Effect of vacuum-aided enzyme infusion on the physicochemical characteristics of peeled musk lime fruits
(Kesan infusi enzim berbantukan vakum terhadap ciri fizikokimia buah limau kasturi terkupas)


Key words: vacuum-aided, enzyme-infusion, physicochemical characteristics, musk lime, *Citrus mitis*

Abstract
The use of enzyme in loosening fruit peel is one of the most intensively studied areas. However, current reports on the use of enzyme in peeling local citrus fruit such as musk lime (*Citrus mitis*) are limited. In this study, musk lime fruits were soaked in the pectinase enzyme solution and kept under vacuum for 15 min before incubation to easy loosen the peel. Vacuum (pressure ranged from 0 to 700 mm Hg) was applied to hasten the penetration of enzyme solution into the peel. Results showed that vacuum pressure did not significantly affect the physicochemical properties (pH, total soluble solids, citric acid, ascorbic acid, moisture and sugar contents) of enzymatic-peeled musk lime. Except at high vacuum pressure (700 mm Hg), the ‘L’ value of puree colour and total pectin content of peel were significantly affected (*p* <0.05). Naringin content in the peel was also affected at very high vacuum pressure. This study indicated that vacuum pressure was not significantly affected the physicochemical characteristics of the peeled musk lime. This indicated that vacuum pressure can be applied to aid the enzymatic peeling of musk lime fruits.

Introduction
The enzymatic technology to loosen or remove the peel of citrus fruit is widely applied in European countries. However, this technology is currently new in Malaysia. Since late 1980s, many studies have been conducted and this technology has renewed interest in the production of citrus sections and whole fruit as reported by Janser (1997). This technology can produce enzymatic prepared grapefruit sections with pleasing appearance, excellent fresh fruit flavour, no excessive juice loss and only requires little hand labour (Berry et al. 1988).

The vacuum-aided enzyme infusion for peeling and sectioning of citrus fruit has resulted in cleaner sections with less adhering albedo and better quality compared with conventionally machine-peeling followed by lye bath as reported by Baker and Bruemmer (1989). In Malaysia, the first application of vacuum-aided in the enzymatic peeling of musk lime was reported by Hazniza et al. (2002).

A local citrus fruit of musk lime (*Citrus mitis*) is a fruit of economic importance in the food industry. The fruit, which is also known as ‘fruit of many uses’,
confronted with many problems in the peeling process and production of its juice. The fruit is small and the very thin skin is strongly attached to the fruit segments and is difficult to peel manually.

Currently, the whole fruit is crushed in a screw press to extract the juice during the juice processing. This conventional method is rather impractical for musk lime because it may extract the bitter compound from the skin and seeds into the juice and thus affect the juice quality.

Market demands for ‘natural’ juices have prompted food scientists to study the quality deterioration of fruit juice during processing. One of the major problems confronted in the production of musk lime juice is the processing operations.

Conventional methods for fruit peeling such as hand peeling and mechanical such as screw press, acid or lye (NaOH) bath, heat and flame usually produce 30–40% of edible waste (Baker and Grohmann 1995). This waste containing live cells is sensitive to the environment and continues changing after processing due to contamination with microorganisms (Setty et al. 1993) and may affect the chemical properties of the fruit.

The chemical composition of lime consists of total soluble solids (10%), total acidity (5.1–7.7%), ascorbic acid (39–62 mg/100 ml), total invert sugar (0.14–0.20%) and pH varies from 1.7 to 3.2 (Swisher and Swisher 1980; Simmonds 1984).

In general practices, fruits are kept and stored at room temperature before processing. However, this application affects the juice quality. A number of deteriorative reactions in the musk lime juice such as development of off-flavour and browning during storage at room temperature has been reported by Kacem et al. (1987). To overcome the problem, enzyme infusion technology with time effective and less hand labour was proposed.

This enzyme infusion technology was introduced in the food industry as an alternative to thermal treatments (Quaglia et al. 1996). This technology can affect the fruit enzyme including their activation, enhancement of activity or possible inactivation and retard the off-flavour and browning reaction (Hayashi 1989). The aid of high pressure at low or moderate temperature in the enzyme infusion technology has been reported by Cheftel (1995) and the aid of vacuum pressure to hasten the enzymatic peeling of citrus fruits has been reported by Janser (1996).

Cheftel (1995) also reported the inactivation of vegetative microbial cells and some enzymes with no spoilage of the organoleptic qualities in the end product using this technology. Therefore in this study the musk lime was peeled using vacuum-aided enzyme infusion and the effect of different vacuum pressures on the physicochemical characteristics of peeled musk lime was studied.

**Materials and methods**

**Source of fruits**

The musk lime (*Citrus mitis*) fruit was of commercial maturity and was purchased from a commercial farm in Hulu Langat, Selangor, Malaysia. Fruit chosen had firm texture with uniform green colour and no signs of spoilage. The fruit was washed thoroughly under running tap water and air-dried. In this study, 100 g of fruit (equivalent to 8–10 fruits) was used for each treatment.

**Preparation of enzyme solution**

The enzyme used was a commercial pectinase (Peelzym II) purchased from Novozyme, Dittingen, Switzerland. The concentration of 1.0% (v/w) enzyme solution was prepared based on the weight of fruits. The method for the preparation of enzyme solution was adopted from Hazniza et al. (2002).

**Preparation of fruits**

The fruit peel was scoured before the peeling process. The scouring was necessary to ensure easy penetration of the enzyme solution into the albedo (white portion). The
scouring was done using abrasive paper at five different parts: one at the distal end and four at equidistant positions along the equatorial region of the fruit. The fruit was carefully scoured without injuring the albedo part of the peel or the segments. This method was adopted from Hazniza et al. (2002).

Vacuum aided enzyme-infusion
The method employed was adopted from Hazniza (2003) based on the work described by Baker and Bruemmer (1989), Rouhana and Mannheim (1994) and Janser (1996) with some modifications. A set of fruit was then immersed in a 500 ml beaker containing 200 ml of 1.0% (v/w) enzyme concentration. The fruit was exposed to different vacuum pressure ranging from atmospheric pressure to 700 mm Hg (0, 100, 300, 500 and 700 mm Hg) and held for 15 min before being transferred (in a beaker) to a shaking incubator at 40 °C and 120 rpm.

In this study, the endpoint for the peeling was defined as 80% of peel removed (visual observation) and resulting in peeled fruits of high quality (free of adhering albedo and without injured or broken segments). The effects of different vacuum pressure on the physicochemical characteristics of peeled musk lime were then determined.

Preparation of sample
Method for the preparation of peeled musk lime was adopted from Hazniza (2003). After the vacuum-aided enzyme infusion peeling process, the fruit was washed under running water to remove the remainder of peel and adhering albedo. The peeled fruit and its peel were washed twice and air-dried, separately. The seed was removed from each segment of the peeled fruit and then washed twice before air-dried.

Each portion of peel, seeds and deseeded fruit segments were blended using a multiple automatic blender (Braun, Germany) at a moderate speed until a fine peel, seeds and puree were obtained, separately. The sample preparation of peel, seeds and puree of peeled musk lime is given in Figure 1.

Physicochemical analysis
The effects of different vacuum pressure on the physicochemical characteristics of peeled musk lime were studied. The colour was measured using Hunterlab Colour Difference meter (Minolta Chroma meter, CR-300, USA) in the reflection mode. The pH and titratable acidity were determined according to AOAC (1990). The pH was measured using a calibrated pH meter model Cyberscan 1000 (Singapore), while titratable acidity for determination of citric acid was estimated by titrating sample with 0.1 M sodium hydroxide and expressed as percentage citric acid.

Ascorbic acid was determined using 2,6-dichloro-phenolindophenol by visual titration (AOAC 1990) and total soluble solids were measured using an ATAGO/
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Moisture content was determined using oven method (Ranganna 1977), while sugars were determined using High Pressure Liquid Chromatography (HPLC) method (Hunt et al. 1977). Tannin was determined using a colorimetric method of AOAC (1980). Total pectin was determined using a modified method of Bluemenkranz and Hansen (1973), whereas naringin was determined using a modified methods described by Salmah et al. (1990) and Hertog et al. (1992).

In this study, puree was used in all analyses except in the analysis of naringin which used the peel, seed and puree. All determinations were carried out in triplicates.

Statistical analysis
Data obtained from this study was analysed using ANOVA. Significant differences between treatments were determined using Duncan Multiple Range Test (DMRT). The statistical program used was Statistical Analysis System software (SAS Inst. 1996).

Results and discussion
Colour
According to Raymond (1992), ‘L’ value is the lightness range from black = 0 to white = 100. A positive ‘a’ value indicates a hue of red-purple; negative ‘a’ of bluish-green, while a positive ‘b’ value indicates a yellow and negative ‘b’ value of blue colour.

No significant difference was found in the colour (Hunter ‘L’, ‘a’ and ‘b’) between the 0 mm Hg (control) and vacuum treated fruits, however, a significant ($p < 0.05$) decrease in ‘b’ value was detected at 700 mm Hg.

The ‘L’, ‘a’ and ‘b’ values of puree colour was not significantly affected ($p < 0.05$) by 100–500 mm Hg vacuum pressure. However the ‘b’ value was significantly affected at high vacuum pressure of 700 mm Hg. The changes in the ‘b’ value could probably be due to the rapid loss of some hydrosoluble components (Quaglia et al. 1996) such as carotenoid pigments upon dehydration (Simpson 1985) during the highly vacuum treatment. According to De Man (1999), colour is an important attribute of citrus juice where the carotenoid content of oranges is useds as a measure of total colour.

pH, citric acid and ascorbic acid contents
There was no significant difference in pH, citric acid and ascorbic acid contents of peeled musk lime when subjected to different vacuum pressures. The results showed quite similar changes on pH values between control and vacuum treatments, and similarly with citric acid and ascorbic acid contents.

Musk lime juice is characterized by its high acidity, as reflected by the high citric acid content. The obtained values for citric acid were within the range given for lemon (4.0–5.0%) as mentioned by Clements (1964), Yamaki, (1989) and Nisperos-Carriedo et al. (1992). Most organic acids are too weak to be affected by pH changes in the 2.4–3.4 range. However, moderately strong organic acid such as citric acid shows a definite pH dependent (Lee 1993). Study showed that vacuum pressure did not appreciably affect the ascorbic acid content of musk lime puree. This finding is similar to that of Ogawa et al. (1992) and Quaglia et al. (1996) who found that high pressure treatment has no significant effect on the ascorbic acid content of citrus juice.

Total soluble solids and moisture content
There was no significant difference in the total soluble solids (TSS) of control and vacuum treated fruits, and similarly in the moisture content of peeled musk lime (Figure 2). The non-significant decrease in TSS, however, could probably be due to the movement of water from the surrounding (enzyme solution) into the fruits, which according to Salunkhe and Desai (1984) is due to osmotic transfer and possibly resulting from the breakdown of carbohydrates. A marked difference in
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The moisture content of peeled musk lime after vacuum treatment was found to be fluctuating. This phenomenon could probably be due to the inherent variability of the fruit itself (Saltveit 2003). The efficiency in losing the water content from the fruit and the infusion of the enzyme solution from the surrounding simultaneously during vacuum treatment was different for each fruit, and therefore causes a difference in moisture content of the peeled fruits (Lizada et al. 1990). The difference in moisture content of the vacuum treated and control, however, was small and was probably negligible from a nutritional standpoint.

**Sugar content**

There was no significant difference in fructose, glucose and sucrose of peeled musk lime when exposed to different vacuum pressure (Figure 3). Sucrose content was the highest as compared to fructose and glucose. Wills et al. (1984) reported a similar trend. In this study, the ratio of fructose, glucose and sucrose was well correlated as reported by Curtis (1997) in citrus fruit, but was differently correlated as reported by Nisperos-Carriedo et al. (1992).

The analysis in this study was conducted in triplicate with three representative samples per replicate. The analysis was conducted in such a way to minimise the variability among the fruit. However, the value of standard deviations obtained in the sugar analysis (fructose, glucose and sucrose) was relatively high. This phenomenon could probably be due to the inherent variability of the fruit itself (Saltveit 2003).

Generally, environmental factors such as high relative humidity and rainfall (Davies and Albrigo 1994) may also affect the physicochemical properties of the fruit and thus render the variability of the fruit. Therefore, it has been taken into consideration to propose a large number of sampling to more than three replicates in order to minimise the inherent variability among the fruit and thus lower the value of the standard deviation in the measured analysis.

**Total pectin and tannin contents**

The total pectin content of peeled musk lime fruit showed that the effect of vacuum infusion was not significantly affected by...
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low to moderate vacuum pressure (100–500 mm Hg). The total pectin content, however, was significantly \( (p < 0.05) \) affected at high vacuum pressure of 700 mm Hg (Figure 4).

The changes in total pectin content at 700 mm Hg is probably due to the concomitant increased in enzyme saturation in the peel (Pretel et al. 1997) along with the increased of vacuum pressure. It can be postulated that the saturation of enzyme in the peel will digest the pectin content in the peel and intact albedo, however, due to a high vacuum (700 mm Hg), some of the enzymes are forced to penetrate into the juice segments and juices. In other words, the increase of vacuum pressure will increase the penetration of enzyme into the peeled fruit and juice segments, and thus increases the amount of total pectin in the peeled fruit.

The changes in tannin content were not significantly different when treated at different vacuum pressure (Figure 4). Different vacuum pressure did not affect the tannin content in the peeled musk lime fruits, but will only be affected by ripening process as reported by Von Loesecke (1949) in citrus fruits.

**Naringin content**

Naringin was found to be high in the peel followed by seeds and puree (Figure 5). The naringin content in the peel was significantly \( (p < 0.05) \) different when exposed to different vacuum pressure, however, no significant difference was found in the seeds and puree. The naringin content in the peel increased from 142.42 µg/g at 0 mm Hg to 218.93 µg/g at 700 mm Hg which indicated that the naringin content in the peel increased with the increased in the vacuum pressure applied.

The high naringin content in the peel is in agreement with those reported by Suri and Radzali (2002) and Caro et al. (2004). Higher amounts of naringin have also been reported in the peel compared to juice from the same fruit by Nogata et al. (1994). A study conducted by Kanes et al. (1993) on the phenolic composition of various tissues of rutaceae species, found that the peel of musk lime contains significantly high amounts of naringin and hesperidin. Others such as isosakuraretin rutinoside, eriocitrin, narirutin, neoeriocitrin, neohesperidin and naringin 6” malonate were also present but only in a small amount.

The kinetics of osmotic dehydration due to high vacuum pressure has been reported by Rastogi and Raghavarao (1996). Osmotic dehydration is the driving force
that is responsible for mass transfer of many variables such as concentration and nature of the fruit and their geometry (Torreggiani 1993). Therefore, the significant increase in naringin contents in the peel at high vacuum pressure of 700 mm Hg could probably be due to the conversion of other flavonoid compounds present in the peel. This conversion could also probably be due to a high osmotic dehydration created during high vacuum pressure which was responsible for the mass transfer of other flavonoid compounds from other portions of musk lime fruit into its peel and therefore increased the naringin compounds in the peel.

**Conclusion**

Application of vacuum in the enzymatic peeling of musk lime has not totally affected the physicochemical characteristics of the peeled fruit. This shows that effect of different vacuum pressure to aid enzymatic peeling of musk lime fruits has not changed most of the physicochemical properties of peeled musk lime, except the ‘b’ value of puree colour and total pectin content in the peel at very high vacuum pressure. However, naringin content in the puree and seeds were not significantly affected by the vacuum pressure, except, in the peel at very high vacuum pressure. These physicochemical changes, however, were only a minor effect of vacuum pressure on musk lime fruits during enzymatic peeling and thus can be controlled. Therefore, most of the physicochemical properties of the peeled musk lime fruit can be improved and maintained.

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**References**


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Abstrak

Penggunaan enzim untuk memudahkan pengupasan kulit buah merupakan salah satu bidang yang sedang giat dikaji. Bagaimanapun, laporan semasa berkenaan penggunaan enzim dalam pengupasan buah sitrus tempatan seperti limau kasturi (Citrus mitis) adalah terhad. Dalam kajian ini, buah limau kasturi direndam dalam enzim pektinase dan divakum selama 15 minit sebelum dieram untuk memudahkan pemisahan kulit. Vakum (tekanan 0, 100, 300, 500 dan 700 mm Hg) digunakan untuk mempercepat kemasukan larutan enzim ke dalam kulit. Hasil menunjukkan vakum tidak mempengaruhi sifat fizikokimia (pH, jumlah pepejal larut, asid sitrik, asid ascorbik, kandungan lembapan dan gula) limau kasturi yang telah dikupas secara enzimatik. Kecuali pada vakum tinggi (700 mm Hg), nilai ‘L’ bagi warna puri dan kandungan pektin bagi kulit dipengaruhi secara signifikan (p <0.05). Kandungan naringin di dalam kulit dipengaruhi oleh tekanan vakum yang tinggi. Kajian menunjukkan tekanan vakum tidak memberi kesan yang signifikan pada ciri-ciri fizikokimia buah limau kasturi terkupas. Ini menunjukkan tekanan vakum boleh digunakan untuk membantu pengupasan berenzim buah limau kasturi.