), total carotenoids (TC), and DPPH value, while black broken line shows that high DM and TSS in raw pumpkin had higher TSS, DM, TFC, TC, DPPH, and FRAP in steamed pumpkins.

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