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Research Article

Effects of Debittering Treatments on the Physical Properties and Antioxidant Capacity of Bitter Gourd Extracts

R. Siti Rashima, M. Maizura and U. Uthumporn
School of Industrial Technology,UniversitiSains Malaysia, 11800 Penang, Malaysia

Abstract: The aim of this study are to investigate the effects of debittering treatments through blanching at 96°C for 3 min, soaking in 3.5% (w/v) sodium chloride (NaCl) solution and addition of carboxymethylcellulose, CMC (0.5%, w/v) and gum arabic (15%, w/v) on physical properties and antioxidant capacity of bitter gourd extract. Addition of CMC was observed to retain the green colour (-*a* value) of bitter gourd extract as compared to other treatments. Viscosity and total soluble solids increased significantly ($p<0.05$) in the bitter gourd extract after treated with NaCl and addition of CMC and gum arabic as compared to the fresh bitter gourd extract. Addition of gum arabic as debittering agent significantly increased the phenolic acid content of bitter gourd extract. Bitter gourd extract added with CMC and gum arabic could retain the catechin and chlorogenic acid in the extract. Flavonoid content was significantly ($p<0.05$) lower in all treated bitter gourd extracts as well as addition with CMC and gum arabic compared to fresh biter gourd extract. This is due to quercetin content that was unstable in presence of oxygen. ABTS scavenging activity of bitter gourd extract incorporated with CMC and gum arabic was observed to increase significantly ($p<0.05$) as compared to fresh bitter gourd extract.

Keywords: Antioxidant capacity, bitter gourd, carboxymethyl cellulose, gum arabic, sodium chloride

INTRODUCTION

Bitter gourd (*Momordicacharantia*) belongs to the Cucurbitaceae family, which are commonly grown in tropical and subtropical region. People of the Asian countries consider bitter gourd as a popular vegetable as they regularly consume it in the form of boiled, fried, pickle or juice. Bitter gourd contains phytochemical substances such as phenols, flavonoids, isoflavones, terpenes, anthroquinones and glucosinolates, which possess high antioxidant activity (Drewnowski and Gomez-Carneros, 2000; Budrat and Shotipruk, 2008; Horax *et al*., 2010). These phytochemicals were reported to reduce cholesterol level in human body (Budrat and Shotipruk, 2008) as well as being anti-diabetic (Joseph and Jini, 2013), anti-tumor and anti-mutagenic (Anilakumar *et al*., 2015), anti-inflammatory and anti-hyperglycemic (Gadang *et al*., 2011).

Presence of phytochemical substances contributes to bitterness of bitter gourd, thus making it less palatable (Snee *et al*., 2011; Joseph and Jini, 2013). Various treatments to reduce bitterness and astringency of fruits and vegetables have been reported, including blanching (Ismail *et al*., 2004; Zhang and Hamauzu, 2004), soaking in salt solution (Xu and Chang, 2008; Guan and Fan, 2010) and addition with polysaccharides (Drewnowski and Gomez-Carneros, 2000; Troszyńska *et al*., 2010) in order to improve palatability.

Blanching is a process of soaking vegetables in boiling water for 2 to 5 min to brighten colour of the vegetables, minimize vitamin loss and inhibit polyphenol oxidase (PPO) enzyme activity responsible for browning reaction (Kim *et al*., 2013; Choo *et al*., 2014). According to Choo *et al*. (2014), blanching retained the vitamin C content of bitter gourd as it inactivates ascorbic acid oxidase responsible for degradation of ascorbic acid. On the other hand, blanching helps to soften vegetables by breaking down the cell walls. This causes seeping of phenolic compound into the water. Degradation of phenolic compounds also occurs under the high temperature used for blanching. Collectively, such loss of phenolic compound was reported to reduce bitterness of bitter gourd, celery and medicinal plants (*Aralia elata*, *Kalopanaxpictus*, *Cedrelasinensis* and *Acanthopanaxsessiloflorus*) (Yao and Ren, 2011; Kim *et al*., 2013; Choo *et al*., 2014).

Soaking in NaCl solution is a method used to preserve and reduce bitterness of vegetables and fruits. Commonly, sodium chloride is used for debittering bitter gourd (Yadav and Singh, 2014; Din *et al*., 2011). During soaking process, osmosis between bitter gourd and salt solution takes place until an equilibrium is

Corresponding Author: Dr. M. Maizura, School of Industrial Technology, UniversitiSains Malaysia, 11800 Penang, Malaysia, Tel.: +6046536216; Fax: +6046573678

reached (Yadav and Singh, 2014). By increasing salt concentration of the soaking medium, bitterness of bitter gourd could be masked with the salty taste (Keast *et al*., 2001).

Carboxymethylcellulose (CMC) and gum arabic are commonly used to increase viscosity and clarity in juice production. Besides being a thickening agent, CMC and gum arabic also help to mask unpleasant taste (Pangborn *et al*., 1973; Gaudette and Pickering, 2012). When dissolved in bitter gourd extract, CMC which is an anionic hydrocolloid, possesses a charged hydroxyl group (OH^-). Interaction between the charged group and phenolic acids in the extract is associated to reduction of bitterness (Troszyńska *et al*., 2010). Previous studies had reported that CMC could reduce astringency taste of tannic acid (Troszyńska *et al*., 2010) and suppress bitterness of caffeine and catechin (Pangborn *et al*., 1973; Gaudette and Pickering, 2012).

Gum arabic, a highly branched polysaccharide containing arabinogalactan polysaccharides and glycoproteins, is capable of producing a viscous mixture when mixed with bitter gourd extract. It forms multiple hydrogen bonds with water due to its polyanionic nature and thus, act as an excellent stabilizing and thickening agent (Dauqan and Abdullah, 2013; Akkarachaneeyakorn and Tinrat, 2015). Besides, gum arabic also shows antioxidant property as it increased the production of free radical in bitter gourd extract (Ali, 2004; Trommer and Neubert, 2005). Several studies (Jimenez *et al*., 2011; Akkarachaneeyakorn and Tinrat, 2015) had been conducted in the past on effects of debittering treatments on physical properties and antioxidant capacity of various fruit juices and coffee-based drinks. However, study on physicochemical and antioxidant properties of bitter gourd after debittering treatment is scarce. Thus, this study attempts to investigate the effects of debittering treatments on physical properties and antioxidant capacity of bitter gourd extract.

MATERIALS AND METHODS

Materials: Fresh bitter gourds which were light green in colour were selected and purchased from a local hypermarket (Tesco, Pulau Pinang, Malaysia) whereas the reagents were obtained from a number of suppliers; The carboxylmethylcellulose (CMC) from Sim Company Sdn. Bhd. (Pulau Pinang, Malaysia). The gum arabic and sodium chloride (NaCl) from Euro Chemo-Pharma (Pulau Pinang, Malaysia). Sodium nitrate ($NaNO_3$) from Bendosen (Selangor, Malaysia) and aluminium chloride ($AlCl_3$) from R&M Chemicals (Essex, UK), 2,2'-Azino-bis 3-ethylbenzothiazoline-6-Sulfonic Acid (ABTS), potassium persulphate, quercetin dehydrate, trolox and catechin from Merck (Darmstadt, Germany). Acetic acid (HPLC grade) from J.T. Baker (USA). Acetonitrile (HPLC grade) and sodium hydroxide (NaOH) from Qrec (New Zealand). Gallic acid, gentisic acid and chlorogenic acid from Sigma-Aldrich (St. Louis, USA).

Preparation of bitter gourd extract: Fresh bitter gourd fruit was washed under running water to remove dirt and cut into half to remove seeds. The bitter gourd is then cut into smaller pieces (2 cm thick). Debittering treatments were then carried out as following:

- Soaking the bitter gourd in 3.5% (w/v) sodium chloride (NaCl) solution (1:5) at room temperature (25°C) for 60 min
- Blanching the bitter gourd at 96°C for 3 min (Yao and Ren, 2011). Upon debittering, juice of the bitter gourd was extracted using a centrifugal juice extractor (JE680, Kenwood, UK) without addition of water. Another method used for debittering was by adding carboxymethylcellulose, CMC (0.5%, w/v) and gum arabic (15%, w/v) into the bitter gourd extract.

Determination of colour, pH, total soluble solids and viscosity of bitter gourd extract: Colour of the bitter gourd extracts was measured using a colourimeter (CM-3500D, Konica Minolta Co. Ltd., Japan). A total of 15 mL of bitter gourd extract was placed in a glass sample cell and the values of *L* (lightness/ brightness), *a* (redness), *-a* (greenness), *b* (yellowness) and *-b* (blueness) were measured. Changes in green colour of the bitter gourd extract were also measured by calculating the ratio of *–a* to *b* value (*-a/b*), which refers to conversion of green to yellow colour of the extract. The pH and total soluble solid of the bitter gourd extract were determined using a pH meter (S40 Seven MultiTM, Metter-Toledo, Switzerland) and Hand Refractomer (Hanna H196801, Romania), respectively. The viscosity of bitter gourd extract (45 mL) was measured by using a Viscometer (Vibro-Viscometer, A and D Co. Ltd., Japan) with Viscometer probe, SV-10. All samples were measured in triplicates.

Determination of phenolic compounds in bitter gourd extracts: Phenolic content in bitter gourd extract was determined according to modified method of Kubola and Siriamornpun (2008) and Horax *et al*. (2005) using High Performance Liquid Chromatography (HPLC), Waters 2690 with Photodiode array detector (PDA). A series of standard solutions for gallic acid, gentisic acid, catechin and chlorogenic acid (20, 40, 60, 80 and 100 mg/L) were prepared by diluting the respective stock solutions in deionized water. Bitter gourd extract and standards were filtered through a 0.45 µm nylon filter (Sortorius, Spain) before injecting 20 µL of the solution into reverse phase C18 Hypersil Gold column, 4.6×250 mm, i.d. 5 µm (Thermo Fisher) at 40°C. The mobile phasescomprised of solvent A (3% acetic acid and 97%

deionized water) and solvent B (3% acetic acid, 25% acetonitrile and 72% deionized water). Gradient was set as following: 0 min, 100% A; 10 min, 82.5% A and 17.5% B; 20 min, 65% A and 35% B; 30 min, 47.5% A and 52.5% B; 35 min, 30% A and 70% B. The flowrate was set at 1.0 mL/min. Standard solutions of phenolic compounds and the bitter gourd extract were measured at a wavelength of 278 nm in triplicates. Concentration of phenolic compounds in the bitter gourd extract was then calculated from the standard curve.

Sample preparation of bitter gourd extract for flavonoid content and ABTS scavenging activity: A total volume of 25 mL of bitter gourd extract was placed in a 50 mL centrifugal tube and centrifuged at 3500 rpm for 10 min at room temperature (25°C). The supernatant was collected and stored at 4°C until further analysis.

Determination of flavonoid content in bitter gourd extract: Flavonoid content in the bitter gourd extract was determined according to Wu and Ng (2008). Bitter gourd extract (0.5 mL) was mixed with 2 mL of distilled water and 0.15 mL of sodium nitrate, $NaNO_3$ (5%) in a test tube and allowed to react for 6 min. A total of 0.15 mL 10% aluminium chloride, $AlCl_3$ was added to the mixture and allowed to react for another 6 min, prior to addition of 2 mL of 4% sodium hydroxide (NaOH). The mixture was then made up to 5 mL with distilled water and left in the dark for 15 min to react at room temperature. Absorbance of the reaction mixture was measured at 510 nm using UV-Vis, 1650 spectrophotometer (Shidmadzu, Japan). A similar procedure was done to prepare a standard curve of quercetin at 2.0, 4.0, 6.0, 8.0 and 10.0 mg/mL. All samples were measured in triplicates and the results were expressed as mg QE/100 mL sample.

Determination of ABTS radical scavenging activity in bitter gourd extract: The ABTS assay of bitter gourd extract was carried out according to modified method of Binsan *et al*. (2008) and Murad *et al*. (2013). ABTS reagent was prepared by mixing 7 mM 2, 2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) with 2.45 mM potassium persulfate which was left in the dark to react for 16 hours at room temperature. The ABTS reagent was then diluted with methanol (1:4) to obtain absorbance of 0.7 at 734 nm using a UV-Vis 1650 spectrophotometer (Shidmadzu, Japan). For the assay, an aliquot of 200 µL bitter gourd extract was mixed with 2000 µL of diluted ABTS reagent in a test tube. The mixture was allowed to react in the dark for 10 min, at room temperature. Absorbance of the reaction mixture was measured at 734 nm. A standard curve was prepared using trolox solutions at different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/mL). Samples were measured in triplicates and results were expressed as mg TE/100 mL sample.

Statistical analysis: Results were reported as means±standard deviation. Analysis of Variance (ANOVA) was performed and comparison of means was done by Duncan's multiple range tests using Statistical Package for the Social Sciences software (SPSS 15.0 for Windows, SPSS Inc., Chicago, IL, USA). Results with mean error probability of $p<0.05$ (95% confidence level) were considered as significantly different.

RESULTS AND DISCUSSION

Colour of bitter gourd extract: Table 1 represents effect of debittering treatments on lightness (*L* value), greenness (*-a* value), yellowness (*b* value) and conversion of green to yellow colour in bitter gourd extract (*-a/b*). Lightness (*L*-value) of the bitter gourd extract after blanching treatment or soaking in 3.5% (w/v) NaCl solution were significantly ($p<0.05$) lower as compared to fresh bitter gourd extract. In contrast, addition of carboxymethylcellulose (CMC) and gum arabic in the bitter gourd extract significantly ($p<0.05$) increased lightness (*L*-value) as compared to fresh bitter gourd extract. All debittering treatments as well as addition of CMC and gum arabic had significant ($p<0.05$) reduced yellow colour (*b*-value) of the bitter gourd extracts, except for the extract obtained after soaking in NaCl solution. Changes in *L* and *b* values of the bitter gourd extract after the treatments, could be due to pheophytin-phyripheophytin conversion or degradation/reaction of other component present in the bitter gourd extract (Weemaes *et al*., 1999).

Green colour (*-a*-value) of the bitter gourd extract is due to chlorophyll pigment. Chlorophyll stability depends on temperature, pH, salts, enzymes and surface-active ions (Nisha *et al*., 2004). Chlorophyll easily degrades during processing. Thus, colour of the bitter gourd extract turns from bright green to olive green during blanching treatment. This process reduced the *-a* value from -4.93 to -1.97 (Table 1). Substitution of magnesium ion residing in the middle of chlorophyll

Table 1: Effect of debittering treatments on the colour properties of bitter gourd extract

Bitter gourd extract	*L* (Lightness)	*-a* (Greenness)	*b* (Yellowness)	*-a/b*
Without debittering treatment (Fresh)	84.01±0.08^{b}	-4.93 ±0.03^{d}	34.26 ±0.15^{b}	-0.14
Blanch in hot water (96 °C)	80.24±0.06^{c}	-1.97±0.05^{a}	31.02±0.16^{c}	-0.06
Soak in 3.5% (w/v) NaCl solution	80.33±0.06^{c}	-4.58±0.05^{c}	35.93±0.34^{a}	-0.13
Addition with 0.5% (w/v) CMC	86.41±0.84^{a}	-4.96±0.13^{d}	30.42±1.26^{c}	-0.16
Addition with 15% (w/v) gum arabic	87.01±0.57^{a}	-2.44±0.04^{b}	19.65±0.62^{d}	-0.12

Means±SD (n = 3) values with different superscript letters within the same column are significantly different at $p<0.05$

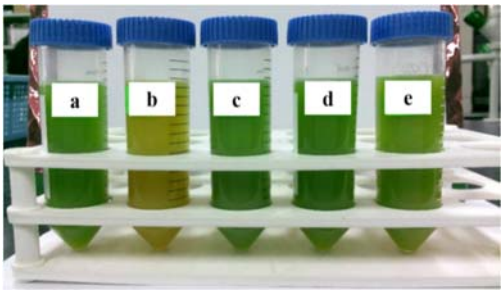

Fig. 1: Colour of bitter gourd extract at different debittering treatments; (a): Bitter gourd without debittering treatment (Fresh); (b): Bitter gourd extract after blanching bitter gourd at 96°C for 3 min; (c): Bitter gourd extract after soaking bitter gourd in 3.5% NaCl solution for 60 min; (d): Addition of 0.5% (w/v) carboxymethylcellulose, CMC into bitter gourd extract and; (e): Addition of 15% (w/v) gum arabic into bitter gourd extract

molecule with hydrogen ion occurs when bitter gourd tissue is damaged during blanching at high temperature, resulting in conversion of green chlorophyll to olive green pheophytin (Heaton and Marangoni, 1996; Erge *et al*., 2008) (Fig. 1). Reduction in green colour intensity of the bitter gourd extract upon blanching was also indicated by the decreasing ratio of -*a* to *b* value (-*a*/*b* parameter) (Erge *et al*., 2008). This observation is associated to conversion from green to yellow colour of the extract during high temperature treatment.

Green colour (-*a* value) of the bitter gourd extract soaked in NaCl solution decreased significantly as compared to fresh bitter gourd extract. The decreasing intensity of green colour (-*a*-value) in bitter gourd treated with 3.5% NaCl solution might be due to destruction of chloroplast and instability of chlorophyll pigment protein complex by the high salinity (Petjukevičs *et al*., 2015). Addition of 0.5% (w/v) CMC in the bitter gourd extract showed no significant ($p>0.05$) changes in yellow colour (*b*-value) compared to fresh bitter gourd extract. During the dissolving of CMC in bitter gourd extract, CMC molecules breakdown into sodium cations and polymer ions through electrolysis process (Yang and Zhu, 2007). Presence of sodium cations had stabilizes green colour of bitter gourd extract as sodium, in a way, may replace magnesium in the middle of chlorophyll (Haismanand Clarke, 1975; Nisha *et al*., 2004). Incorporation of gum arabic into bitter gourd extract resulted in significant ($p<0.05$) decrease of green colour (-*a* value). Loss of green colour of the bitter gourd extract might be due to dilution of chlorophyll pigments present in the extract with addition of high concentration of yellowish gum arabic (Tellis and Martinez-Navarrete, 2009; Dauqan and Abdullah, 2013).

pH, total soluble solids and viscosity of bitter gourd extract: pH, total soluble solids and viscosity of bitter gourd extract at different debittering treatments are presented in Table 2. The pH values of the bitter gourd extract were in range of 5.44 to 5.92. Total soluble solids of the bitter gourd extract were in the following order of debittering treatments: Addition of gum arabic> Soaking in 3.5% NaCl solution = Addition of CMC > Fresh > Blanching (90°C, 3 min). Total soluble solids in blanched bitter gourd extract was observed to have reduced significantly ($p<0.05$) as compared to fresh extract. An increase in cell wall modification of bitter gourd during high temperature of blanching might cause the loss of total soluble solids (Luna-Guevara *et al*., 2015). Total soluble solid of other debittering treatments was found to be significantly higher as compared to fresh bitter gourd extract.

Total soluble solids of bitter gourd extract were found to be significantly ($p<0.05$) higher after soaking in NaCl solution. An increasing soluble solids content of bitter gourd extract after soaking process could be associated to the movement of salt molecules from the high concentration region (soaking medium) to the low concentration region of bitter gourd slices which subsequently enhance the total soluble solids of the bitter gourd extract (Rahman and Lamb, 1991; Karathanos *et al*., 1995). Workneh *et al*. (2014) reported that total soluble solids increase in pumpkin slices after soaked in 10% salt solution for 10 min. Addition of CMC and gum arabic also had significantly increased the total soluble solids of the bitter gourd extract.

Viscosity of the bitter gourd extract after blanching process increased significantly ($p<0.05$), as per shown in Table 2. During blanching, cell walls of the bitter gourd soften and release its pectin, which then increase viscosity of the extract. Viscosity of the bitter gourd extracts with addition of 0.5% (w/v) CMC and 15% (w/v) gum arabic showed significant ($p<0.05$) increased to 8.70 and 14.13 mPas, respectively compared to fresh bitter gourd extract (1.05 mPas). Both CMC and gum arabic have polyelectrolyte properties and act as thickening agent that increase viscosity of the bitter gourd extract (Saha and Bhattacharya, 2010). Addition of CMC to the bitter gourd extract resulted in electrolysis of the CMC molecule; causing it to split to sodium cation and polymer anions. These ions interact with each other via electrostatic forces. Water molecules and hydroxyl groups (-OH) in CMC molecules exhibit electric dipole, which is capable of forming hydrogen bonds in the extract. These hydrogen bonds help to thicken bitter gourd extract (Yang and Zhu, 2007).

In addition, the highly branched polysaccharides of gum arabic contribute to large molecular charge (OH^-) resulting in more hydrogen bonds forms in the extract solution. The hydrogen bond network formed reduces movement of water and other free moving molecules which results in an increase of viscosity in the bitter

Table 2: Effect of debittering treatments on pH, total soluble solids and viscosity of bitter gourd extract

Bitter gourd extract	pH	Total soluble solids (°Brix)	Viscosity(mPas)
Without debittering treatment(Fresh)	5.96	3.13±0.06^{c}	1.05±0.03^{d}
Blanch in hot water (96 °C)	5.62	2.80±0.10^{d}	1.67±0.08^{c}
Soak in 3.5% (w/v) NaCl solution	5.44	4.00±0.00^{b}	1.05±0.02^{d}
Addition with 0.5% (w/v) CMC	5.92	3.77±0.06^{b}	8.70±0.18^{b}
Addition with 15% (w/v) gum arabic	5.58	13.30±0.26^{a}	14.13±0.06^{a}

Means±SD (n=3) values with different superscript letters within the same column are significantly different at p<0.05

Table 3: Effect of debittering treatments on the polyphenols compounds in bitter gourd extract (mg/ 100 ml extract)

Bitter gourd extract	Gallic acid	Gentisic acid	Catechin	Chlorogenic acid
Without debittering treatment(Fresh)	3.78±0.05^{b}	6.54±0.38^{b}	1.05±0.19^{c}	0.27±0.01^{c}
Blanch in hot water (96 °C)	3.63±0.02^{c}	3.40±1.56^{c}	1.08±0.19^{c}	0.24±0.00^{d}
Soak in 3.5% (w/v) NaCl solution	2.83±0.04^{d}	3.65±0.80^{c}	2.20±0.04^{a}	0.44±0.00^{b}
Addition with 0.5% (w/v) CMC	4.47±0.24^{a}	2.36±0.86^{c}	1.78±0.26^{b}	0.63±0.05^{a}
Addition with 15% (w/v) gum arabic	3.79±0.03^{b}	8.36±0.40^{a}	1.85±0.12^{b}	0.62±0.02^{a}

Means±SD (n = 3) values with different superscript letters within the same column are significantly different at p<0.05

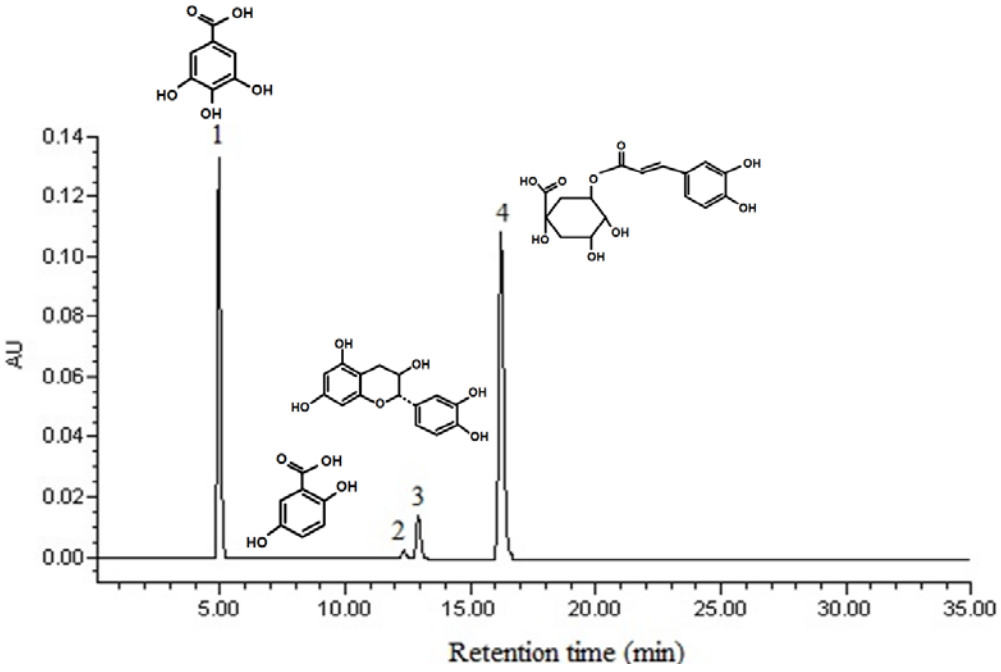

Fig. 2: HPLC chromatogram of standard phenolic compounds. 1) Gallic acid; 2) Gentisic acid; 3) Catechin and; 4) Chlorogenic acid

gourd extract (Akkarachaneeyakorn and Tinrat, 2015; Sworn, 2004). Increase in viscosity and hydrogen bonding between hydrocolloids and polyphenols in the bitter gourd extract containing CMC and gum arabic could also be associated to decrease in bitterness, as interaction between the polyphenols and tongue receptors was reported to weaken (Ares *et al*., 2009; Troszyńska *et al*., 2010). Addition of CMC and gum arabic into the bitter gourd extract is not only as a debittering treatment, but also to increase viscosity and to maintain homogeneous suspension of the extract by increasing its mass fraction (%). Hydrocolloids are added to stabilize turbidity in production of viscous juices. However, caution has to be taken as it may cause the juice to become too viscous and unacceptable to consumers (Akkarachaneeyakorn and Tinrat, 2015; Pangborn *et al*., 1973).

Phenolic compounds in bitter gourd extract: Phenolic compounds, namely gallic acid, gentisic acid, catechin and chlorogenic acid are available abundantly in bitter gourd (Horax *et al*., 2005). Therefore, this study attempts to determine effect of debittering treatment on these four major phenolic compounds. High Performance Liquid Chromatography (HPLC) chromatograms for the four phenolic compound standards are presented in Fig. 2. Phenolic content of the bitter gourd extract for the different debittering treatments was determined using the standards as relative (Table 3). Phenolic acids in bitter gourd extract present in the form of hydroxycinnamicacid (chlorogenic acid) and hydroxybenzoic acids; which are aromatic compounds comprising of gallic acid and gentisic acid (Thammapat *et al*., 2015). The flavonoid compound which is Catechin (flavon-3-ol) is also found

Table 4: Effect of debittering treatments on the flavonoid content and ABTS scavenging activity of bitter gourd extract (mg/ 100 ml extract)

Bitter gourd extract	Total flavonoid content mg QE/ 100 mlextract	ABTS assaymg TE/ 100 mlextract
Without debittering treatment(Fresh)	318.24±18.16[a]	0.42±0.04[d]
Blanch in hot water (96 °C)	197.94±2.57[b]	8.10±0.05[b]
Soak in 3.5% (w/v) NaCl solution	134.84±4.59[c]	0.39±0.11[d]
Addition with 0.5% (w/v) CMC	182.99±11.99[b]	1.64±0.25[c]
Addition with 15% (w/v) gum arabic	183.54±7.03[b]	13.26±0.22[a]

Means±SD (n=3) values with different superscript letters within the same column are significantly different at $p<0.05$

partially as the bitter gourd's phenolic content assuming the structure of polyphenols. Fresh bitter gourd extract possesses highest content of gentisic acid (6.54 mg/100 mL extract), followed by gallic acid (3.78 mg/100 mL extract), catechin (1.05 mg/100 mL extract) and chlorogenic acid (0.27 mg/100 mlextract). Loss of phenolic compounds after the blanching of bitter gourd extract, mainly due to dissolution of phenolic acids in water (Myojin *et al*., 2008).

There was a significant ($p<0.05$) increase of chlorogenic acid and catechin content in the bitter gourd extract after soaking in 3.5% (w/v) NaCl solution. This indicates that sodium chloride (NaCl) increase the biosynthesis of these compounds. It could be caused by salt stress condition which is responsible for reducing oxidative processes (Ali and Ismail, 2014; Thammapat *et al*., 2015). Similarly, soaking black nightshade berry (Elhaak *et al*., 2015) and tomato (Ali and Ismail, 2014) in NaCl solution, had also increased phenolic acid compounds through activation of fruits' oxidative system.

Addition of carboxymethylcellulose (CMC) and gum arabic in bitter gourd extract showed an increase of catechin and chlorogenic acid, suggesting that CMC and gum arabic are able to retain phenolic compounds in the extract. Similar finding was reported by Jimenez *et al*. (2011), Serrano-Cuz *et al*. (2013) and Siti Rashima *et al*. (2017) in which polyphenols content of roselle, blackberries and bitter gourd juice had increased after addition with CMC or gum arabic.

Antioxidant capacity of bitter gourd extract: Total flavonoids content and ABTS scavenging activity of bitter gourd extract at different debittering treatments are shown by Table 4. Fresh bitter gourd extract was observed to be a good source of flavonoids, containing 318.24 mg quercetin·100 mL^{-1} extract. Quercetin (3, 5, 7, 3', 4'-pentahydroxyflavone) and its derivatives (isoquercitrin, quercitrin, hyperoside, rutin and spiraeoside) are natural flavonols which contribute to antioxidant and photoprotection properties in plants (Zhou and Sadik, 2008; Dall'Acqua *et al*., 2012). Although quercetin is stable against UV and visible spectrum, degradation of its structure can easily occur in presence of oxygen as well as without enzymatic catalysis or light irradiation (Zenkevich *et al*., 2007). As expected, total flavonoid content in treated bitter gourd extract was observed to decrease significantly ($p<0.05$) indicating that degradation of quercetin had taken place in presence of air bubbles (Zenkevich *et al*., 2007).

In fruits and vegetables, quercetin is normally present in the form of glycosides derivatives which is bound to sugar group. High temperature of blanching treatment degrades quercetin glycosides to aglycone (quercetin) (Bentz, 2009), noticeable by the high quercetin content (197.94 mg QE/100 mL extract) in the blanched bitter gourd extract. Similar study had reported that blanching white saffron with citric acid and distilled water had hydrolyzed quercetin-3-rutinoside into quercetin aglycone (Pujimulyani *et al*., 2012). Several studies also reported the loss of phenolic acid and flavonoid contents in broccoli (Zhang and Hamauzu, 2004), spinach, kale, cabbage, shallots (Ismail *et al*., 2004) and bitter gourd (Choo *et al*., 2014) due to boiling or blanching treatment. Total flavonoids content of bitter gourd extract obtained upon soaking in NaCl solution was found to be the lowest among others debittering treatments (Table 4). Osmosis process occurs during soaking of bitter gourd slices in the salt solution. Movement of salt molecules from soaking medium into the bitter gourd slices leads to increase of salt-stress condition in the fruit which subsequently cause degradation of quercetin in the bitter gourd extract (Cazado and Pinho, 2016). The high content of flavonoid in bitter gourd extract after addition with carboxymethylcellulose (CMC) and gum arabic was due to ability of these polysaccharides to encapsulate and protect quercetin (Jimenez *et al*., 2011; Serrano-Cuz *et al*., 2013).

ABTS assay is a method to measure capacity of hydrogen-donating antioxidant to scavenge ABTS radical ($ABTS^{\cdot+}$) through electron transfer (Re *et al*., 1999). Antioxidant activity of bitter gourd extract at different debittering treatments was determined based on the capacity to reduce of blue/green $ABTS^{\cdot+}$ radical cations and expressed as Trolox Equivalent Antioxidant activity (TEAC) (Table 4). Blanching of bitter gourd slices inactivate polyphenoloxidase enzyme (PPO) which is responsible for browning reaction and non-enzymatic Maillard reaction. Maillard reaction which involves interaction of sugar and amino acids in bitter gourd lead to formation of more sugar-amino acid products and hence cause browning of the extract (Ajandouz *et al*., 2008; Wei *et al*., 2013). Result showed that high flavonoid content might contributed to the high ABTS scavenging activity. The ABTS scavenging activity of bitter gourd extract obtained after soaking in NaCl solution was not significantly

($p>0.05$) different with fresh bitter gourd extract. Addition of polyelectrolyte hydrocolloids (0.5%, w/v carboxymethylcellulose (CMC) and 15%, w/v gum arabic) into bitter gourd extract increased the ABTS scavenging activity to 1.64 and 13.26 mg TE/100 mL extract, respectively (Table 4) as compared to fresh bitter gourd extract. This is due to increase of charged molecules (OH^-) in the bitter gourd extract upon addition of CMC and gum arabic, which resulted in reduction of more $ABTS^{\cdot+}$ radical cations (Akkarachaneeyakorn and Tinrat, 2015). Other study found that the increasing of polyphenols content in spray-dried blackberries (*Rubusfruticosus*) juice after incorporated with gum arabic (Jimenez *et al*., 2011) which suggesting the role of gum arabic as a protective agent towards polyphenols compound present in the juice.

CONCLUSION

Addition of carboxymethylcellulose (CMC) could retain the green (-*a*-value) colour of bitter gourd extract. Viscosity of bitter gourd extract containing CMC and gum arabic was enhanced with an increase in total soluble solids content. Increase in viscosity of blanched bitter gourd extract is due to presence of pectin gels in the extract after blanching. Addition of CMC and gum arabic to the bitter gourd extract could retain the polyphenol content which contributed to a significantly higher in ABTS scavenging activity of bitter gourd extract.

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