




Research Article

The Effect of Thermal Processing on the Saponin Profiles of *Momordica charantia* L.

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Saponins from *Momordica charantia* L. are a class of triterpenoid glucoside molecules that contribute to the bitter flavour of the plant and possess pharmacological properties. However, little is known about how the bioactivity and bitter flavour of saponins are affected by thermal processing. We established saponin profiles in bitter gourd extracts using a UPLC-ESI-MS/MS method. Seven saponins including momordicoside F₁, momordicoside F₂, momordicoside I, momordicoside K, momordicoside L, 3 β ,7 β ,25-trihydroxycucurbita-5,23(E)-dien-19-al, and momordicine I were monitored for the effects of thermal processing on their stabilities. The results showed that both 3 β ,7 β ,25-trihydroxycucurbita-5,23(E)-dien-19-al and momordicoside L were extremely sensitive to heat treatment, particularly when they were heated at 100°C for more than 10 mins and under 121°C for 20 mins. Other saponins were reduced significantly by autoclaving, but they remained unchanged at lower temperatures. In conclusion, specific bitter gourd saponins are affected by thermal treatment, which may modify the bioactive components or bitter flavour of the bitter gourd extracts.

1. Introduction

Bitter gourd (*Momordica charantia* L.) is a member of the cucurbitaceae family. The most well-known functional properties of *M. charantia* L. are the hypoglycaemic effects and antidiabetic activities [1–5]. In Asian countries, bitter gourds are usually cooked by steaming, microwave, or boiling, while studies have shown these various preparation and cooking processes can affect the nutritional quality of the bitter gourd [6–8]. Shreds of evidence also shown that thermal treatment can alter the nutritional profiles by changing the stability and functionality of the ingredients in vegetables [6, 9]. However, pressure cooking and frying were reported to retain the maximum antioxidant potential of *M. charantia* L. fruit in total phenol content, total flavonoids,

tannin content, carotenoid content, and antioxidant activity measurements, when compared to microwave cooking [8, 9].

Interestingly, the major active components of the *Momordica charantia* L. extracts are cucurbitane-type triterpenes and related glycosides, also known as bitter gourd saponins [10–13]. Currently, more than 100 bitter gourd saponins have been isolated and identified from different parts of this plant, including the fruit, stems, or leaves. It is reported that the bitter gourd fruit is rich in momordicoside I, momordicoside F₂, and momordicoside F₁ [14]. The antidiabetic component, 3 β ,7 β ,25-trihydroxycucurbita-5,23(E)-dien-19-al [15], has been used as the functional ingredient for dietary supplements extracted from the bitter gourd [16]. However, only some bitter gourd saponins,

including momordicoside K, momordicoside L, momordicine I, and momordicine II are related to the bitter taste or bitterness of the bitter gourd [17]. The presence of these bitter taste-related saponins may limit the consumption of bitter gourds. Thus, potential food processing methods that could remove some of the bitter taste-related saponins while retaining the functional component could make the bitter gourd acceptable to consumers.

Although saponins are considered the iconic compounds of *M. charantia* L., little is known about the effects of cooking methods on changes in the saponins of the bitter gourd. Tan et al. discussed the effect of food processing, including temperature, time, and water-to-sample ratio, on the stability of saponins in bitter gourds. The results indicated that the total saponin content of the bitter gourd is affected by temperature [18]. Donya et al. specifically explored the effects of six treatments, including frying, blanching, sun drying, oven drying, and freeze-drying on the proximate compositions and contents of momordicoside K and momordicoside L. It was reported that momordicosides K and momordicosides L were not detected by HPLC-ESI/MS in the blanched samples [19]. In addition, momordicoside L, but not momordicoside K, was retained in the hot water ($98^{\circ}\text{C} \pm 2$, 3 min).

Due to the complexity of the saponin structure in *M. charantia* L. fruit, the detection of individual cucurbitane-type triterpene glycoside in *M. charantia* fruit is challenging. Recently, Hu et al. developed a method for the quantitative determination of cucurbitane-type triterpenes and triterpene glycosides in bitter gourd juices using UHPLC [20]. Apart from momordicoside K and momordicoside L, there is no literature available regarding the thermal stability of the bitter saponins in *M. charantia* L. Thus, the objective of this study was to establish a UPLC-ESI-MS/MS method to simultaneously determine seven bitter gourd saponins and to evaluate the profile changes of these saponins in *M. charantia* L. var. Hualien No. 3 before and after thermal treatment.

2. Materials and Methods

2.1. Materials and Reagents. Hualien No. 3 wild bitter gourd was bred through the Hualien District Agricultural Improvement Station, Taiwan. The whole wild bitter gourd fruits purchased from a local farm were dried at 45°C and ground into a fine powder. Bitter gourd powder was vacuum packaged and stored at 4°C in the dark before use. Methanol (LC-MS grade) was purchased from J. T. Baker (Mallinckrodt Baker, Phillipsburg, NJ, USA). Methanol (HPLC grade) was purchased from Macron Fine Chemicals (Center Valley, PA, USA). Formic acid, analytical grade 99.7% purity, was purchased from Riedel-de Haen (Seelze, Germany). Saponin standards, including momordicoside L, $3\beta,7\beta,25$ -trihydroxycucurbita-5,23(E)-dien-19-al, momordicoside K, momordicine I, momordicoside I, momordicoside F₂, and momordicoside F₁, were purchased from Starsci Biotech Co., Ltd.

(Taipei, Taiwan) with purities of 94.3%, 95.6%, 92.3%, 95.2%, 98.1%, 95.3%, and 92.2%, respectively.

2.2. Sample Preparation. Bitter gourd powders (0.5 g) were sonicated in 40 mL of 100% methanol for 30 min, followed by centrifugation for 15 min at 4000 rpm. The supernatant was transferred to a flask. This procedure was repeated five more times. The combined supernatants were evaporated in a Rotavapor (BÜCHI Labortechnik AG, Flawil, Switzerland) until dry.

2.3. Standard Preparation. Stock solutions of each standard saponin (1.0 mg/mL) were prepared in methanol and stored at -30°C . Different calibration levels were prepared by diluting the stock solutions with methanol. Seven stock solutions were mixed with methanol to prepare a working solution (1 $\mu\text{g}/\text{mL}$ each). The working solution was further diluted to six concentration levels for the calibration curve within the range of 20–200 $\mu\text{g}/\text{mL}$.

2.4. Thermal Stability Test. Methanol extracts of bitter gourds were dissolved in 40 mL of water and were used as a background control. The aqueous solution (5 mL) was placed in a 50 mL test tube and boiled in a water bath at various temperatures, including 30°C , 60°C , and 100°C for periods of 5, 10, and 20 mins, respectively. Samples were also autoclaved at 121°C for 20 minutes. After heating, samples were immediately placed in an ice box to cool down.

2.5. UPLC-ESI-MS/MS Analysis. Methanol extracts of the bitter gourds and the aqueous solution were purified by reversed-phase C-18 solid-phase-extraction cartridges (Agela, China) using 10–100% methanol as the elution buffer. The total eluted solvent was evaporated until dry. The residues were reconstituted to 1 mL with methanol and filtered using a 0.22 μm nylon membrane filter before UPLC-ESI-MS/MS analysis. UPLC-ESI-MS/MS analysis was performed using a Waters ACQUITY ultra performance LC system (Waters, Milford MA, USA) equipped with a TQS triple quadrupole MS/MS system (Waters, Milford, MA, USA). The reversed-phase Waters BEH RP-18 column (2.1 mm \times 100 mm i.d., particle size 1.7 μm) (Waters, USA) was maintained at 35°C with the flow rate of 0.3 mL/min. The mobile phase consisted of (A) water and (B) methanol, both containing 0.01% formic acid. Gradient elution was performed as follows: 0–2 min, 20% B; 2–6 min, 70% B; 6–15 min, 70% B; 15–19 min, 72% B; 19–22 min, 72% B; 22–25 min, 80% B; 25–28 min, 80%. Two microliters of the sample were injected by an autosampler. The ESI parameters were set as follows: capillary voltage was 3.0 kV, cone voltage was 39 V, desolvation temperature was 200°C , desolvation gas flow was 800 (L/hr), and cone gas flow was 150 (L/hr). Data were acquired and processed using the Mass Lynx 4.1 software (Waters, Milford, MA, USA).

2.6. Statistical Analysis. The data were presented as the mean \pm standard deviation (SD) from three independent experiments. The data were analysed using one-way ANOVA followed by Tukey's honest significant difference (HSD) test. Statistical significance is determined by $P < 0.05$.

3. Results

3.1. Identification and Quantification of Cucurbitane Triterpenoids. We first established the platform to analyse the specific saponins from the wild bitter gourd and investigated the effect of thermal processing on these functional or bitter taste-related compounds (Figure 1).

Individual saponins were detected using tandem mass spectrometry with the multiple reaction monitoring (MRM) mode. For each compound, the strongest MRM transition was selected as the quantification ion and the other transition was used for qualification. To distinguish compounds with the same MRM transitions, such as $3\beta,7\beta,25$ -trihydroxycucurbita-5,23(E)-dien-19-al and momordicine I, m/z 495 ($[M + Na]^+$) $> m/z$ 477 ($[M + Na - H_2O]^+$), or momordicoside I and momordicoside F_2 , m/z 641 ($[M + Na]^+$) $> m/z$ 337 ($[M + Na - \text{hexose-side chain}]^+$), the retention time of the individual compound was determined according to the corresponding standard (Table 1). These identification profiles of individual saponins helped quantify the bitter gourd saponins.

3.2. The Specific Saponin Content of the Bitter Gourd. Calibration curves were established by independently diluting individual stock standard solutions. The concentrations (20–200 ng/mL) of the target saponins were tested. The results showed that linearity was obtained for all analytes throughout the concentration range, and the coefficient of correlation (r) was greater than 0.9970 (Table 2). The specific triterpenoid contents in the methanol extract of bitter gourd were analysed and calculated based on the standard curves. The results revealed that the bitter gourd extracts contained high amounts of momordicoside L ($420.94 \pm 18.17 \mu\text{g/g}$) and momordicine I ($458.78 \pm 35.08 \mu\text{g/g}$) compared to other analysed saponins (Table 3). The bitter gourd extracts also contained a high amount of momordicoside F_2 ($218.28 \pm 26.43 \mu\text{g/g}$), momordicoside I ($200.45 \pm 26.71 \mu\text{g/g}$), and $3\beta,7\beta,25$ -trihydroxycucurbita-5,23(E)-dien-19-al ($190.00 \pm 9.63 \mu\text{g/g}$) (Table 3). Momordicoside K ($5.23 \pm 0.79 \mu\text{g/g}$) and momordicoside F_1 ($1.91 \pm 0.24 \mu\text{g/g}$) were minor components in our extract (Table 3).

3.3. The Effect of Thermal Processing on the Saponin Content. To study the effect of thermal processing on the stability of the bitter gourd saponins, we treated the bitter gourd extracts with an extreme autoclaving condition at 121°C for 20 mins as a control. The results showed that this dramatically reduced the levels of all saponins (Figure 2). We also heated the bitter gourd extracts at various temperatures, including 30°C , 60°C , and 100°C , and

collected the samples at 5 min intervals for saponin analysis. It was surprising that the levels of momordicoside L, momordicoside K, momordicoside I, and momordicoside F_1 remained stable during the 20 mins of treatment at different temperatures (Figures 2(a)–2(d)). However, heating at 100°C might change the levels of momordicoside F_2 (Figures 2(e)) compared to those at lower temperatures. The bioactive component, $3\beta,7\beta,25$ -trihydroxycucurbita-5,23(E)-dien-19-al and the bitter taste compound, momordicine I, were less affected after 30°C and 60°C treatment for 20 mins. However, the levels of $3\beta,7\beta,25$ -trihydroxycucurbita-5,23(E)-dien-19-al were significantly reduced after 5 mins at 100°C and almost eliminated after 20 mins of high-temperature treatment (Figures 2(f)). Momordicine I was the most sensitive to high temperature. It was barely detected after 10 mins at 100°C (Figures 2(g)).

4. Discussion

In this study, we identified that specific triterpenoids, including the bioactive component, $3\beta,7\beta,25$ -trihydroxycucurbita-5,23(E)-dien-19-al, and the bitter taste compound, momordicine I, in the methanol extracts of bitter gourd were extremely sensitive to high temperature (100°C) treatment. Others, including momordicoside L, momordicoside K, momordicoside I, and momordicoside F_1 , were relatively stable under the same conditions. We also observed that autoclaving had profound effects on the levels of all 7 saponins studied. Indeed, Tan et al. reported that thermal treatment exceeding 40°C would decrease total saponin content in the bitter gourd [18], but no specific components were identified. Interestingly, Donya et al. could not detect momordicoside K and momordicoside L from the blanched bitter gourds. It was speculated that momordicosides might increase the loss in the blanching water or be degraded at the high blanching temperatures [19]. Here, we demonstrated that momordicoside K and momordicoside L are less affected by heating at 100°C for 20 mins, which was more stringent than the blanching in other experimental conditions.

It is known that the bitter gourd fruit is rich in bioactive and bitter flavour-related saponins [15, 16]. The profiles of saponins may vary in different breeds of bitter gourds. In this study, we determined the levels of specific saponins in a hybrid bitter gourd, Hualien No. 3, in Taiwan. Interestingly, we found that momordicoside I and momordicoside L were abundant in our sample. Wang et al. indicated that the amount of momordicoside L was the highest in the Fujian bitter gourd, while kuguaglycoside C and goyaglycoside D were higher in the Guangdong bitter gourd [2]. However, others showed that momordicoside L and momordicoside F_2 were the major components, compared to other saponins, in the bitter gourd extracts [21]. Thus, the content of these saponins in the bitter gourd samples may be related to environmental regions, species [2], or the extraction methods. Notably, $3\beta,7\beta,25$ -trihydroxycucurbita-5,23(E)-dien-19-al, has been used as the functional

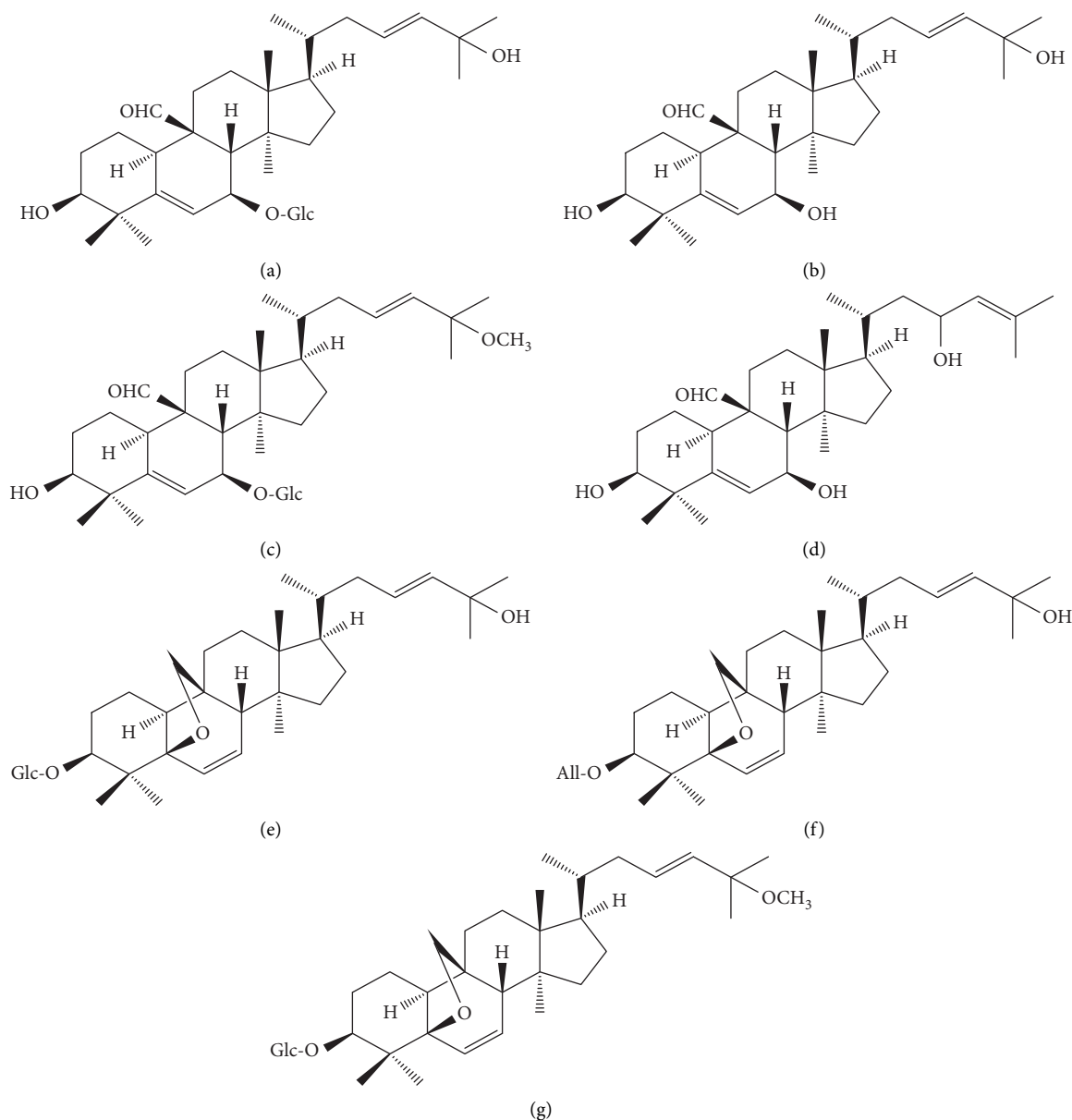


FIGURE 1: Structures of specific bitter melon saponins. (a) Momordicoside L, (b) 3 β ,7 β ,25-trihydroxycucurbita-5,23(E)-dien-19-al, (c) momordicoside K, (d) momordicine I, (e) momordicoside I, (f) momordicoside F₂, and (g) momordicoside F₁. Glc denotes a glucose molecule. All denotes allose. Structures were drawn by MDL ISIS/Draw 2.5.

TABLE 1: Retention times and fragment ions of bitter melon saponins.

Bitter melon saponins	Retention time (min)	MW	Parent ion (m/z) > daughter ion (m/z)	
			Quality	Quantity
Momordicoside L	9.52	634	657 > 203	657 > 477
3 β ,7 β ,25-trihydroxycucurbita-5,23(E)-dien-19-al	11.51	472	495 > 477	495 > 495
Momordicoside K	14.55	648	671 > 203	671 > 491
Momordicine I	15.24	472	495 > 477	495 > 495
Momordicoside I	15.46	618	641 > 337	641 > 203
Momordicoside F ₂	15.80	618	641 > 337	641 > 203
Momordicoside F ₁	24.03	632	655 > 337	655 > 625

TABLE 2: Calibration curve for specific bitter gourd saponins by UPLC-ESI/MS/MS.

Bitter gourd saponins	Linear range (ng/mL)	Calibration curve	<i>r</i>
Momordicoside L	20–200	$y = 397.55x - 1373.1$	0.9995
3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al	20–200	$y = 8175.7x - 160.56$	0.9997
Momordicoside K	20–200	$y = 236.32x - 700.75$	0.9991
Momordicine I	20–200	$y = 1997x - 5541$	0.9988
Momordicoside I	20–200	$y = 27.691x - 149.9$	0.9993
Momordicoside F ₂	20–200	$y = 29.61x - 133$	0.9970
Momordicoside F ₁	20–200	$y = 122.42x - 272.49$	0.9994

TABLE 3: Contents of specific bitter gourd saponins in bitter gourd extracts.

Bitter gourd saponins	Bitter gourd methanol extracts (μg/g)
Momordicoside L	420.94 ± 18.17
3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al	190.00 ± 9.63
Momordicoside K	5.23 ± 0.79
Momordicine I	458.78 ± 35.08
Momordicoside I	200.45 ± 26.71
Momordicoside F ₂	218.28 ± 26.43
Momordicoside F ₁	1.91 ± 0.24

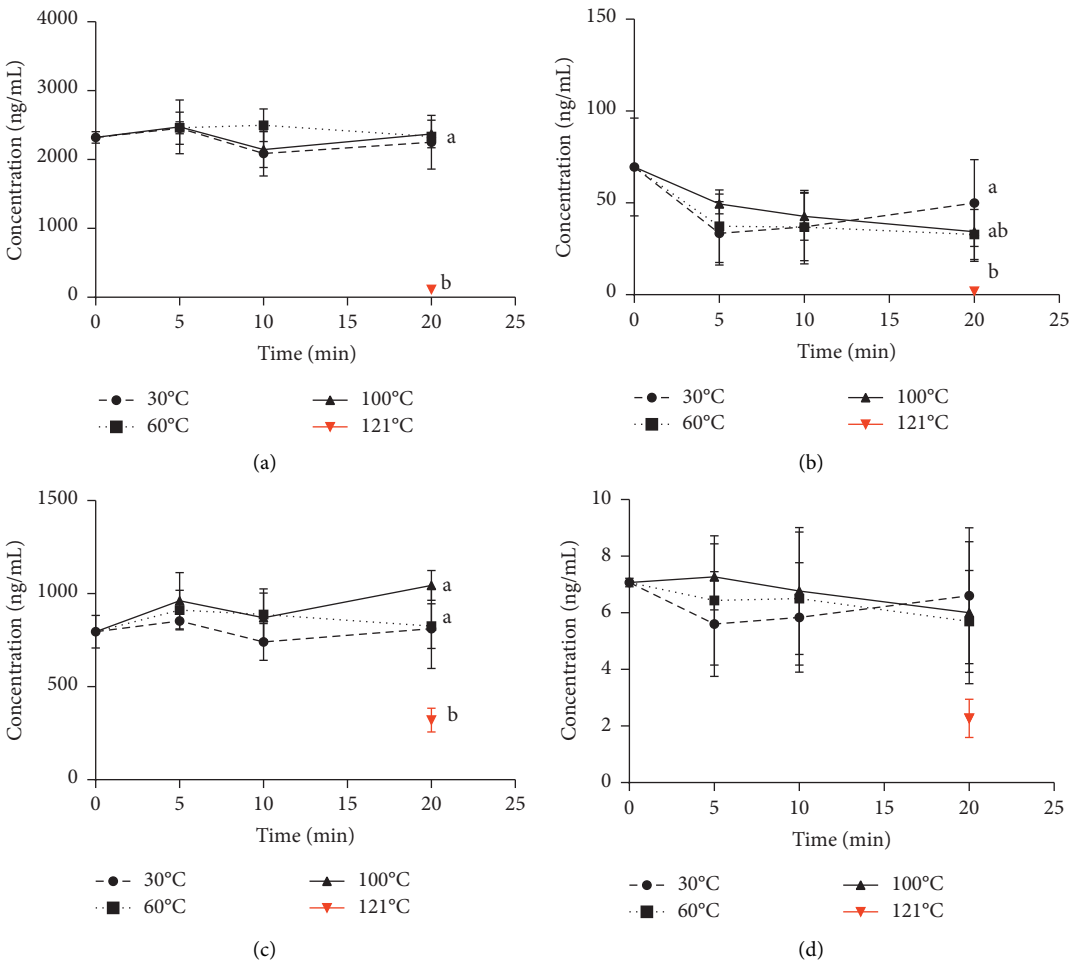


FIGURE 2: Continued.

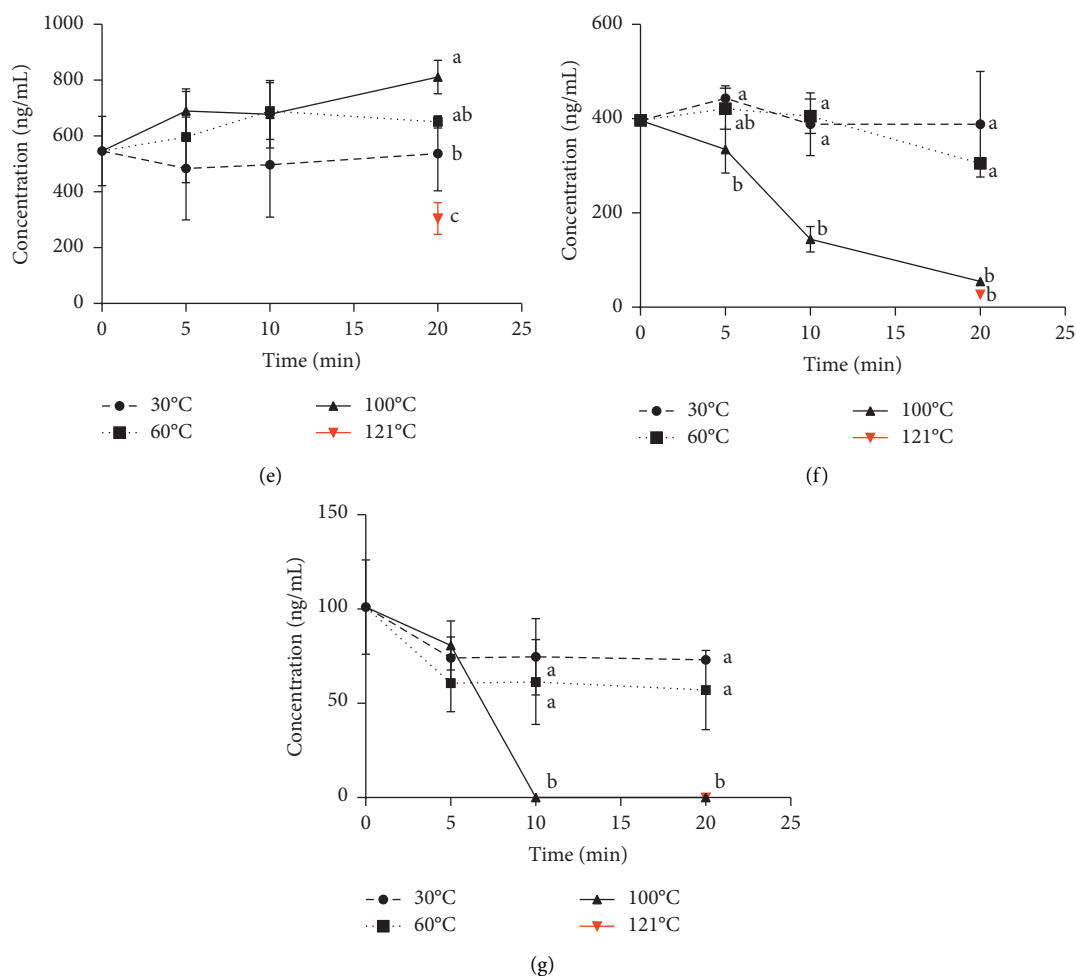


FIGURE 2: The effect of thermal processing on the contents of bitter gourd saponins. Bitter gourd extracts were treated at 30°C (filled circle), 60°C (filled square), 100°C (filled triangle), or 121°C (inverted triangle). (a) momordicoside L, (b) momordicoside K, (c) momordicoside I, (d) momordicoside F₁, (e) momordicoside F₂, (f) 3, 7, 25-trihydroxycucurbita-5,23(E)-dien-19-al, and (g) momordicine I were analysed by UPLC-ESI-MS/MS. Different letters represent significant differences. The error bars represent the standard deviation ($n=3$).

ingredient for dietary supplement extracted from the bitter gourd [16]. Our study revealed that 3 β ,7 β ,25-trihydroxycucurbita-5,23(E)-dien-19-al is unstable after heating at high temperatures or autoclaving. Thus, this compound could be a good indicator of functional ingredients and a quality control for bitter gourd saponins in the future.

5. Conclusions

In conclusion, our results demonstrate that cooking at high temperatures may partially eliminate the bitter taste of saponins of bitter gourds. However, it could also result in the reduction of the bioactive components in the bitter gourd. These could provide insights to modify methods for bitter gourd processing in the future.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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