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A new approach for reduction of chemical compounds causing undesirable odour in *Morinda citrifolia* fruit juice

(Pendekatan baru untuk pengurangan sebatian kimia yang menyebabkan bau yang tidak diingini dalam jus buah *Morinda citrifolia*)

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Abstract

A specific selective technique for reduction of chemical compounds that caused undesirable odour from fresh *Morinda citrifolia* fruit juice has been developed. A polysaccharide derivative known as Sephadex LH-20 has been used as the stationary phase to separate the chemical compounds that caused undesirable odour from the juice. Eight common chemical compounds responsible for the undesirable odour have been reduced and some have been successfully removed from the *M. citrofolia* juice. Based on analysis using GCMS (Gas Chromatography Mass Spectrometer), the eight major compounds and groups of compounds that contributed to undesirable odour were identified as hexanoic acid, octanoic acid, trimethylsilyl ester, tridecane, methyl ester, 4-octanol, undecane and methyl arachate.

Introduction

Morinda citrifolia or *mengkudu* is a popular fruit which has long been used as a traditional remedy for many ailments. These include hypertension, diabetes, liver inflammation, indigestion, arthritis, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, atherosclerosis, blood vessel problems, and drug addiction as well as revitalizing mothers after childbirth (Morton 1992; McClatchey 2002; Wang et al. 2002; Kamiya et al. 2004). Currently, *M. citrifolia* is popularly taken as a functional food, hence making the products palatable is critical. The fruit has a foul smell or odour when fully ripe thus making it undesirable to some consumers (Anh et al. 2006).

Therefore, one of the challenges in processing the fruits in recent years is to reduce the undesirable odour from *M. citrifolia* fruit juice. Levland and Larson (1979) reported that low molecular weight compounds identified from the fruit that produce the odour include asperulosidic, hexanoic and octanoic acids. While Farine et al. (1995) reported that the main chemical compounds which contributed to the undesirable smell or odour were acidic compounds such as octanoic and hexanoic acids. These compounds are volatile and can be used as biopesticides, especially octanoic and hexanoic acids, to repel insects (Farine et al. 1995).

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Previous studies on reduction of odour from *M. citrifolia* juice had been carried out by Norma et al. (2006) and Sharmella et al. (2007) and ozone reaction by commercial industries (Duguet et al. 1990). In these studies, calcium carbonate ($CaCO_3$) was used to reduce the undesirable odour from *M. citrifolia* juice. The calcium carbonate ($CaCO_3$) was mixed with the *M. citrifolia* juice at different amounts and reduction of volatile compounds was monitored using gas chromatography. Another method which was developed by the juice industry involved electrophilic reactions using O_3 and known as ozonation (Duguet et al. 1990). Both methods were based on chemical reactions with no specific target compounds to reduce (Farine et al. 1995). Duguet et al. (1990) reported that ozone reaction could destroy some phenolic and aromatic compounds that could lead to decrease of phytomedicinal benefits.

Another method developed by Norma et al. (2006) did not involve chemical reaction. In this study, activated charcoal powder was used to absorb the smelly compounds. Although this method did not involve any chemical reaction, the separation process was not specific to any target compounds. The final product or output was a clear *M. citrifolia* juice in which most of the valuable phytochemicals had been absorbed by the activated carbon. Therefore, the objective of this study was to develop a specific method for reduction of chemical compounds that caused undesirable odour from *M. citrifolia* juice.

Materials and methods

Sample collection and juice extraction

Fresh *M. citrifolia* fruits at three quarters maturity (yellowish) as described by Rohani and Rosalizan (2006) were obtained from MARDI Muadzam Shah, Pahang. The fruits were cut into small pieces and finally ground using the food processer (Panasonic MK5080M). The juice was extracted using the centrifugal juicer MH-819A High Speed at 13,000 rpm for 30 min to separate the juice from the pulp.

Polysaccharide derivative treatment

The method was developed according to Farine et al. (1995). Most of the chemical compounds that caused undesirable smell or odour are small molecular weight types of compounds. Therefore, theoretically, size exclusion filtration method is suitable to separate all chemical compounds that cause undesirable smell or odour from *M. citrifolia* juice. A size exclusion type filtration method known as Sephadex LH-20 was selected as the filtration agent to separate the odour causing compounds from *M. citrifolia* juice.

Sephadex LH-20 (manufactured by Sigma Aldrich) is a highly specialised gel filtration synthetically derived from polysaccharides or glycans which are relatively complex carbohydrates. They are one type of polymer made up of many monosaccharides chained by glycosidic linkages. They are very large, often branched molecules, tend to be amorphous, insoluble in water, and have no sweet taste. They could be used in polar organic solvents and aqueous solvent mixtures. Sephadex LH-20 was used in the column chromatography (CC) according to the method described by Lee et al. (2010), with modification of the mobile phase to separate the undesirable odour causing compounds from *Morinda citrifolia* fruit juice. The separation concept for Sephadex LH-20 is based on the size of the compounds. The larger chemical compounds will easily pass through while the smaller chemical compounds will slowly move through the CC system.

Forty grams of LH-20 powder was mixed with 100% distilled water and placed into a glass column, 3.0 cm in diameter and 40 cm in height. Pure distilled water was used as the mobile phase isocratically as eluent in the prepared Sephadex LH-20 CC system. A volume of 250 ml of *M. citrifolia* juice was allowed to pass through the

Sephadex LH-20 CC system. Then 250 ml distilled water was added on top of the system after the juice had fully entered the column. Fractions were collected from the CC system and each fraction contained 100 ml solutions. Five fractions were collected for further analysis using the gas chromatography mass spectroscopy (GCMS) for detection of compounds causing the undesirable smell or odour. Fractions that showed similar profile in GCMS spectrum will be combined together as a fraction.

GCMS analyzer

Gas Chromatography, GCMS, (Shimadzu Version 3 GC-17A) with a single quadrupole mass spectrometer was used to analyse the compounds. The column used was a BPX5 with specifications of 30 m x 0.25 mm id and 0.22 μm film thickness. An aliquot of 5 μl sample was injected into the system operating with a split flow of 12.6 ml/min. The column was set isothermally at 80 °C for 3 min, then increased to 50 °C per/min until 220 °C and held for 17 min programmed. The injection and detector temperatures were 200 and 260 °C respectively. The chemical compounds that caused undesirable smell or odour were identified by comparing the chromatogram obtained with the library of National Institute of Standards and Technology (NIST) Virtual Library, Gaithersburg.

Results and discussion

GCMS profile of fresh M. citrifolia juice

Morinda citrifolia fruits are green during the young stage and rapidly change to a light yellow and translucent white during the matured stage (fully ripe). Fruit scent varies, with some varieties being virtually odourless but commonly, the vigorous growing varieties having a stronger smell of butyric acid when fully ripe (McClatchey 2002).

Figure 1 shows the GCMS profile of fresh *M. citrifolia* juice. The chromatogram shows the presence of major compounds that contributed to undesirable smell or odour in the *M. citrifolia* juice. The identification for all chemical compounds was done by comparing the chromatogram obtained with the library of National Institute of Standards and Technology (NIST) Virtual Library, Gaithersburg, based on mass and retention time (RT). Three major peaks could be seen at retention times 6.29, 8.05 and 8.50 min

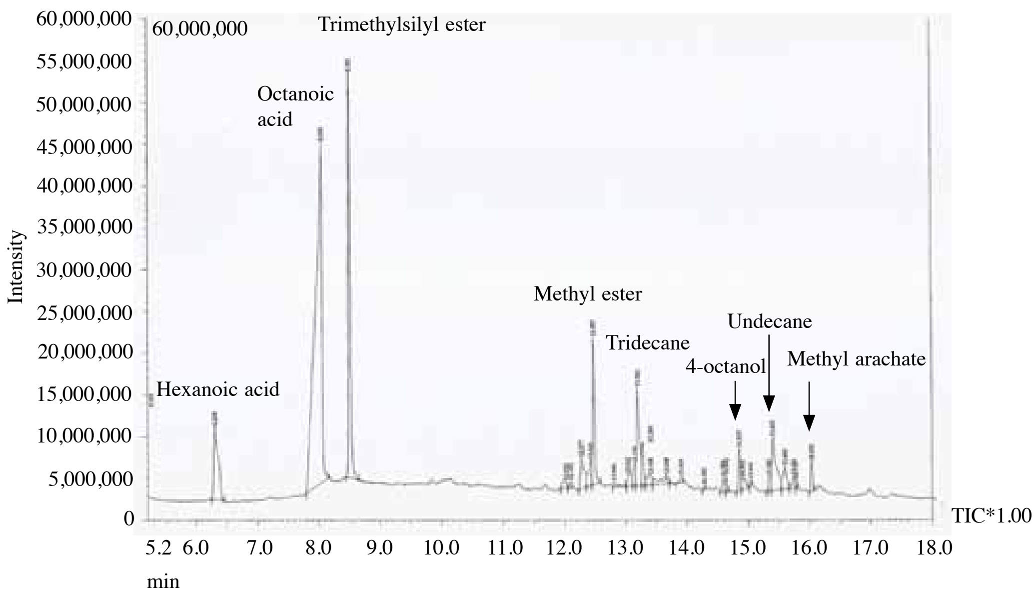

Figure 1. Chromatogram of compounds identified in fresh Morinda citrifolia fruit juice using GCMS

with high intensity compared to other compounds. According to GCMS analysis, these compounds are known as hexanoic acid, octanoic acid and trimethylsilyl ester. The compounds showed intensity at 11 million counts per second (cps) for hexanoic acid, 46 million cps for octanoic acid and 54 million cps for trimethylsilyl ester. The results showed that all the compounds were major contributors to undesirable smell or odour. The results were in agreement with previous reports by Farine et al. (1995) and Sharmella et al. (2007) that major contributors to undesirable smell or odour were based on acidic compounds especially octanoic acid.

Other types of chemical compounds detected and identified by GCMS shown in *Figure 1* are methyl ester (RT 12.49 min), tridecane (RT 13.20 min), 4-octanol (RT 14.85 min), undecane (RT 15.40 min) and methyl arachate (RT 16.03 min). These compounds also contributed to undesirable smell or odour besides hexanoic, octanoic acid and trimethylsilyl ester that react as major contributors (Farine et al. 1995).

GCMS profile of M. citrifolia after filtration with Sephadex LH-20

Five aqueous fractions of fresh pure *M. citrifolia* juice that passed through the Sephadex LH-20 CC system were collected and analysed using GCMS. Their profiles were compared against major compounds that contributed to undesirable smell or odour as reported by Farine et al. (1995).

Based on the results of GCMS analysis, fractions 1, 2, 3 and 4 obtained from Sephadex LH-20 separation or filtration system showed similar chemical profiles. Therefore all the fractions were combined as one. From the results of GCMS analysis (*Figure 2*), a number of peaks were not detected and identified. A proper inspection and comparison on chromatograms from *Figures 1* and *2* revealed that several undesirable chemical compounds were successfully removed from *M. citrifolia* fruit juice. All the undesirable compounds previously detected at retention time ranging from 5 to 11 min in fresh fruit juice sample were lost or disappeared after being treated with Sephadex LH-20 filtration system.

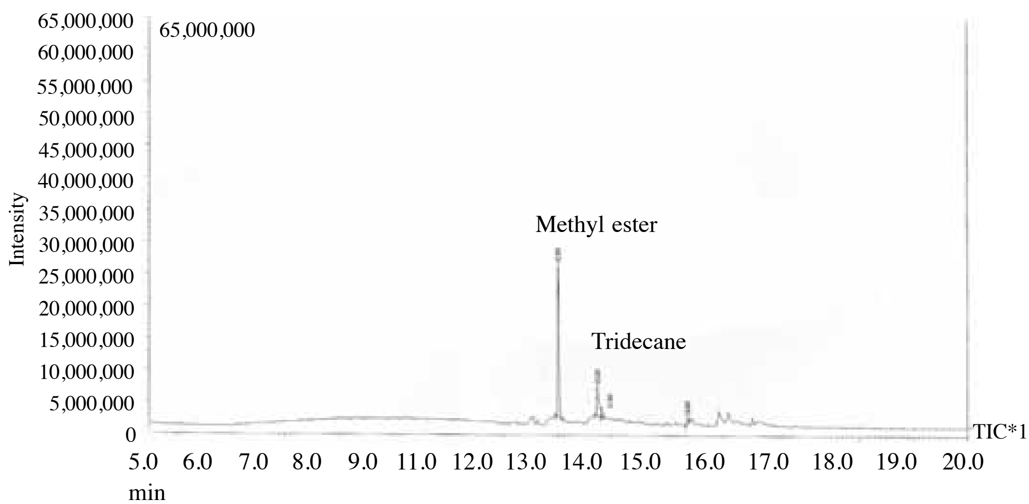

Figure 2. GCMS chromatogram of fractions 1 to 4 collected from Sephadex LH-20 filtration system

The major compounds that contributed to undesirable smell or odour such as hexanoic acid, octanoic acid and trimethylsilyl ester (Farine et al. 1995; Sharmella et al. 2007) were successfully removed by the filtration technique and not detected in fractions 1, 2, 3 or 4.

Fraction 5 was not combined with other fractions because the GCMS profile was different from the other 4 fractions (*Figure 3*). Eight peaks were observed and all the peaks were detected in retention time ranging from 7 to 17 min. The major peaks were detected at 7.01, 8.46 and 8.86 min. Identification based on mass using the GCMS library at National Institute of Standards and Technology (NIST) Virtual Library, Gaithersburg showed that these compounds were hexanoic acid, octanoic acid and trimethylsilyl ester. Other compounds that contributed to undesirable odour were detected at retention times of 10.02, 10.57, 11.15, 12.06 and 16.63 min. These compounds were identified as methy ester, tridecane, 4-octanol, undecane and methyl arachate respectively.

Although the retention times of the compounds and group of compounds were different from *Figures 1* and *2*, the presence of these compounds and groups of compounds were confirmed based on mass analysis from the GCMS library at NIST Virtual Library. Fraction 5 was in semi purified form compared to fractions 1, 2, 3 and 4, which were a crude mixture of complex matrix. Previous study by Hajslova and Cajka (2007) also showed that a complex matrix contains multiple analytes in crude form that could lead to various difficulties at injector and detector sides especially for time detection due to the presence of interferences.

Advantages of the Sephadex LH-20 filtration system

Table 1 shows the comparison in mechanisms between the 4 methods that have been developed for reducing the undesirable odour from *M. citrifolia* fruit juice. There is an advantage in using the Sephadex LH-20 filtration system compared to the other methods. The mechanism used in this method did not involve any chemical reactions and more specific towards the target compounds. The methods used in the previous studies were not specific to the major compounds that contributed to the undesirable smell or odour as reported by

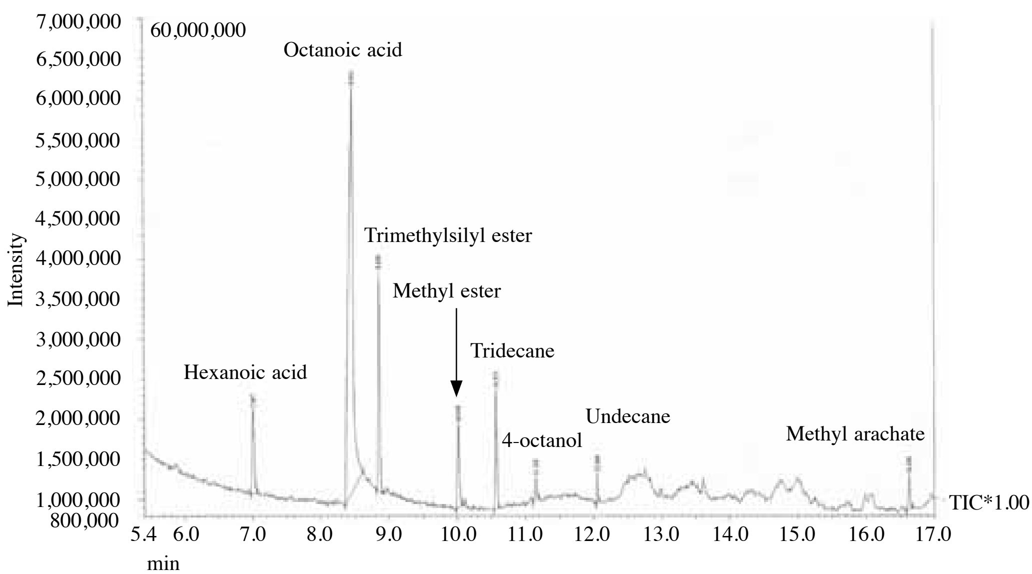

Figure 3. GCMS chromatogram of fraction 5 collected from Sephadex LH-20 filtration system

Table 1. Comparison between techniques developed for removal of undesirable odour from *Morinda citrifolia* juice

Technique	Method used	Mechanism	Target compounds
Commercial	Ozone (O_3)	Chemical reaction	Non specific
Sharmella et al. 2007	Calcium carbonate ($CaCO_3$)	Chemical reaction	Non specific
Norma et al. 2006	Activated carbon	Filtration with absorption	Non specific
Sephadex LH-20 filtration system	Dextrin polysaccharides	Specific separation or filtration according to size of the compounds	3 major chemical compounds with 5 minor compounds that contribute to undesirable smell or odour

Farine et al. (1995). The method developed by the juice industry involved electrophilic reactions such as ozone reaction. Duguet et al. (1990) showed that ozone reaction could destroy some phenolic and aromatic compounds. Similar results were obtained with calcium carbonate reaction (Sharmella et al. 2007) but the destruction of phenolic and aromatic compounds was much lower than ozone reaction. The main problem in calcium carbonate reaction is due to excess of calcium from supplements, fortified food and high-calcium diet, which can cause the "milk alkali syndrome," which is very toxic and can be fatal (Ami and Stanley 2010). The use of activated carbon for filtration or separation for major compounds was not selective and causes decrease in phytochemical contents in *M. citrifolia* juice (Norma et al. 2006).

The Sephadex LH-20 filtration system is based on specific separation or filtration according to the size of the chemical compounds found in *M. citrifolia* juice. The larger chemical compounds will easily pass through the column while the smaller chemical compounds will move slowly through the system. This processing method can be applied for industrial *M. citrifolia* juice processing for production of an innovative product.

Sephadex LH-20 has been used in many food separation or filtration applications. It has been used in preparative separation of oligostilbenes from *Vitis thunbergii* var. *taiwaniana* using centrifugal partition chromatography followed by Sephadex LH-20 filtration (Lih and Ching 2009). Sephadex LH-20 has also been used on rapid large scale purification of ellagitannins from pomegranate husk, a by-product of the commercial juice industry (Seeram et al. 2005).

Conclusion

A more specific method for reduction of chemical compounds that caused undesirable odour from *M. citrifolia* juice has been developed from this study. Sephadex LH-20, a polysaccharide derivative successfully reduced most of the chemical compounds that caused undesirable odour from fresh *M. citrifolia* fruit juice. Three major chemical compounds were identified by comparing the chromatogram obtained with the GCMS library at NIST Virtual Library, Gaithersburg. The compounds were identified based on mass and retention times as hexanoic acid, octanoic acid and trimethylsilyl ester groups. Five other compounds and group of compounds that contributed to undesirable odour were also identified as methyl ester, tridecane, 4-octanol, undecane and methyl arachate. The separation technique is specific based on the molecular size of the compounds. Since no chemical reaction was involved, there is no reduction or destruction in phenolic and aromatic compounds as well as in the phytomedicinal benefits. Therefore, there is a great advantage in this method of filtration for production of odourless *M. citrifolia* fruit juice or any other products.

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Abstrak
Satu teknik spesifik yang selektif telah dibangunkan bagi mengurangkan sebatian kimia yang menyebabkan bau yang tidak diingini daripada jus buah *Morinda citrifolia*. Satu terbitan polisakarida yang dikenali sebagai Sephadex LH-20 telah digunakan sebagai fasa pegun bagi memisahkan sebatian kimia yang menyebabkan bau yang tidak diingini daripada jus tersebut. Lapan sebatian kimia yang bertanggungjawab terhadap bau yang tidak diingini dalam jus *M. citrifolia* telah berjaya dikurangkan dan sebahagiannya telah dihilangkan. Berdasarkan analisis GCMS (*Gas Chromatography Mass Spectrometer*), lapan sebatian kimia dan kumpulan sebatian kimia yang menyebabkan kehadiran bau telah dikenal pasti sebagai asid heksanoik, asid oktanoik, trimetilsilil ester, tridekana, metil ester, 4-oktanol, undekana dan metil aracidat.

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