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Effects of Processing Methods on the Proximate Composition and Momordicosides K and L Content of Bitter Melon Vegetable

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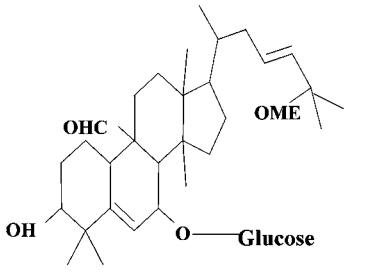
Bitter melon (*Mormodica charantia* L.) has been associated with health benefits such as hypoglycemic, antiatherogenic, and anti-HIV activities. The vegetable, however, has an unpleasant bitter taste. The purpose of this research was to establish the effect of various processing methods on the moisture, lipid, and protein content of the Sri Lanka variety of bitter melon and to determine the effect of the processing methods on momordicosides K and L contents. The processing methods used were frying, blanching, sun drying, oven drying, freeze drying, and bitter masking with five different commercial bitter masking agents. Moisture, lipid, and protein analyses were done using standard AACC methods. Drying decreased moisture content from 92% to 9.5−10.2%. Frying lowered moisture content to 0.8% while increasing lipid content from 3.6 to 67%. Protein content remained unaffected by treatments. A liquid chromatography−electrospray ionization−mass spectrometry (LC/ESI/MS) method was used to identify momordicosides K and L in methanolic extracts of fresh and processed samples. Only extracted ion chromatographs for blanched bitter melon and bitter melon with MY 68 agent showed the absence of momordicosides K and L.

KEYWORDS: Bitter melon; frying; blanching; bitter masking; freeze drying; oven drying; sun drying; momordicosides; LC/ESI/MS

INTRODUCTION

Asian cuisine has become very popular in several places outside Asia. This is partly because several of the fruits and vegetables used in this cuisine fall in the functional food category. Bitter melon (*Mormordica charantia* L.) is a vegetable from the Curcubitaceae family used in some Asian cuisine. It has hypoglycemic, antitumor, antiatherogenic, antioxidant, antiviral, and even anti-HIV activities (*1*−*7*). Bitter melon is also high in fiber, phenols, iron, and ascorbic acid (*8*). Yen and Hwang (*9*) also found the pulp around the seeds of the mature ripe fruit to be a good source of lycopene. Despite its health benefits, bitter melon is unpopular outside of Asia because it contains two triterpenes glycosides, momordicosides K and L (**Figure 1**) which make the vegetable bitter (*10*). The bitter compounds, mormodicosides K and L, have not been found to have any health benefits of their own.



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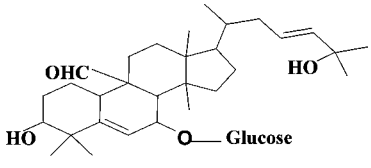

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Figure 1. Structures of momordicoside K (**1**) and momordicoside L (**2**).

The health benefits, nutritional constituents, and chemical composition of bitter melon have been studied (*2*, *10*, *11*). A

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few studies determined the effect of blanching, pickling, and fermentation of bitter melon on its bitterness and overall acceptability *(12, 13)*. Very few studies have, however, been done to determine the effect of debittering processing methods on mormordicoside K and L contents. This study was designed to investigate the effects of sun drying, oven drying, freeze drying, bitter masking, blanching, and frying on proximate composition and mormordicoside K and L contents of bitter melon. The hypothesis for this study was that treatment may cause the removal of these bitter compounds or their modification making them undetectable by liquid chromatography electrospray ionization mass spectrometry.

MATERIALS AND METHODS

Materials. Bitter melon (Sri Lanka variety) was grown at the University of Arkansas farm. Bitter masking agents, MR 15 masking type Fl all Nat, MZ 70 Masking N&A FF PWD, and MY 68 masking type Fl all Nat were obtained from Virginia Dare, Brooklyn, NY. Masking agents Mag-nifique Green Away and Mag-nifique Green Away powder were both obtained from Wixon Inc, St. Francis, WI. Analytical grade methanol, chloroform, petroleum ether, and sulfuric acid were obtained from VWR International (West Chester, PA). Kjeldahl tablet catalyst was obtained from Fisher Chemicals (Morris Plain, NJ).

Methods. *Sample Preparation.* Bitter melons were washed and sliced with a Leifheit kitchen slicer adjusted to give uniformly thin slices. The slices were mixed and divided into 11 portions each weighing approximately 500 g. The portions were subjected to one of the following treatments: oven drying, sun drying, freeze drying, blanching, frying, and bitter masking. The bitter masking was done with five different masking agents. The last portion was left untreated and was used as the control treatment. Moisture content and extraction of momordicosides were done immediately.

Oven Drying. Slices of bitter melon were dried in a harvest saver commercial dehydrator (R-4 Syst Inc., Eugene, OR) set at 55 °C until there was no change in weight between two consecutive readings

Sun Drying. Slices of bitter melon (one-layer thick) were placed on trays and were sun dried to dryness. Exposure to sun was for 8 h each day (10:00 am to 6:00 pm) during the summer of 2005. In between drying times, samples were brought indoors and were kept at room temperature. Average temperature and humidity during the duration of drying were 35.8 °C and 42%, respectively. Drying was continued until no change in weight was observed between two consecutive readings.

Freeze Drying. Samples were dried in a 25LE freeze dryer (The Virtis Company, Inc., Gardiner, NY). Drying was done at −40 °C and at pressure of less than 200 mTorr.

Blanching. Slices of bitter melon were placed on a wire basket and were completely immersed in water held at 98 ± 2 °C for 3 min. The basket was removed and set aside until the water was drained off.

Frying. Preliminary testing of the frying procedure was done to determine the temperature and time of frying required to achieve cooking end point. Frying was done using a Model LLF 14 thermostatically regulated commercial deep fat fryer, (Liteline-Wells MFG.Co., Verdi, NV). The oil used was Great Value vegetable oil (soybean oil) and the bitter melon to oil ratio was maintained at 1:50 w/v. Samples were introduced into the oil on a wire basket so as to ensure uniform frying. Frying temperatures used for the trial were 80, 90, 100, 110, and 120 °C for 10 min. After frying, samples were placed on trays lined with adsorbent paper. A fresh sample of oil was used for each trial. From this preliminary testing, the temperature required to give a desirable golden brown color and crisp texture was found to be 110 °C .

Bitter Masking. For each bitter masking procedure, 500 g of bitter melon slices was thoroughly mixed with 0.5 g of the masking agent to obtain 0.1% percent bitter masking agent in the final product as recommended by the manufacturers. For MZ 70 and Magn-ifique Green Away powder, however, 2.0 g of bitter masking agents was used to obtain a product with 0.4% bitter masking agent as recommended by the manufacturers.

Preparation of Samples for Proximate Analysis. Each sample was ground using an M20 mill, (IKA Works Inc. Wilmington, NC) and was passed through a no. 60 mesh sieve. Grinding was done after determination of moisture content.

Moisture Content. Moisture was determined by the air oven method (AACC method 44.19). Aluminum moisture dishes with lids were preheated for 1 h at 135 °C and were cooled in a desiccator for 15 min. After cooling, the weights of the aluminum moisture dishes were taken, and approximately 2.0 g of the test sample was weighed into them. The moisture dishes and contents were subjected to oven drying at a temperature of 135 °C for 2 h after which they were removed and cooled in a desiccator for 15 min. The moisture content was calculated as percent moisture content by the equation

$$\% \text{ moisture} = \text{loss in weight X100/weight of sample}$$

Three determinations were made for each of three replicates.

Lipid Content. Lipid content was determined by the Soxhlet method (AACC method 30.26). A dry Soxhlet flask was first weighed, and the weight was recorded. Approximately 2 g of sample was then weighed and transferred into a filter paper folded in the shape of an envelope. This was placed in a thimble and was covered with adsorbent cotton making sure to prevent escape of sample. The thimble with the sample was then set in a butt-type extension apparatus and was extracted into the previously weighed Soxhlet flask with 30 mL petroleum ether for 5 h. After cooling, the extension flask was disconnected from the apparatus, and the residual petroleum ether was allowed to evaporate overnight under a fume hood. The Soxhlet flask with the extracted fat was then weighed. The percentage oil on dry matter basis was calculated by the formula

$$\text{oil } \% = \text{wt of oil} \times 100/\text{wt of sample}$$

$$\%\text{oil (dry matter basis)} = F(100 - \%\text{moisture content})/100 - \%\text{moisture content}$$

where F = oil % in sample.

Three determinations were made for each of three replicates.

Protein Content. The protein content was determined by the automated Kjeldahl method. A Kjeldahl 2006 Digester (Foss Tecator, Hoganas, Sweden) was used to digest samples. Concentrated sulfuric acid and a Kjeldahl tablet (catalyst) were used for digestion. Temperature and time of digestion were 420 °C and 1 h, respectively. A 2300 Kjeltec Analyzer Unit (Foss Tecator, Hoganas, Sweden) was used for the nitrogen content analysis, and the protein content was automatically calculated using a factor of 6.25. Three determinations were made for each of three replicates.

Extraction of Momordicosides K and L. Extraction of momordicosides K and L was done by the method of Okabe et al. (*10*) with slight modifications. Five hundred grams of freshly sliced bitter melon was soaked in methanol (1:2, w/v) for 1 day with constant stirring. After 24 h, the bitter melon was homogenized in the methanol, and the mixture was filtered. The filtrate was concentrated in a rotary evaporator to $^1/_{10}$ of its original volume. The resultant solution was extracted with $CHCl_3$ (1:1.5, v/v) in a separating funnel overnight. The chloroform extract obtained was concentrated to dryness in rotary evaporator. Twenty milliliters of hot 70% methanol was used to resolubilize the dry extract. Upon cooling, the solubilized extract was passed through a polystyrene resin column. The extraction was repeated for treated samples using equivalent weights on dry matter basis. Extraction and LC/MS were done in duplicates.

Column Chromatography. An HP 20Ag, 50−100 mesh polystyrene resin obtained from Supelco Bellefonte, PA, was prepared into a slurry according to the manufacturers' directions. Fifty grams of the dry resin was transferred into a 250 mL beaker, and sufficient methanol was added to completely cover the resin bed by 2.0−5.0 cm. The resin−methanol mixture was then stirred gently for 1 min to ensure complete mixing. After stirring, the mixture was allowed to stand for 15 min to ensure complete wetting of the resin. The methanol was then decanted and replaced with deionized water. The resin-deionized water suspension was stirred for 1 min and was kept for 5 min to obtain the slurry. The slurry was slowly loaded into a column containing about 2.5−5.0

Table 1. Effect of Processing Treatments on Moisture, Fat, and Protein Contents of Bitter Melon

treatment	moisture[a]	fat[a]	protein[a]
blanched	94.0a	3.6b	14.9a
bitter masking (MR15)	93.4ab	3.5b	14.7a
bitter masking (234A)	93.3abc	3.6b	14.2a
bitter masking (MZ70)	93.0abcd	3.4b	14.2a
bitter masking (MY68)	92.5bcd	3.7b	14.2a
bitter masking (6100)	92.3cd	3.7b	14.3a
fresh	92.0d	3.6b	14.2a
freeze-dried	10.2e	3.0b	14.6a
oven-dried	10.1e	4.3b	14.5a
sun-dried	9.5e	3.1b	14.7a
fried	0.8f	67.0a	14.0a
<chgrow;lp;4q>standard error	0.2	0.2	0.3

[a] Values are percent means. Means of each attribute followed by the same letter indicate that they are not significantly different ($p < 0.05$) according to Tukey's HSD test.

cm deionized water. As loading proceeded, excess water was drained from the bottom of the column. The column was then washed with methanol and was equilibrated with 70 mL of deionized water. The internal diameter of the column was 15 mm, and the final packed length was 45 cm. The product obtained from sample extraction as described above was then introduced into the column and was eluted at a rate of 0.5 mL/min with 20 mL methanol. The column was then washed with 20 mL methanol. The combined eluent was concentrated in a rotary evaporator to 5 mL and subsequently was subjected to mass spectrometry for identification of mormordicosides K and L.

Identification of Momordicosides K and L. Liquid chromatography−electrospray ionization−mass spectrometry (LC/ESI/MS) in the positive ion mode was used to identify momordicosides K and L. A system composed of an Esqire quadruple ion trap multiple mass spectrometer (Bruker ESQIRE LC/MS, Billerica, MA) was used. A 250 nm × 4.6 Synergi 4 Fusion reverse-phase C 18 column (Phenomenex, Torrance, CA) was used for the HPLC separation. Two elution solvents were used, A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). For the first 5 min, elution was done with 80% A and 20% B. This was followed by a linear gradient in 25 min to 60% B and was held for 5 min; the percentage of B was increased to 100% within the following 5 min. Flow rate was maintained at 0.7 mL/min. Between runs, the column was washed with 100% of solvent A for 5 min and was equilibrated with 80% A and 20% B for 15 min. Column temperature was maintained at 40 °C throughout the analyses. Interface settings for mass spectrometer were as follows: Capillary voltage, 4200 V; drying or vaporizing temperature, 300 °C; nebulizing pressure, 32 psi; flow rate of dry gas, 12 L/min; and skim voltage, 19.4 V. Mass spectrometry data was collected at full scan mode from *m*/*z* 50 to 1000.

Effect of debittering treatments on momordicosides of interest was assessed by determining the absence or presence of momordicosides K and L on an extracted ion chromatograph.

Statistical Analysis. Statistical analysis for the proximate composition of was done using JMP 6.0 software (SAS, Cary, NC). Group means were compared to test the null hypothesis that protein, moisture, and lipid content are the same for all treatments. Analysis of variance (ANOVA) and Tukey HSD were used for means comparison.

RESULTS AND DISCUSSION

Moisture Content. The moisture content observed for fresh bitter melon ranged from 91 to 93% with a mean of 92% (**Table 1**). This compares well with moisture content determined by Wills et al. (*14*). who determined moisture content to be 93.8%. Pal et al. (*15*) also reported the moisture content of Pusa hybrid-1 and Pusa Vishesh varieties of bitter melon to be between 91.3% and 92.2% moisture. As was expected, moisture content decreased considerably on drying.

There was no significant difference in moisture content ($p < 0.05$) among samples dried by different methods indicating that the efficiency of drying was comparable among drying methods. Even though sun drying remains the predominant way of drying in most tropical countries because of its relatively low cost and simplicity, it has the disadvantage of being quite uncontrollable. In addition, it exposes the material to contamination from dust and microorganisms (*16*−*18*) and may therefore not be the preferred option of drying.

The fried sample had lower moisture content than the other samples ($p = 0.0001$); this is because deep frying expels most of the moisture and replaces it with fat (*19*−*20, 21*). This also accounts for the very high total lipid content observed for the fried sample. This confirms what various other studies have shown indicating that oil uptake during frying is directly related to initial moisture content and is inversely related to water removal (*19*, *21*, *22*).

Blanched bitter melon had higher moisture content than fresh bitter melon (**Table 1**) which could be a result of the tissues imbibing water during blanching. This is possible because of the partial destruction in cell membrane structure (*23*). Even though it has been reported that blanching does result in dehydration in some cases where cell membrane destruction is extensive, this was not observed in this case probably because the blanching time was not long enough to result in extensive damage and turgidity loss.

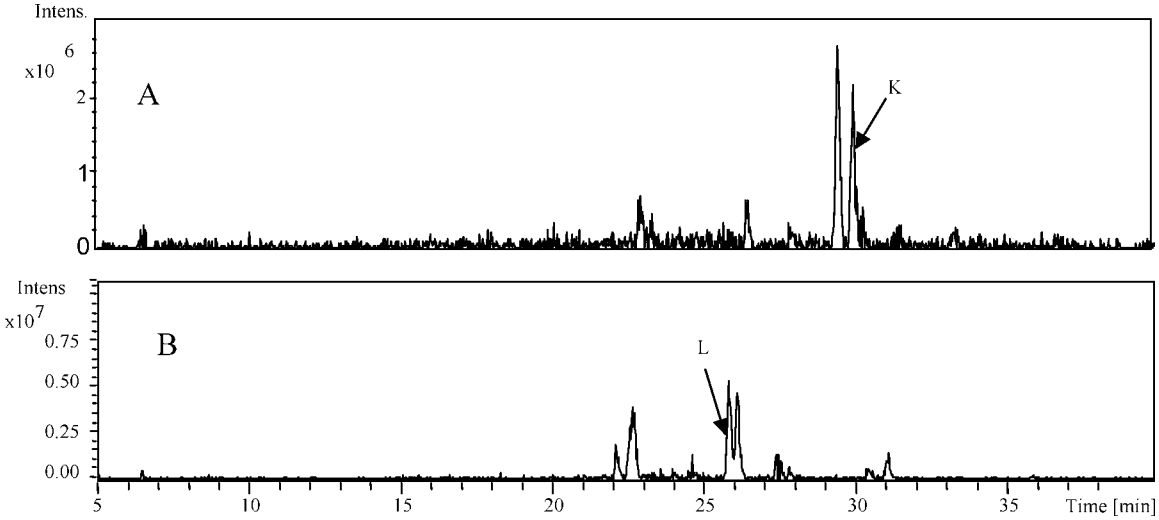

Figure 2. LC/ESI/MS (+) extracted ion chromatogram (EIC) of (**A**) *m*/*z* 649.5 (labeled K) and (**B**) *m*/*z* 635.4 (labeled L) from methanolic extract of the immature fresh bitter melon vegetable.

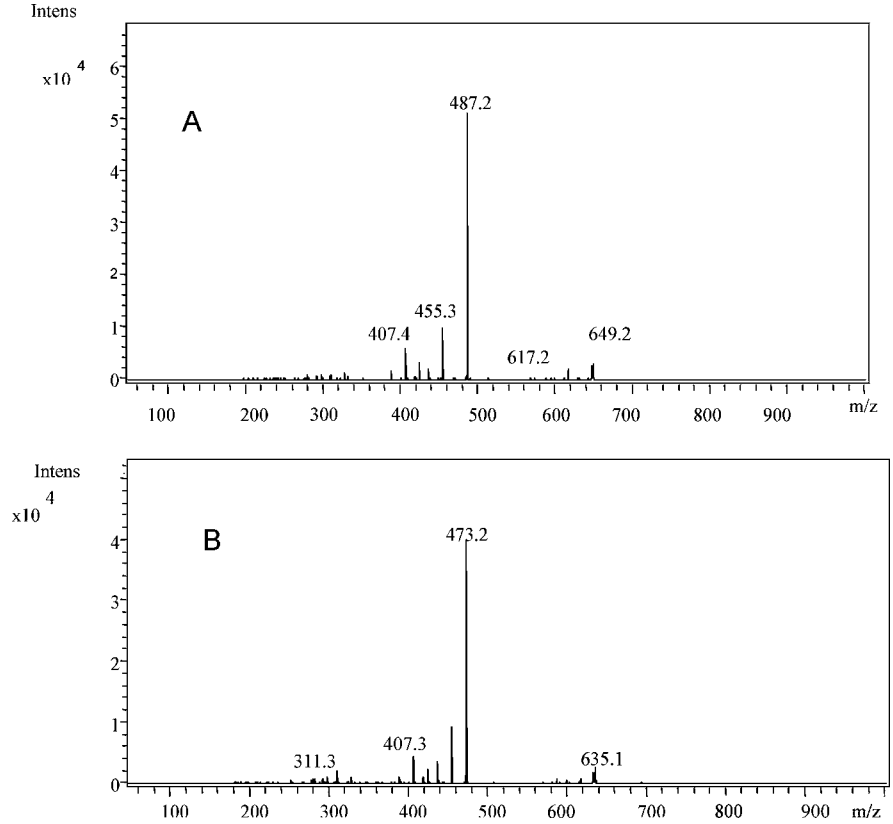

Figure 3. Fragmentation pattern of (**A**) peak K (Figure 2) and (**B**) peak L (**Figure 2** after MS/MS).

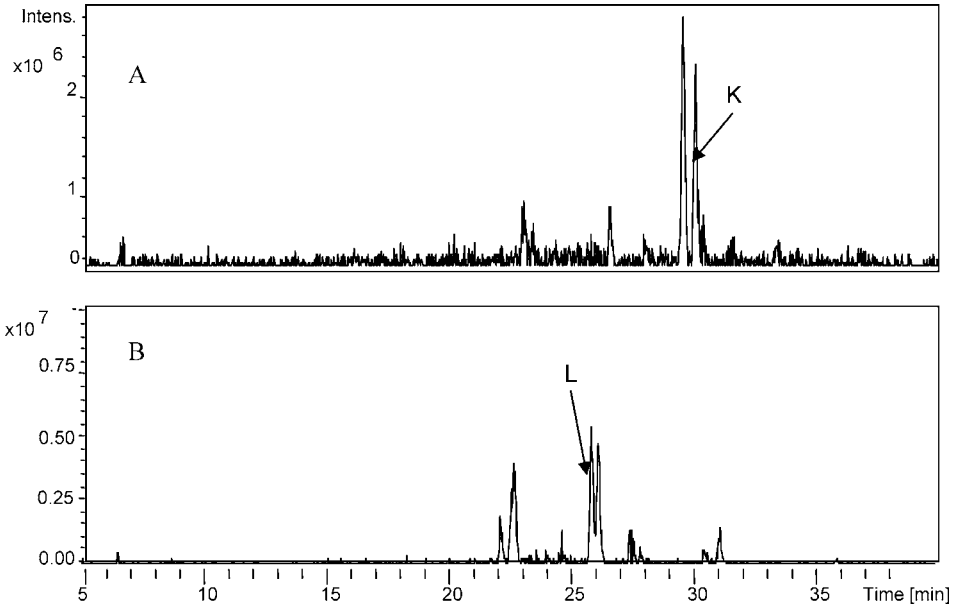

Figure 4. LC/ESI/MS (+) extracted ion chromatogram (EIC) of (**A**) *m*/*z* 649.5 and (**B**) 635.4 from methanolic extract of sun-dried bitter melon vegetable.

Lipid Content. The lipid contents of all samples on dry matter basis were identical (**Table 1**) except for the fried sample ($p = 0.0001$) where the lipid content increased from 3.6 to 67% because deep frying results in the expulsion of water and the absorption of fat. The amount of fat absorbed is not exactly equal to the amount of water lost because other factors affect the absorption of oil (*20*, *21*). The total lipid for all samples, however, was different from that observed by Wills et al. (*14*) who found bitter melon to contain 0% fat. The differences in fat determined by the two studies could be a result of differences in sample preparation. Whereas Wills et al. removed all seeds, this study used the whole immature vegetable. The use of the whole immature vegetable may have increased the lipid content. This is because even though seeds have not fully developed in the immature vegetable, there is the possibility that some lipid could have been stored in anticipation of seed development. Suzuki et al. (*24*) observed that the undeveloped seeds of the Gunsei variety of bitter melon contained 1.4% total lipids. Differences in lipid content could also be due to varietal differences. Varietal differences have been found to be a reason for lipid content differences in other types of plants (*25*, *26*).

Protein Content. Protein content on dry matter basis of nontreated bitter melon was 14.2%. This compared well with other studies conducted by Wills et al. (*14*) and Assubaie and

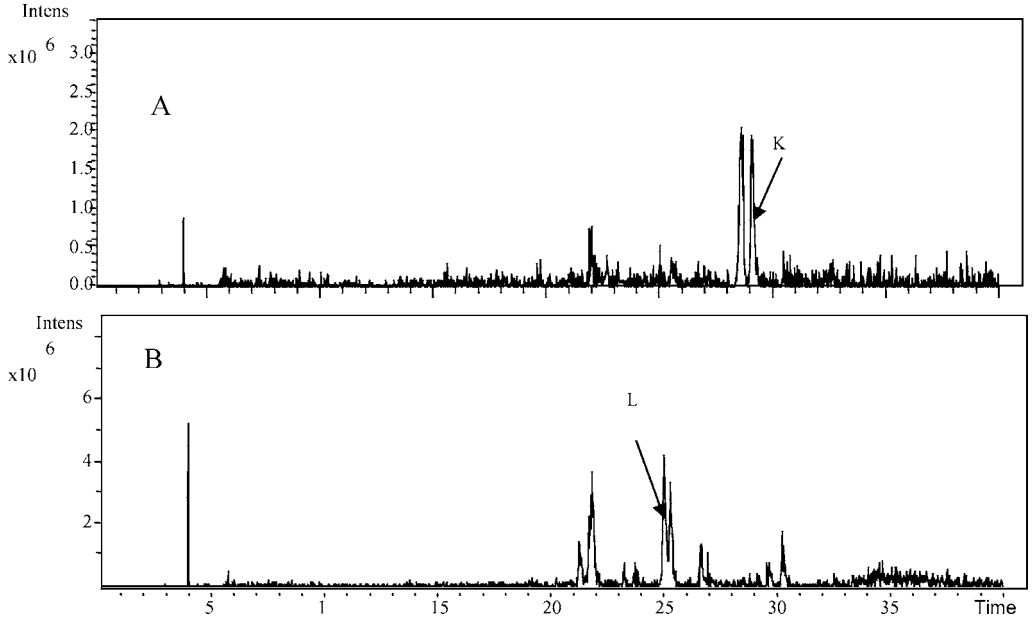

Figure 5. LC/ESI/MS (+) extracted ion chromatogram (EIC) of (**A**) *m*/*z* 649.5 and (**B**) 635.4 from methanolic extract of bitter melon masked with MR 15.

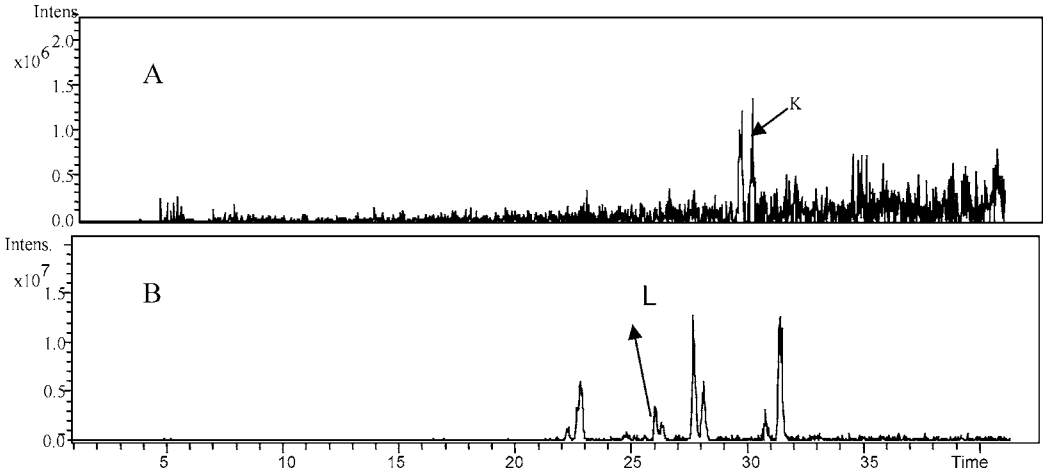

Figure 6. LC/ESI/MS (+) extracted ion chromatogram (EIC) of (**A**) *m*/*z* 649.5 and (**B**) 635.4 from methanolic extract of fried bitter melon.

El-Garawany (*8*) that reported bitter melon to contain 0.9% protein on fresh weight basis (14.5% dry matter) and 14.4% dry matter, respectively. All other samples had similar protein contents.

Identification of Mormordicosides K and L in Fresh Bitter Melon. The method used in this study to identify momordicosides K and L was an HPLC mass spectrometry method. This method was used because both momordicosides lack chromophores and have no commercial standards, making it impossible to use the conventional HPLC method of detection and identification. Because of these difficulties, this study used three characteristic features of the momordicosides in the identification process. These features are molecular ions (*m*/*z* 649.5 for momordicoside K and *m*/*z* 635.4 for momordicoside L), their corresponding aglycone fragments (*m*/*z* 487.4 for momordicoside K and *m*/*z* 473.4 momordicoside L), and their retention times.

Figure 2 shows the extracted ion chromatograms (EIC) of *m*/*z* 649.5 and 635.4 ions from the total ion chromatogram (TIC) of the methanolic extract of the fresh bitter melon. The *m*/*z* 649.5 extracted ion chromatogram shows two major peaks while the EIC for *m*/*z* 635.4 show several peaks.

An MS/MS of the peak labeled K shows peaks at *m*/*z* 649.5, 487.2, and 455 (**Figure 3A**). This is consistent with the expected fragmentation pattern of momordicoside K where the *m*/*z* of 649.5 is the expected *m*/*z* of the intact molecular ion, 487.2 is the expected *m*/*z* of the aglycone, and *m*/*z* 455 is expected for the a-glycone without the methoxy group.

Figure 3B shows the MS/MS of the peak labeled L in **Figure 2**. This shows a peak at *m*/*z* 635 consistent with the *m*/*z* of intact protonated momordicoside L. It also shows a peak at *m*/*z* 473.2 consistent with the aglycone of momordicoside L. From the MS/MS analyses, the peaks that were found to have fragmentation patterns consistent with momordicosides K and L were found to have retention times of 30.0 and 26.0 min, respectively. These retention times were used to identify momordicosides K and L in subsequent samples. This study recognizes the fact that it is not possible to absolutely differentiate momordicosides K and L from their isomers or other possible compounds with similar molecular structures. For this reason, all peaks identified as K or L are actually momordicoside K or momordicoside L-like compounds.

Identification of Momordicosides K and L in Treated Samples. All dried samples showed the presence of both momordicosides (**Figure 4**).

All the samples that were treated with bitter masking agents (**Figure 5**) showed EIC similar to the EIC observed for the unprocessed bitter melon. This is because the main mode of

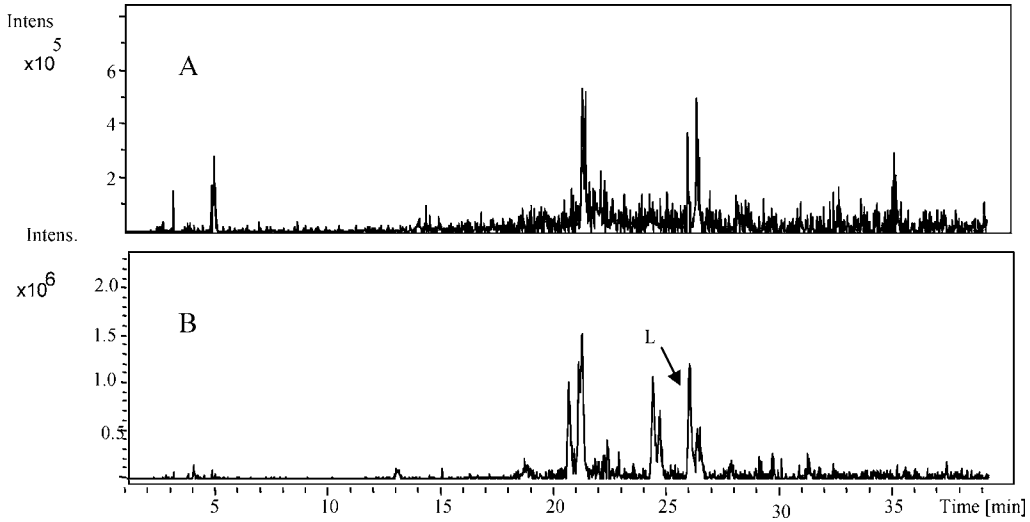

Figure 7. LC/ESI/MS (+) extracted ion chromatogram (EIC) of (**A**) *m*/*z* 649.5 and (**B**) 635.4 from water used for blanching.

operation of most bitter masking agents is not to remove or change the conformation of the bitter compound it is masking but rather to compete for receptor sites on the tongue with the bitter tasting stimulus. EIC for sample masked with MY 68 (data not shown) did not show peaks for momordicosides K and L as expected. At this point, we believe this is either because there is some interaction between the bitter masking agent and the momordicoside making them unable to elute at their expected retention times or the masking agent interferes with the extraction of both momordicosides. Conclusive explanation may only be possible after sensory evaluation.

Both momordicosides were absent from the EIC for blanched sample (data not shown). This indicates that the momordicosides may either be water soluble and hence extracted in the water used for blanching or may have been degraded at the high blanching temperatures.

Even though the EIC for the fried bitter melon shows some traits of momordicosides K and L (**Figure 6**), the spectrum in general shows some difference in its appearance, and this may be due to residual lipids from the frying oil that may have been extracted in the momordicoside extract. Since high temperatures are used for frying, it is expected that if the high temperatures degrade the momordicosides, they should not be observed in the EIC for fried bitter melon. The fact that the momordicosides are observed in the EIC for fried bitter melon suggests that high temperatures do not degrade the momordicosides. The absence of both momordicosides from the EIC of the blanched bitter melon is thus more likely to be a result of the momordicosides being extracted into the blanching water. The momordicoside L is observed in the EIC for the blanching water (**Figure 7**) but not on EIC of the aqueous extract made at ambient temperature (data not shown). Momordicoside K is not observed in either EIC. This suggests that at elevated temperatures and in an aqueous medium, momordicoside L may be extracted from bitter melon. The presence of momordicoside L in the blanching water indicates that during blanching, momordicoside L is extracted into the water and is thus removed from the blanched bitter melon. The absence of momordicoside K in both the blanching water and the blanched bitter melon can, however, not be explained from the observed data because there is no available literature on the effect of debittering treatments on the stability of the bitter compounds.

Only blanching of bitter melon at 98 ± 2 °C for 3 min resulted in the removal of mormodicoside L from methanolic extract of bitter melon. Blanching may therefore be the most appropriate way of reducing the bitterness of the bitter melon vegetable, hence making it more acceptable to more consumers. This treatment would allow more people to enjoy the health benefits of the bitter melon. All other treatments used in the study did not affect momordicosides K and L contents. It is not yet clear why bitter masking with MY 68 results in the absence of momordicosides K and L. More research would have to be done to give conclusive explanations.

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