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Bitterness of Papaya Milk Is Related to Protein and Free Amino Acid Contents, with Phenylalanine and Tyrosine/Tryptophan Levels Being the Most Important

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Abstract. **Papaya milk, a mixture of papaya pulp and dairy milk, is one of the most popular beverages in Taiwan. However, the enzymes present in papaya can cause accumulation of hydrophobic amino acids, resulting in a bitter taste of papaya milk. Thus, it is important to select papaya cultivars without the potential to form the bitter taste, but it is difficult to select these papaya cultivars using a sensory test. The purpose of this research was to investigate the relationship between the intensity of the bitterness with the contents of proteins and free amino acids. The results indicated that neither milk nor papaya alone tastes bitter. Heating the milk or the papaya before mixing and mixing only papaya latex with milk confirmed that an enzyme in papaya causes the bitter taste in papaya milk. The intensity of bitterness positively correlated with the contents of total soluble protein, free amino acids and the phenylalanine and tyrosine/tryptophan contents. Analyses using different papaya accessions in different seasons showed that tyrosine/tryptophan (r = 0.613***) and phenylalanine (r = 0.612***) correlate more strongly with bitterness intensity than the total soluble protein (r = 0.258*) or free amino acids (r = 0.38**). In this drink, milk provides the substrates to form the bitter substances, but the enzymes in the papaya are needed for the reaction to occur. The levels of the amino acids phenylalanine and tyrosine/tryptophan showed the highest correlation with the intensity of bitterness.**

The taste of a food (sweet, salty, bitter, sour) is an important aspect determining consumer acceptance of a product (Drewnowski 1997). Among these tastes, bitterness is the least desirable among consumers (Drewnowski and Gomez-Carneros 2000). *Carica papaya* is a tropical fruit that can be eaten when ripe or unripe. In Taiwan, consumers enjoy eating papaya fruit and consume blended/macerated papaya mixed with milk, which is known as papaya milk. However, there is a downside to this beverage. Once blended, a bitter taste can form within 2 h at room temperature. In preliminary research, if papaya fruits are heated to a high temperature before mixing with milk, the bitter taste did not develop, suggesting that this flavor may be associated with heat-sensitive compounds within the papaya flesh that form stable latex dispersions. In commercial preparations of papaya milk, the papaya pulp is heated to a high temperature or its pH is changed, which inhibits enzymatic activity to avoid a bitter taste (Huet et al. 2006). Papaya cultivars also show differences in papain content, total protein content, and enzyme activities (Chovatia et al. 2010; Manjunath et al. 2014) and are therefore important in the agri-food sector (e.g., meat tenderizer). Although sensory analysis is the usual method for studying taste, it can be an obstacle due to many factors, such as the number of panelists needed and variation in taste and training. Therefore, research into new and effective screening methods is required to support papaya breeding.

In papaya, ~80% of the protein in the latex belongs to the family of high-temperature cysteine proteases (Azarkan et al. 2003; El Moussaoui et al. 2001; Sumner et al. 1993). These cysteine proteases have a wide range of substrate specificity, mostly hydrophobic amino acids (Juan et al. 2009; Michael et al. 2000; Zucker et al. 1985). Among the hydrophobic amino acids, tyrosine (0.017%), phenylalanine (0.069%), and tryptophan (0.133%) are the most bitter (Kunisuke et al. 2010).

The levels of these amino acids can easily be measured using a spectrophotometer. Tyrosine and tryptophan (Tyr/Trp) can be detected together using the Folin phenol reagent (Lowry et al. 1951), which may also be used to detect total protein levels. One way to separate the protein and tyrosine/tryptophan detection is addition of copper during this reaction. In the absence of copper, the Folin phenol reagent is highly reactive to tyrosine/tryptophan. However, this specificity is lost after the addition of copper into the reaction, which can now be used to detect the protein (Lowry et al. 1951). Therefore, this study also investigated the total soluble protein content. The phenylketonuria disease identification method was used to analyze the phenylalanine content (Ambrose 1969). The objective of this research was to determine whether there is any correlation between the bitter taste and the amino acid (tyrosine/tryptophan and phenylalanine), free amino acid, and protein contents in papaya milk and to evaluate whether any of these compounds are suitable as screening tools for papaya breeding.

Materials and Methods

Preparation of papaya drinks. Papaya fruit from the cultivar 'Tai-Nong No. 2' (TN-2) were harvested at 100% yellowed skin maturity in Gaoshu Township, Pingtung County (22°49′32″N, 120°36′5″ E), Taiwan. Ripe papaya fruits were cut into small pieces (50 g/pack) and stored at –20 °C until use. Fresh whole cow's milk was bought from the Weichuan Company.

Collection of latex from different papaya fruit lines and cultivars. The latex of the papaya lines/cultivars ML × PPI, 23, TN-2, Ekso, Thai, TN-10, Mex × Ekso, X-2, and 4-14 were collected from fruits growing during both the warm season (6 Oct) and the cool seasons (6 Jan). All papayas were grown in a net house, with the exception of TN-2, which was grown in a net house and a plastic house. Latex was harvested from fruits at stage 1 of fruit development (eighth node from flower blooming) by scratching the fruit peel then freeze-dried and stored at –20 °C until use. Latex was sampled from lines/cultivar with three replicates for determination of their bitterness.

Different proportions of papaya fruit in 250 mL of milk. Papaya milk drinks were made in four proportions (fresh frozen papaya/milk): 2:5 w/v, 3:5 w/v, 6:5 w/v, and papaya juice (1:0). One proportion was also made after heating the papaya pulp for 8 min using a 1270-W microwave: 150 g/250 mL (3:5). All mixtures were blended with 50 mL of water and incubated at 25 °C for 75 min. The bitter taste analysis was made at 0, 45, 60, and 75 min. Each treatment consisted of three replicates.

Different volume of milk with 200 g of papaya fruits. Papaya milk drinks were made in four proportions (fresh frozen papaya/milk): 4:3 w/v, 4:5 w/v, 4:7 w/v, and papaya juice (1:0). One proportion was also made after heating the

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papaya pulp for 8 min: 200 g/250 mL (4:5 w/v). All the treatments were blended with 50 mL of water. The papaya milk mixtures incubated for 0, 45, 60, and 75 min at 25 °C before tasting. Each treatment consisted of 3 replicates.

Heat treatment of papaya flesh before mixing with fresh milk. Two hundred fifty grams of frozen fresh papaya flesh was mixed with 50 mL of water and put in the microwave for 8 min. The heated papaya slurry was divided into five treatments: 1) heated papaya slurry directly mixed with 250 mL milk, 2) 50 g of heated papaya slurry cooled for 5 min and then blended with milk, 3) heated papaya slurry only, 4) papaya slurry without heating, and 5) papaya milk mixture made with 3:5 proportions (fresh frozen papaya/milk) without heating the papaya first. All treatments were tasted for bitterness after 1 h at 25 °C. Each treatment consisted of three replicates.

Heat treatment of fresh milk before mixing with papaya fruit or latex. Two hundred fifty milliliters of milk was microwaved at 1270 W for 4 min, then blended either directly with papaya juice (250 g papaya + 50 mL water) or the hot milk was cooled for 5 min before mixing with papaya juice. In the other treatment, 250 μL of 2% latex was mixed with 250 mL milk without heating. The controls included pure latex, nonheated milk, heated milk, and papaya milk (250 mL milk + 250 g papaya + 50 mL water). The bitter taste was observed after 1 h at 25 °C. Each treatment consisted of 3 replicates.

The preparation different concentrations of latex from 'Tai-Nong No. 2' papaya. Freeze-dried latex (0.1 g) was diluted in 5 mL of distilled water and divided into three groups to mix in different ratios with milk: 0.25, 0.5, and 0.75 mL of latex were mixed with 150 mL of milk. The bitter taste was observed after 30, 45, and 60 min at 30 °C. Each treatment consisted of 3 replicates.

The preparation of latex from different papaya lines and cultivars: freeze-dried latex (0.05 g) from each of the papaya lines and cultivars was dissolved in 5 mL of distilled water. This latex solution (0.24 mL) was mixed with 75 mL of milk and incubated at 30 °C for 45 min before tasting for bitterness. Each treatment consisted of three replicates.

Bitter taste analysis. The bitterness was analyzed at the Department of Horticulture (Postharvest group), National Chung Hsing University. The panelists, bitter taste rate and replicates as described in Jioe et al. (2022). Ten panelists (four women and six men, average age 20–25 years) participated in this study. The bitterness was rated at one of five levels: 0 (no bitterness), 1 (mildly bitter), 2 (bitter), 3 (very bitter), and 4 (extremely bitter). Each treatment had three replicates.

The bitter taste was calculated using the following equation:

$$\frac{\Sigma(\text{Bitter taste level} \times \text{number of people choosing that bitter taste level})}{\text{total people}}$$

Supernatant preparation of papaya milk and latex. After observation of the bitter taste, 2 mL of each treatment was mixed with 2 mL of 6% trichloroacetic acid (TCA) to stop the reaction (Yu et al. 2014). This mixture was centrifuged at 13,000 g_n for 3 min. The supernatant was collected and stored at 1 °C until analysis.

Total soluble protein (TSP) measurement: The content of total soluble proteins was determined based on Lowry et al. (1951). The supernatant (2 mL) was mixed with 5 mL of reagent A (2 g sodium carbonate, 1 mL of 2% potassium tartarate, 1 mL of 1% $CuSO_4$, 1 mL 1 N sodium hydroxide and 90 mL water) and incubated for 10 min. Then, 0.5 mL of reagent B (Folin reagent/water = 1:1) was added and incubated for 30 min at room temperature. The reaction was measured by ELISA Reader (FLUOstar Omega) at wavelength 660 nm. The standard was 0.25 mg/mL BSA. The amount of protein was reported as mg/100 mL papaya milk or μg/mL milk. Each treatment consists of three replicates.

Free amino acid measurement. The content of the free amino acids (FAA) was determined following Rosen (1957). The supernatant (1 mL) was mixed with 1 mL of ninhydrin reagent, boiled for 10 min, cooled with ice water, and then mixed with 5 mL of color diluent and was measured at 570 nm. The standard was 1 mM α-alanine. The amount of free amino acids was reported as mmol/100 mL papaya milk or μmol/mL milk. Each treatment consists of three replicates.

Tyr/Trp measurement. The level of combined tyrosine/tryptophan (Tyr/Trp) was determined using the method from Lowry et al. (1951). The supernatant (1 mL) was mixed with 5 mL sodium carbonate (4% w/v) and 1 mL Folin ciocalteu reagent (20% v/v) and incubated for 20 min at 37 °C. The absorbance of the solution was measured by ELISA Reader (FLUOstar Omega) at wavelength 660 nm. The standard was 0.025 g tyrosine/100 mL. The Tyr/Trp level was reported as mg/100 mL papaya milk or mg/100 mL milk. Each treatment consists of 3 replicates.

Phenylalanine measurement. Phenylalanine (Phe) measurement followed Ambrose (1969). One milliliter of supernatant was added with 2.5 mL of 0.06 N trichloroacetic acid (TCA) and divided into three groups. For the sample, 0.5 mL of dilution sample was added with 1 mL ninhydrin-peptide mixture (4 mL 0.5 M phthalate buffer pH 5, 8 mL 0.03 M ninhydrin, and 8 mL 0.005 M L-leucyl-L-alanine). For the dilution sample blank, 0.5 mL of dilution sample was added with 1 mL peptide control mixture (3 mL 0.5 M phthalate buffer pH 5, and 2 mL 0.03 M ninhydrin). The blank was 0.5 mL 0.06 N TCA mixed with 1 mL ninhydrin-peptide. All groups were incubated for 16 min at 85 °C then cooled for 10 min at 30 °C. After cooling, 6 mL of pyrophosphate reagent were added to the dilution sample and dilution sample blank; 6 mL ddH_2O was added for the blank. All supernatants including the blank were incubated at 30 °C for 30 min, then incubated again at 85 °C for exactly 6 min and recooled for 15 min at 30 °C and were analyzed with a Fluorometer Spectrophotometer f-2500 (Hitachi, Tokyo, Japan) at wavelength 360-nm activation and 495-nm emission. A volume of 1.65 mg Phe/100 mL in 0.06 N TCA was set as the standard. The Phe level was reported as mg/100 g DW. Each treatment had three replicates.

Statistical analysis. Bitter taste analysis, total soluble protein content, free amino acid content, and tyr/try and Phe contents were showed in triplicate, and the mean values were calculated. The data were subjected to analysis of variance and Duncan's test in CoHort software (CoStat v. 6.101) were used to assess the differences between the means. A significant difference was presumed at a level of $P < 0.05$. The correlation between a compound content and the bitter taste was reported at the following levels of significance: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS = not significant.

Results

Bitterness intensity in papaya milk of different proportions. The bitterness of papaya milk increased by increasing the incubation time (Fig. 1) at three proportions of papaya to milk (2:5, 3:5, and 4:7). The mixtures with the lowest papaya content (2:5) had the highest bitter taste after incubation for 75 min (intensity of 2.63) compared with the higher papaya proportions (1–1.78). A similar trend was seen when the amount of milk was changed compared with the amount of papaya (Fig. 2B). The papaya juice by itself and the papaya milk containing heated papaya showed no bitter taste after 75-min incubation (Fig. 1A and B).

Heating the papaya fruits before mixing with milk showed that either hot or recooled papaya fruits yielded a papaya milk with low bitterness intensity (0.2) compared with papaya milk prepared with unheated fruit (Fig. 1C). On the other hand, heating the milk before mixing with the papaya fruit created papaya milks with a bitterness of 1.395 (heated milk) and 2.23 (heated then cooled milk) compared with the papaya milk (1.46). The highest bitter taste level was the papaya latex milk mixture (3.8) (Fig. 1D). However, papaya latex, papaya juice, and milk each showed low or no bitter taste (0.1, 0.0475, and 0) (Fig. 1C and D).

Bitterness intensity in papaya latex from different accession harvested during warm and cool seasons. Latex taken from fruit of the cultivar 'Tainung No. 2' was mixed with milk and then incubated for up to 60 min. Higher concentrations of papaya latex in milk did increase the bitter taste intensity over time (Fig. 2A). This showed that the bitterness is related to latex content.

Fruits from different papaya accession were harvested in both the warm and cool season. Papaya harvested in the warm season yielded latex with higher bitterness intensities in all accession except 4-14 (Fig. 2B) The bitterness of the latex from cultivar 4-14 did not significantly differ with season. Of the fruits harvested in the warm season, the most bitter were 'Ekso' followed by ML×PPI, and 'TN-10' (bitterness intensities of 0.98, 0.68, and 0.68, respectively). Of the fruit harvested during the cool season, only TN-2 (grown in a plastic house), X-2, and 4-14 showed much

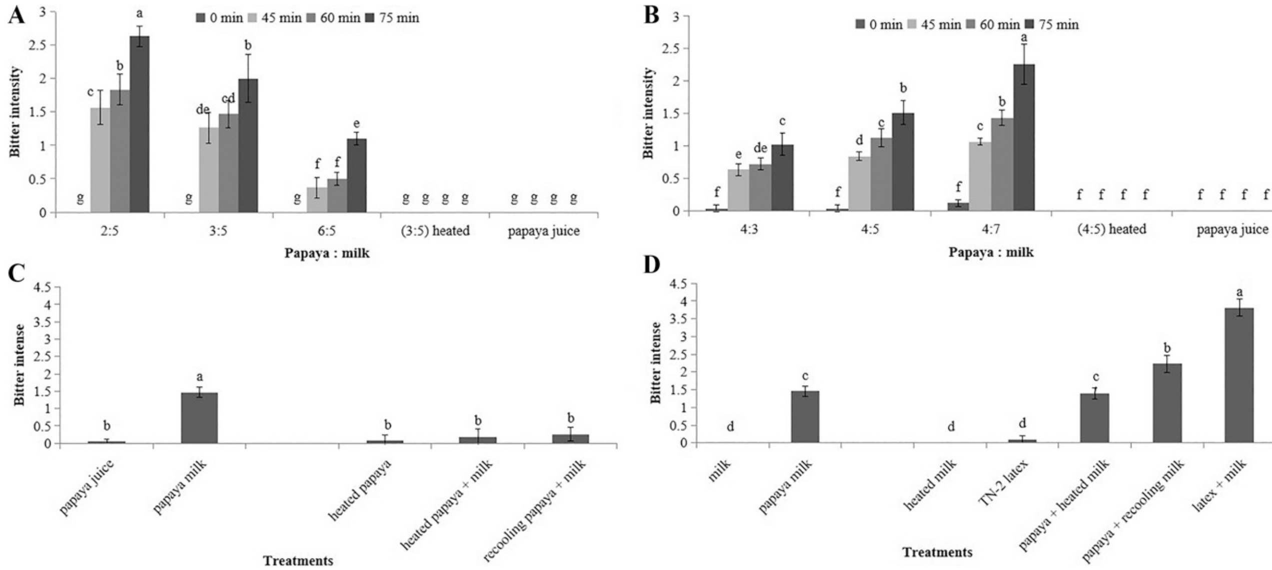

Fig. 1. Bitterness intensity of various papaya milk mixtures: (**A**) papaya content increasing in 250 mL fresh milk; (**B**) volume of fresh milk increasing with 200 g papaya; (**C**) with or without heating the papaya fruit before mixing with milk; (**D**) with or without heating the milk and papaya latex. Data are mean ± *SD*, n = 3. * Mean statistical data within a column were obtained by Duncan's test at the 5% level (same letters means no significant difference).

bitterness (0.25, 0.23, and 0.16), whereas other lines/cultivars showed little to no bitter taste (Fig. 2B).

Total soluble protein content in papaya milk and latex/milk mixtures. The different papaya milk mixtures as well as the latex/milk mixtures were analyzed for total soluble protein (TSP) (Fig. 3). The papaya milk at all proportions showed an increase in TSP content after incubation for 75 min except the mixtures made with heated papaya milk or papaya juice, in which no TSPs were detected (Fig. 3A and B). Comparison of the TSP content with the bitterness intensities in the different papaya content in 250 mL of fresh milk showed no or a similar tendency. For example, after 75 min, the 4:5 papaya-to-milk mixture showed the lowest total soluble protein but the second highest bitterness intensity (Figs. 1B and 3B).

In the mixtures containing TN-2 latex and milk, the TSP content increased with incubation time and with latex concentration in milk (Fig. 3C). The latex mixtures showed a similar tendency in TSP content and bitter intensity with incubation time (Figs. 1A–C and 3A–C).

Comparison of the latex from different papaya accessions showed that most of the lines/cultivars had higher TSP content in warm seasons, except Ekso, X-2, and 4-14. Ekso had a high TSP content during the cool season and X-2 and 4-14 showed no significant differences between the warm and cool season samples (Fig. 3D).

FAA content in papaya milk and mixtures. The different papaya milk mixtures as well as the latex/milk mixtures were analyzed for FAA (Fig. 4). The FAA content of the various papaya milk blends increased during incubation in trends similar to their bitterness intensity (Figs. 1A and 4A). On the other hand, the FAA content decreased as the proportion of milk increased (Fig. 4A), which was opposite to the increase in bitterness (Figs. 1B and 4B). Among the latex samples, the FAA content in the latex of TN-2 (plastic house), X-2, and 4-14 showed opposite tendencies compared with the bitterness. The latex from 'Mex × Ekso' and ML × PPI showed no significant different in FAA between seasons but had a high bitter taste in warm seasons. The latex from most of the lines/cultivars showed similar tendencies in the FAA content and bitterness level (Fig. 4D).

Tyr/Trp content in papaya milk and latex/milk mixtures. Analysis of the Tyr/Trp content revealed that increasing either the papaya content or the milk volume decreased the tyrosine Tyr/Trp content (Fig. 5A and B). Furthermore, the bitterness intensity correlated with the Tyr/Trp content with increasing the amount of papaya but not the different milk volumes (Figs. 1A, 1B, 5A, and 5B).

The FAA content showed a similar trend to bitterness in the latex samples (Fig. 5A). Most of the papaya lines/cultivars between seasons also showed similar tendencies in FAA content and bitterness. In samples from the same season, there was little difference. For example, the Tyr/Trp content between 4-14 and TN-2 (plastic house) harvested during the warm season were similar, but the TN-2 (plastic house)

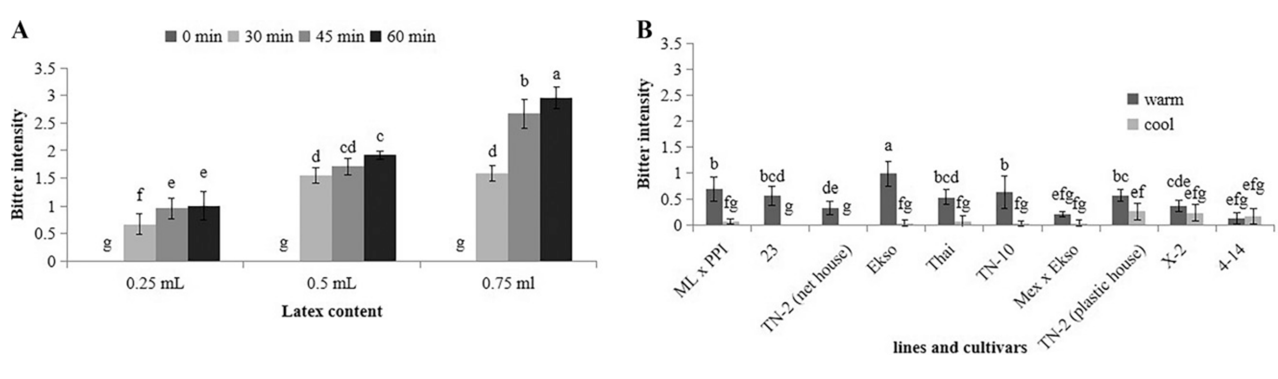

Fig. 2. The investigation of bitterness intensity in the latex of papaya fruit. (**A**) Latex from 'Tainung No. 2' (TN-2) was mixed at different concentration with milk and incubated at 30 °C for 0, 30, 45, or 60 min. (**B**) Papaya latex from nine lines or cultivars were harvested during the cool season and the warm season. TN-2 papaya were also grown in a plastic house. Data are mean ± *SD*, n = 3. * Mean statistical data within a column were obtained by Duncan's test at the 5% level (same letters mean no significance).

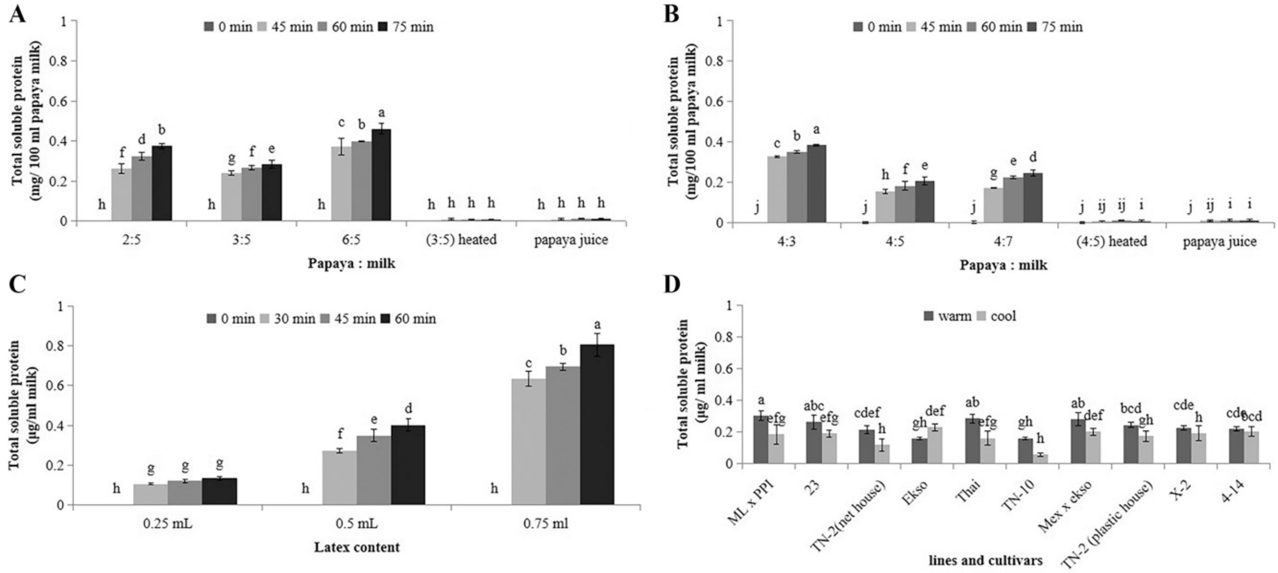

Fig. 3. The investigation of total soluble protein content in papaya milk made with (**A**) varying papaya content in 250 mL fresh milk, (**B**) varying volume of fresh milk with 200 g papaya, (**C**) different concentrations of 'Tainung No. 2' (TN-2) latex in milk, and (**D**) papaya latex from different lines/cultivars harvested during cool or warm seasons. Data are mean ± *SD*, n = 3. * Mean statistical data within a column were obtained by Duncan's test at the 5% level (same letters mean no significance).

sample had a more bitter taste than the 4-14 sample. The Tyr/Trp content of 'Ekso' did seem to correlate with bitterness because the latex from 'Esko' had the highest bitterness intensity and the second highest Tyr/Trp content.

Phe content in papaya milk and latex/milk mixtures. Phe content in the various papaya milk mixtures increased during incubation for all proportions. Interestingly, the heated papaya milk and papaya juice showed little to no accumulation in Phe (Fig. 6A and B). In the TN-2 papaya latex in milk samples, the Phe content increased with incubation time or with increasing latex concentration (Fig. 6C). Among the latex samples from the different papaya accessions, 'Ekso' had the highest Phe content in both the warm and cool seasons. The TN-10 latex samples had no detectable Phe content when harvested in the cool season. The TN-2 (plastic house) and X-2 samples showed no Phe accumulation during the warm season. 'Thai', 'Mex × Ekso' and 4-14 showed no significant difference in Phe content between the warm and cool seasons (Fig. 3D).

Correlation between bitterness intensity of papaya milk/latex milk mixtures and TSP, FAA, Tyr/Trp, and Phe contents. The correlation between bitterness intensity and the protein and amino acid content is shown in Table 1. All four content measures showed a strong positive correlation with bitterness intensity in the papaya milk made with different papaya-to-milk proportions and in the latex/milk mixtures with different concentrations of papaya latex. The correlation coefficients ranged from 0.637 to 0.96.

The harvest season (warm or cool) showed more variation with bitterness level. The TSP and FAA content in the different papaya accessions showed weak correlation in both the warm and cool seasons. Combination of the levels of Tyr/Try and Phe from the warm and cool season correlated (r = 0.613 and 0.612) with bitterness, whereas TSP and FAA had correlation coefficients of 0.258 and 0.38, respectively.

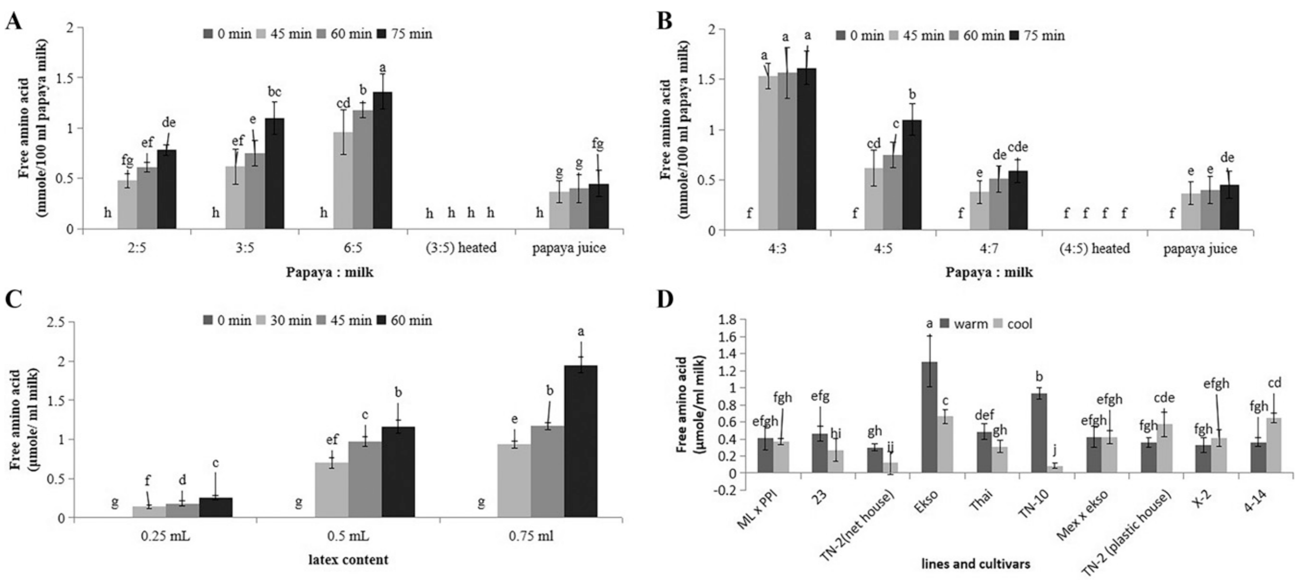

Fig. 4. Free amino acid content in papaya milk made with (**A**) varying papaya content in 250 mL fresh milk, (**B**) varying volume of fresh milk with 200 g papaya, (**C**) different concentrations of 'Tainung No. 2' (TN-2) latex in milk, and (**D**) papaya latex from different lines/cultivars harvested during cool and warm seasons. Data are mean ± *SD*, n = 3. * Mean statistical data within a column were obtained by Duncan's test at the 5% level (same letters mean no significance).

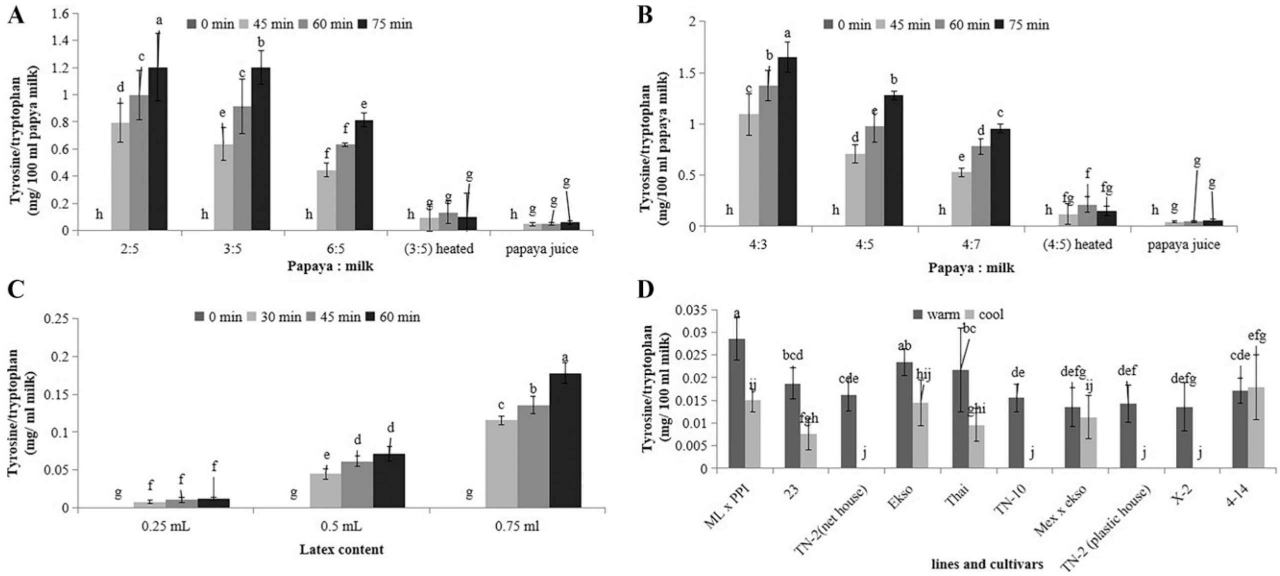

Fig. 5. The investigation of tyrosine/tryptophan content in papaya milk with (**A**) varying papaya content in 250 mL fresh milk, (**B**) varying volume of fresh milk with 200 g papaya, (**C**) different concentration of 'Tainung No. 2' (TN-2) latex in milk, and (**D**) papaya latex in fruit during cool season and warm seasons. Data are mean ± *SD*, n = 3. * Mean statistical data within a column were obtained by Duncan's test at the 5% level (same letters mean no significance).

Discussion

The bitterness intensity in the different papaya milk mixtures revealed that a higher papaya content or a lower milk content decreased the bitterness intensity (Fig. 1A and B). This suggested that sweetness may interfere during the sensory analysis and oppose the bitterness in papaya milk, acting as a masking agent (Gous et al. 2019; Talcott and Howard 1999). These results are similar to observations in carrot in which the threshold of the bitter taste substance in carrot (coumarin) was ∼20 mg/kg in water (Czepa and Hofmann 2003), but in carrot puree, coumarin content had a threshold of ∼94 to 100 mg/kg (Talcott and Howard 1999; Yoshino et al. 1993). Another possibility is the lower levels or lack of bitter tasting compounds, as can be seen in Fig. 1A and B where low milk content lowered the bitterness intensity compared with high milk content. These findings indicated that bitter taste in papaya milk mixture is caused by papaya enzymes degrading milk protein.

However, the milk did not taste bitter (Fig. 1D) and only became bitter after mixing with papaya fruit or latex. Furthermore, heating the papaya fruit or latex before mixing with milk inhibited the bitter taste in the papaya milk (Fig. 1C and D). Azarkan et al. (2003) and El Moussaoui et al. (2001) found that ∼80% of the latex protein is cysteine proteases and suggested that these enzymes cause the bitter taste in papaya milk beverage by hydrolyzing milk proteins (Fig. 2A). Furthermore, preliminary research for determining the microwave duration (4 and 8 min) for papaya juice found that papaya juice microwaved for 4 min (temperature ∼70 °C) was still able to induce the bitter taste after mixing with milk. On the other hand, papaya juice microwave for 8 min (the juice was boiling for the last 2 min) showed no bitter taste. These experiments suggested that latex enzymes were stable at high temperatures. These results are in

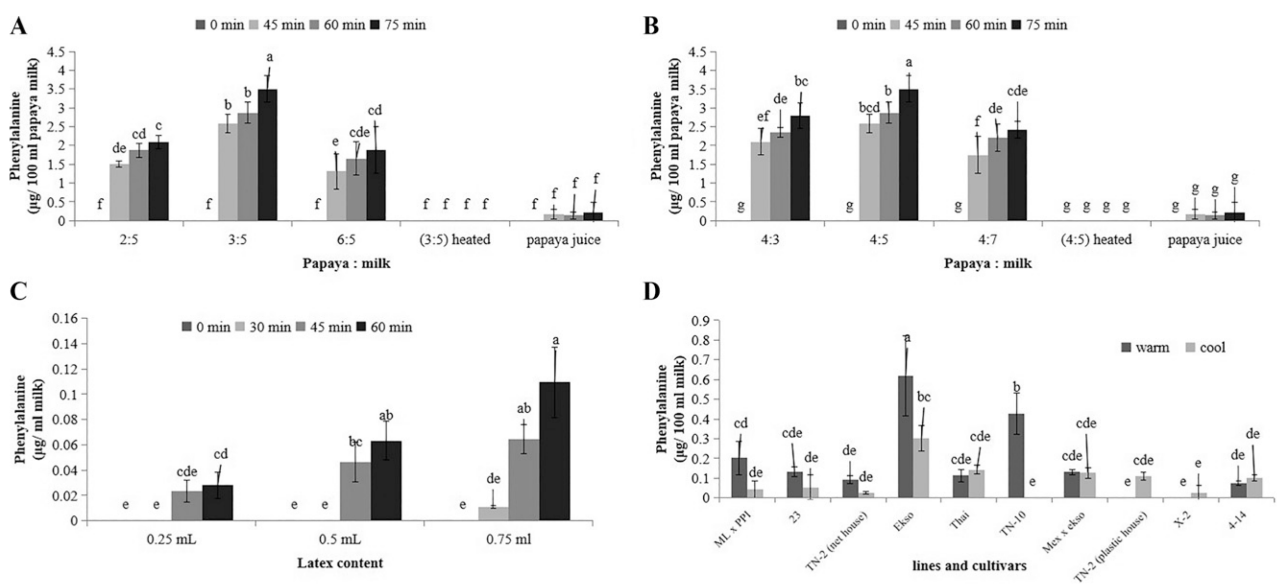

Fig. 6. The investigation of phenylalanine content in papaya milk with (**A**) different papaya content in 250 mL fresh milk, (**B**) different volume of fresh milk in 200 g papaya, (**C**) different concentrations of TN-2 latex in milk, and (**D**) papaya latex from different accessions harvested during cool and warm seasons. Data are mean ± *SD*, n = 3. * Mean statistical data within a column were obtained by Duncan's test at the 5% level (same letters mean no significance).

Table 1. Correlation of bitterness intensity with total soluble protein (TSP), free amino acid (FAA), tyrosine/tryptophan, and phenylalanine content.

	Bitterness intensity (*r*)			
Bitterness compound	Different papaya wt	Different milk volume	Different concn of 'Tai-Nong No. 2' papaya latex	Warm seasons × cool seasons
Total soluble protein/TSP	0.755***	0.738***	0.882***	0.258*
Free amino acid/FAA	0.651***	0.637***	0.895***	0.38**
Tyrosine/tryptophan content	0.96***	0.756***	0.9***	0.613***
Phenylalanine content	0.84***	0.83***	0.76***	0.612***

NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, or 0.001, respectively.

agreement with those of Esmat (2021) and Sumner et al. (1993): the cysteine proteases of papaya denatures at high temperature (80 to 89.2 °C). Although high temperature prevented the development of the bitter taste in the papaya milk, the taste preference of papaya milk decreased as a result (Supplemental Fig. 1), suggesting that some compounds had been oxidized during heating (Dulce et al. 2010; Zhou et al. 2021). In addition, The color of the heated papaya milk mixture also changed (data not recorded), possibly from oxidization of the carotenoids, especially lycopene, which is abundant in red-fleshed papaya (Julius 2012; Ralf et al. 2011; Wall 2006). Together, the data show that the bitter taste forms through the reaction between papaya latex and milk.

The experiments mixing various concentrations of latex in milk (Fig. 2A) suggest that the development of the bitter substances could be related to the enzymes present in the papaya latex. The papaya latex enzymes papain, chymopapaine, and caricain mainly cleave proteins at hydrophobic amino acids. (Jacquet et al. 1989; Juan et al. 2009; Michael et al. 2000; Zucker et al. 1985). Previous research suggests that hydrophobic peptides in cheese and sake and that hydrophobic amino acids themselves taste bitter (Koka and Weimer 2000; Maeda et al. 2011). These previous studies were supported in this experiment, where TSP and FAA contents increased in latex/milk mixtures during incubation time (Figs. 3A–C and 4A–C) with correlation coefficients ranging between 0.637 and 0.895 (Table 1). However, the correlation between bitterness intensity and harvest season was a weak positive correlation ($r = 0.258$ and 0.38) (Table 1). The findings could be linked to the nonspecific methods used in this research Lowry et al. (1951) and Rosen (1957).

The investigation into the Phe and Tyr/Try content in different papaya milk formulations showed similar increased with the TSP and FAA during incubation (Figs. 5A–C and 6A–C). However, the correlations between Phe and Tyr/Trp and bitter intensity ($r =$ 0.759–0.96) were slightly higher than those of TSP and the FAA (0.637–0.755) (Table 1). These differences were bigger in the papaya accession/harvest seasons observations, where Phe and Tyr/Try content showed a strong positive correlation ($r = 0.612$ and 0.613), whereas TSP and FAA showed a weak positive correlation ($r = 0.258$ and 0.38) (Table 1), suggesting that the source of bitter taste in papaya milk was related to the hydrophobic amino acids (Kunisuke et al. 2010; Rudolph et al. 2018; Yin et al. 2019). Although hydrophobic peptides are one of the factors, the study of bitter peptides was difficult due to the various combinations of hydrophobic amino acids (Ishibashi et al. 1988; Matoba and Hata 1972). In cool seasons, either TSP, FAA, Phe, and Tyr/Trp content showed no significant differences (data not shown), suggesting that the papaya produce less enzymes during the cool season. Nevertheless, the correlation of bitterness intensity with TSP, FAA, Phe, and Tyr/Trp content showed a decline in the correlation coefficient (Table 1), implying that there were different compositions of papaya enzymes among the lines/cultivars. However, this study did not observe these differences. Additionally, study of one cultivar, TN-2, grown in different conditions (net house and plastic house) showed different results, indicating that light also plays an important role in latex biosynthesis. Overall, this study showed that Tyr/Try and Phe content had strong correlation with bitter taste.

Conclusions

In conclusion, the different proportions of papaya and milk ($r =$ 0.651–0.755) and latex and milk ($r =$ 0.882–0.895) showed that there is a strong positive correlation between protein and amino acid content and the intensity of the bitterness. There was less correlation between bitterness intensity and papaya accessions ($r = 0.258$ and 0.38). In the analysis of Phe and Tyr/Trp, the correlation coefficients for the different papaya lines/cultivars also decreased but still achieved a high positive correlation with the intensity of bitterness ($r = 0.612$ and 0.613), which suggests that those amino acids had a role in the bitterness of papaya milk.

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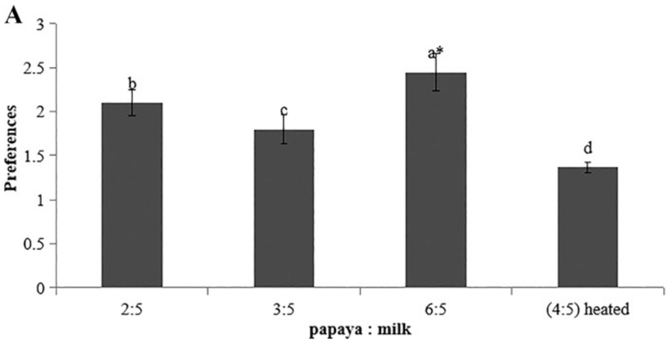

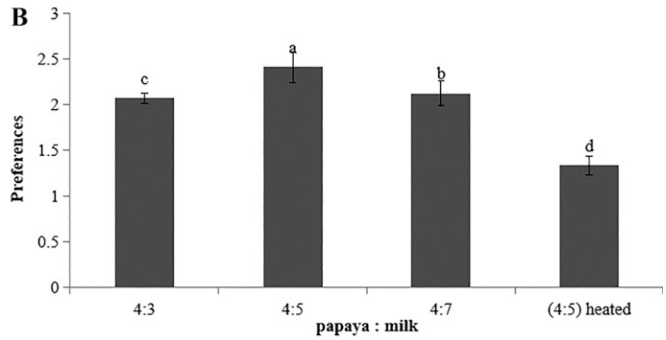

Supplemental Fig. 1. Preference levels of papaya milk prepared with (**A**) varying papaya content in 250 mL fresh milk and (**B**) varying volume of fresh milk with 200 g papaya. Data are mean ± *SD*, n = 4. * Mean statistical data within a column were obtained by Duncan's test at the 5% level (same letters mean no significance). The preference level was divided into 5 categories: 0 (very bad), 1 (bad), 2 (acceptable), 3 (good), 4 (very good). The bitter taste was calculated using the following equation:

$$\frac{\Sigma(\text{Preferences level} \times \text{number of people chose the preferences level})}{\text{total people}}$$