Factors Affecting Bitterness in Papaya (*Carica papaya* L.) Milk

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Summary

Papaya milk is one of the most popular beverages in Taiwan. However, fresh papaya milk is easy to form bitter substances after storing at room temperature for more than two hours. The purpose of this study is to investigate the causes of bitter taste in papaya milk. Our results indicated that milk or papaya alone did not taste bitter. Furthermore, heated milk or milk that's been heated and then cooled mixed with papaya showed stronger bitter taste than heated papaya or papaya that's been heated and then cooled mixed with milk. Moreover, papaya latex mixed with milk showed the strongest bitter taste. These results suggested that the bitter taste in papaya milk may be involved with the enzymatic reaction between papaya enzymes in papaya fruit and their substrates in the milk.

Introduction

Taste (sweet, salty, bitter, sour) is the important index to determine consumers' acceptance (Drewnowski *et al*., 1997). Among these tastes, bitter taste usually becomes a consumer's refusal (Drewnowski and Gomez-Carneros, 2000) and this phenomenon can be found in many beverages and fruits. Papaya is a tropical fruit and can be eaten at ripe or unripe condition. In Taiwan, despite eating as fruit, papaya also can be drunk together with milk known as papaya milk. However, fresh papaya milk must be drunk within two hours due to the formation of bitter taste. In order to prevent the formation of bitter taste, papaya pulps need to be cooked before mixing with milk. Wolff (1978) observed that the papaya pulp had to be blended at 91^0C for 25 min to avoid bitter

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taste and unpleasant smell. This heating process might cause some flavor-loss and affect the consumer preferences. Up to now there is no information about how bitter taste was formed in papaya milk.

Thus, the purpose of this research is to observe the bitter taste in different proportion of papaya milk and find out how the bitter taste is formed.

Material and Methods

1. Preparation of 'Tai-Nung No. 2' papaya fruits

'Tai-Nung No. 2' fully ripen papaya fruits were cut into small pieces (50 g/ packet) and stored at -20^{0}C till its usage.

2. Papaya milk prepared from different weights of papaya and 250 mL of milk

Papaya milks were prepared by using three different papaya and milk mixing ratios (papaya/milk): 100 g / 250 mL (2:5), 200 g / 250 mL (4:5), and 300 g / 250 mL (6:5). They were then individually mixed with 50 mL of water. After that, preference index and bitter intensity were immediately recorded. A heating treatment by microwaving 200 g of papaya fruit for 8 minutes and then blended directly with 250 mL of milk and 50 mL of water was also included in this experiment. In another set of experiment, the above-mentioned papaya milks were incubated at 25^{0}C for 75 min after mixing with 50 mL of water. In each papaya milk, preference index and bitter intensity were recorded at 0, 45, 60, and 75 min at 25^{0}C after mixing with water. Each measurement consists of three replicates.

3. Papaya milk prepared from various amount of milk and 200 g of papaya

In another study, papaya milk was prepared with another three different papaya and milk mixing ratios (papaya/milk): 200 g / 150 mL (4:3), 200 g / 250 mL (4:5), and 200 g / 350 mL (4:7). They were then individually mixed with 50 mL of water. After that, preference index and bitter intensity were immediately recorded. A heating treatment by microwaving 200 g of papaya fruit for 8 minutes and then blended directly with 250 mL of milk and 50 mL of water was also included in this experiment. In another set of experiment, the above-mentioned papaya milks were incubated at 25^{0}C for 75 min after mixing with 50 mL of water. In each papaya milk, preference index and bitter intensity were recorded at 0, 45, 60, and 75 min at 25^{0}C after mixing with water. Each measurement consists of three replicates.

4. Different heating conditions of papaya fruit

Papaya fruit (250 g) was blended with 50 mL of water and microwaved for 8 minutes. The papaya juice thus obtained was treated in three different ways: First, the heated papaya juice was

directly mixed with 250 mL of milk. Secondly, the heated papaya juice was cooled for five minutes and then mixed with 250 mL of milk. Thirdly, the heated papaya juice was not mixed with any milk. Papaya juice and papaya milk (prepared as previously mentioned) without heating treatment were used as control. In all treatments, the bitter intensity was recorded at 25^0C one hour after mixing with milk. Each treatment consists of three replicates.

5. Different heating conditions of milk with or without adding latex

Milk (250 mL) was heated with microwave for 4 minutes and then mixed directly or five minutes after microwaving with papaya juice (prepared as previously described). In another treatment, 250 μL of 2% papaya latex was mixed with 250 mL milk. Pure latex, non-heated or heated milk and papaya milk (250 mL milk + 250 g papaya + 50 mL water) were used as control. Bitter intensity was recorded at 25^0C one hour after mixing. Each treatment consists of three replicates.

6. Determination of preference index and bitter intensity

At least ten consumers were asked to taste the papaya fruits and record the bitter intensity and preference index of the fruits. Bitter intensity was measured on a scale of 0-4: 0 (no bitter), 1 (mild bitter), 2 (bitter), 3 (very bitter), 4 (extreme bitter). Preference index was also determined on a scale of 0-4: 0 (very bad), 1 (bad), 2 (acceptable), 3 (good), 4 (very good). The following formula was used to calculate the average bitter intensity or preference index:

$$\frac{(N_0*0)+(N_1*1)+(N_2*2)+(N_3*3)+(N_4*4)}{\text{total people}}$$

Ni= number of people choose the scale i.

i = 0, 1, 2, 3, or 4.

Result

In this study, the preference indexes observed in papaya milk containing either different papaya weights or milk volumes and measured immediately after mixing with 50 mL of water were all around 2 but the values dropped to about 1.5 when these papaya milks were prepared from 200 g of microwaved papaya (Fig. 1). If these papaya milks were incubated at 25^0C for 0-75 min, the preference index decreased as the incubation time increased (Fig. 2 and 3). Comparison among papaya milks containing different weights of papaya indicated that after 75 min of incubation, papaya milk with a mixing ratio of 6:5 (papaya : milk) had the highest preference index with a value of 2 followed by those with mixing ratios of 2:5 and 4:5 with values of 0.76 and 0.87, respectively (Fig. 2). Results from papaya milk containing different

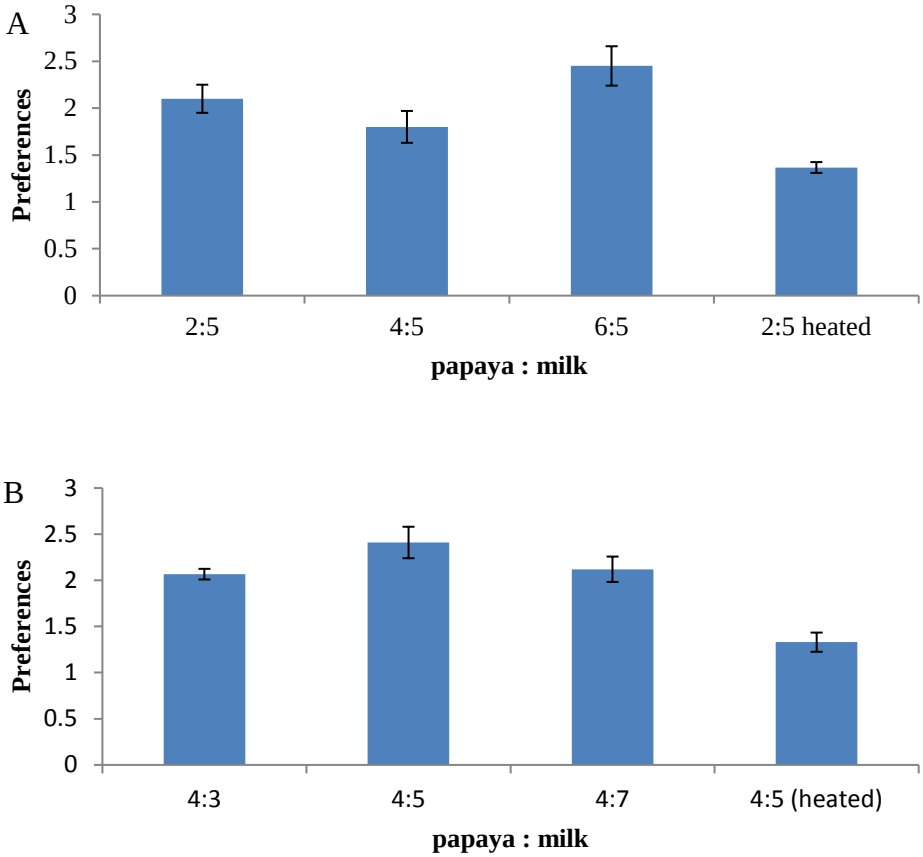

Fig. 1. The preference index of papaya milk prepared from (A) different weights of papaya and 250 mL milk, and (B) various amounts of milk and 200 g of papaya.

[h] 200 g of papaya was microwaved for 8 minutes and then mixed directly with 250 mL of milk and 50 mL of water.

volumes of milk showed that a mixing ratio of 4:7 has the lowest preference index with a value of around 1.09 after incubation at 25^0C for 75 min (Fig. 3).

On the other hand, bitter intensity was negatively correlated with preference index in all treatments and increased with the incubation time (Fig. 2 and 3). Results from papaya milk containing different weights of papaya showed that a mixing ratio of 6:5 had the lowest bitter intensity during the 75 min incubation period (Fig. 2). Furthermore, results from papaya milk containing different volumes of milk showed that the highest bitter intensity was observed in a mixing ratio of 4:7 with a value of 2.26 followed by mixing ratios of 4:5 and 4:3 with values of 1.51 and 1.02, respectively (Fig. 3).

Results of papaya milk made from papaya heated at different conditions revealed that the bitter intensity of papaya milk was around 0.2 while the bitter intensity in non-heated papaya milk was about 1.46 (Fig. 4). In contrast, papaya milks made from milk heated at different conditions showed completely different results whereas heated milk or milk that's been heated and then cooled (recooling milk) resulted in stronger bitter taste compared to that of papaya milk made from heated papaya fruit (Fig. 4 and 5). In addition, papaya milk containing papaya latex had highest bitter intensity with a value of about 3.8 but the bitter intensity of latex itself was only about 0.1 (Fig. 5). Furthermore, the bitter intensity in heated milk and papaya fruit was around 0 and 0.09, respectively (Fig. 4 and 5).

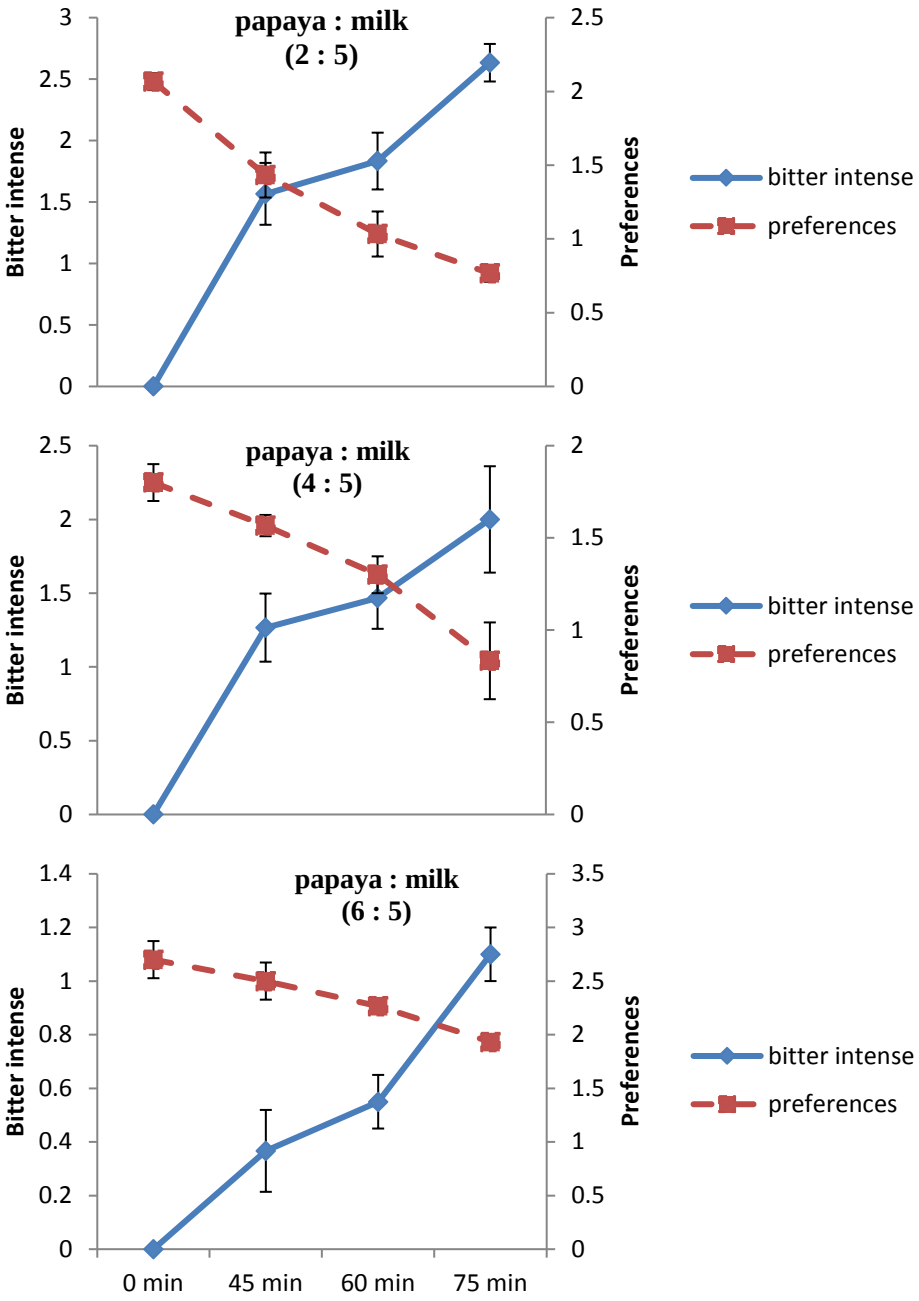

Fig. 2. The bitter intensity and preference index of papaya milk prepared from different weights of papaya and 250 mL milk during 0-75 min of incubation at 25^0C.

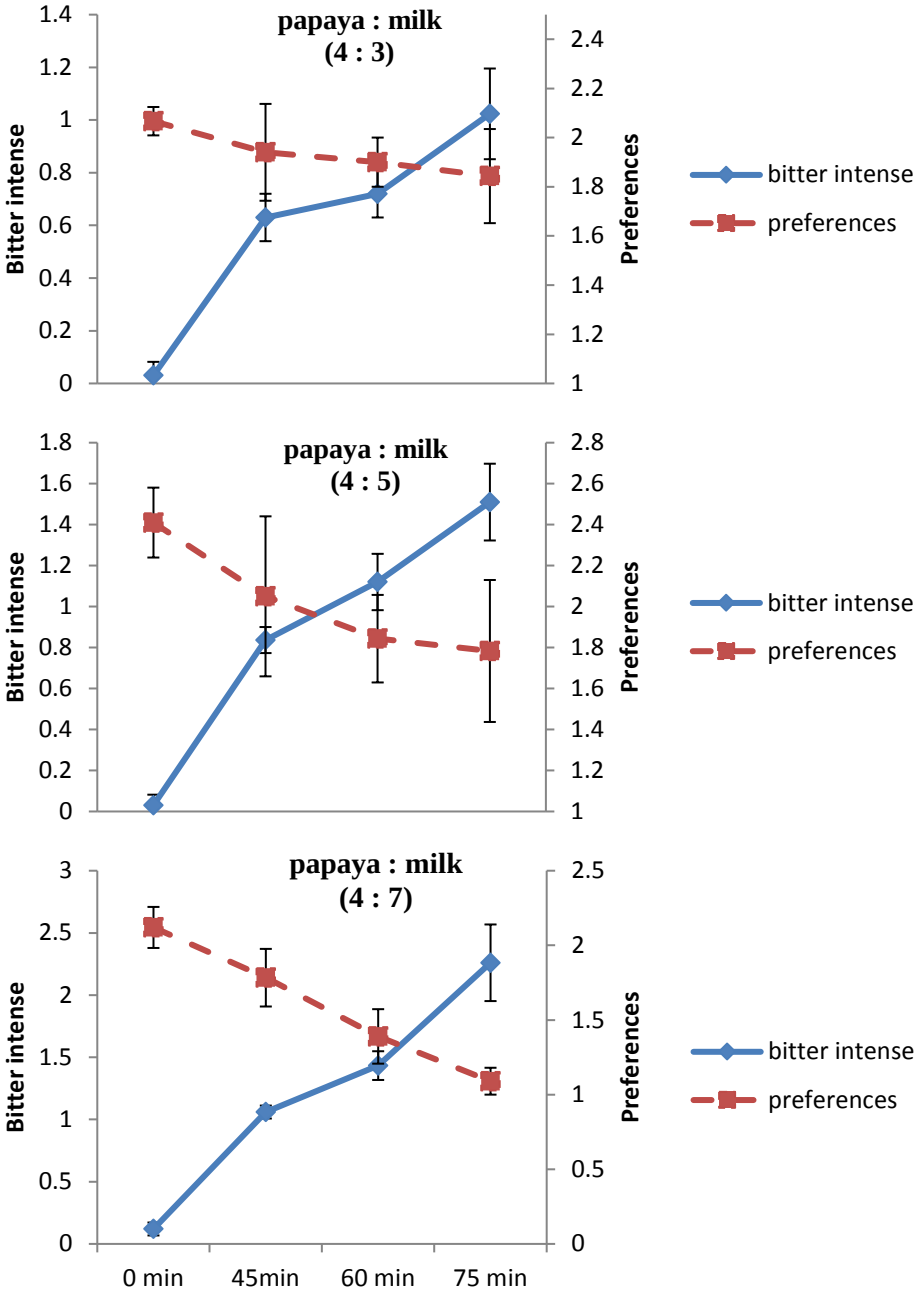

Fig. 3. The bitter intensity and preference index of papaya milk prepared from different amounts of milk and 200 g papaya during 0-75 min of incubation at 25^0C.

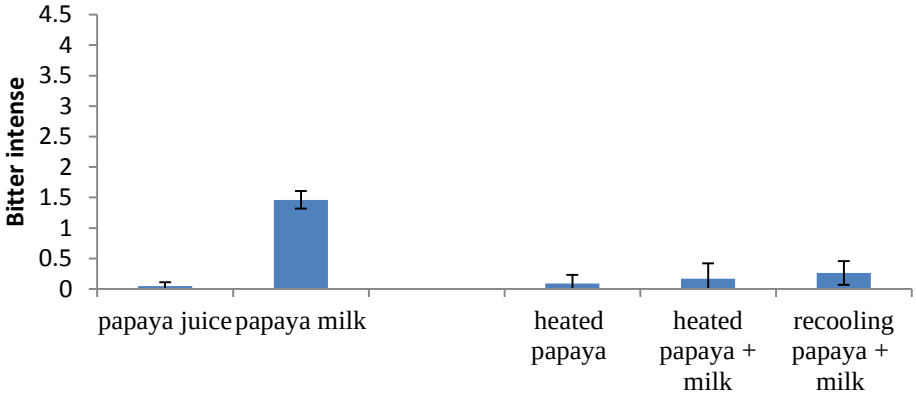

Fig. 4. The bitter intensity of papaya juice, papaya milk, heated papaya and papaya milk containing papaya heated at different conditions. All test samples were used one hour after been prepared.

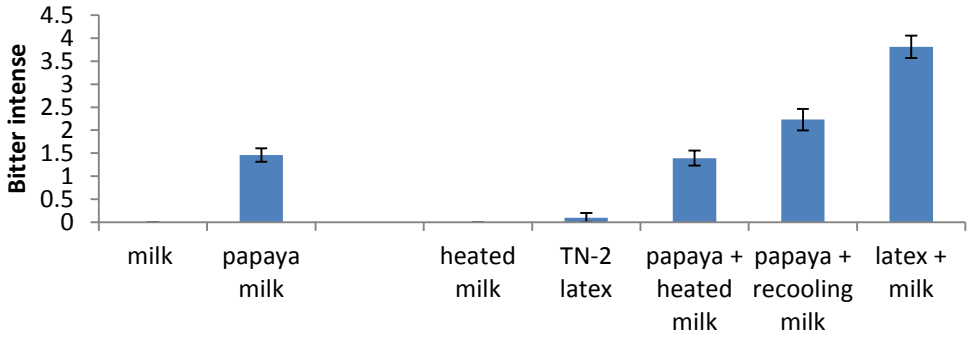

Fig. 5. The bitter intensity of milk, papaya milk, heated milk, ‘Tai-Nung No. 2’ papaya latex, papaya milk containing milk heated at different conditions and papaya latex mixed with milk. All test samples were used one hour after been prepared.

Discussion

Talcott and Howard (1999) studied the carrot puree and found that consumer preferences in choosing the products had close relationship with the content of its secondary compound. In the present study, we showed that papaya milk prepared from heated papaya fruit has lower preference index compared with papaya milk made from unheated papaya fruit (Fig. 1a and b). These results may be explained by previous reports indicating that some chemicals such as terpenes and phenolic compounds can be oxidized during heating process (Jurgen *et al*., 1984 and Dulce *et al*., 2010). In addition, heated papaya milk also showed some changes in color (data not shown) suggesting that heating also resulted in the oxidation of the carotenoids such as lycopene which is abundant in red-fleshed papaya such as papaya 'Tai-Nung No. 2','Sunrise' and 'SunUp' (Ralf *et al*., 2011; Julius, 2012 and Wall, 2006).

Our results indicated that consumer preference will decrease as incubation time increase due to the increment of bitter intensity (Fig. 2 and 3). This result was consistent with data reported in carrot and cruciferous vegetables (Drewnowski, 1997; Talcott and Howard, 1999). Furthermore, sweetness can also interfere with the bitterness in papaya milk causing an increase in consumer preference as indicated by the value of preference index recorded in papaya milk with high papaya and low milk content (Fig. 2 and 3). Cabanax and Duclaux (1970) suggested that sweet taste can affect the consumers' preferences though not all products showed a link between sweetness and preferences (Drewnowski, 1997). Another possibility that caused an increase in preference index was low amount of bitter substances in papaya milk (Fig. 2 and 3) whereas low milk content resulted in lower bitter intensity in papaya milk as compared to that with high milk content. These results suggested that the milk may be a source of bitter substances.

Interestingly, milk and papaya juice alone without mixing together showed no bitter taste (Fig. 4 and 5). This result suggested that bitter substances were formed due to the chemical reaction between papaya juice and milk. The papaya enzymes may be one of the key factors that cause bitter taste in papaya milk. This assumption was supported by the results obtained from the papaya milk prepared from heated papaya (heated or recooling papaya juice) in which papaya milk containing heated papaya had lower bitter taste (Fig. 4 and 5). It is possible that papaya enzymes in papaya fruit were denatured during heating process in which the temperature may rise over 100^0C.

Meanwhile, results from heated milk experiment indicated that bitter intensity remained high in papaya milk prepared by using the papaya juice mixed directly with boiled milk (Fig. 5). Under this circumstance, papaya enzymes may still be functional due to the decreasing temperature of boiled milk after mixing with papaya juice. In addition, papaya milk prepared from milk that's

been heated and then cooled could enhance bitter intensity (Fig. 5). Azarkan *et al.* (2003) and Moussaoui *et al.* (2001) found that around 80% of the papaya latex protein in fruit are enzymes belonged to the cysteine protease family which consists of 4 types: papain, chymopapain, glycyl endopeptidase and caricain. Moreover, according to Sumner *et al.* (1993), cysteine proteases have a very high thermal stability, for example, papain remain stable for five mins above 75^0C under a pH value of four. This might explain why heated milk or milk that's been heated and then cooled still had strong bitter taste compared with heated papaya juice or papaya juice that's been heated and then cooled (Fig. 4 and 5).

Our results also indicated that the formation of bitter substances in papaya latex mixed with milk may be due to the degradation of milk protein by enzymes contained in the papaya latex (Fig. 5). Previous studies indicated that cysteine protease especially papain, chymopapain and caricain may target and cleave various hydrophobic amino acids (Michael, *et al*., 2000; Juan *et al*., 2009; Sigma-Aldrich, 2015; Zucker *et al*., 1985). Furthermore, studies in cheese and sake showed that hydrophobic amino acid forming peptide is the major source of bitter taste (Koka and Weimer, 2000; Maeda *et al*., 2011). These research findings supported the idea that bitter taste in papaya latex mixed with milk may be caused by the reaction of papaya latex and milk.

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影響木瓜(*Carica papaya* L.)牛奶中苦味物質之探討

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關鍵字：苦味指數、番木瓜、木瓜牛奶、喜愛度

摘要：木瓜牛奶是台灣最受歡迎的飲品之一，然而，新鮮木瓜牛奶在室溫下無法放置超過 2 小時，因有苦味產生。因此，本試驗擬探討木瓜牛奶苦味發生之原因。結果顯示，單獨以牛奶或木瓜果肉打汁皆無苦味。進一步調查顯示「加熱過之牛奶與常溫木瓜」、「加熱過回溫之牛奶與常溫木瓜」之苦味指數高於「常溫牛奶與加熱過之木瓜」與「常溫牛奶與加熱並回溫之木瓜」。此外，木瓜乳汁與牛奶混合後具有最高之苦味指數。本試驗推測：木瓜牛奶苦味之形成是由木瓜果實提供酵素去分解牛奶中之物質所導致。

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