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# Chemical composition and antioxidant properties of ivy gourd (*Coccinia grandis*) wines prepared with different pretreatment techniques

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***Abstract:***

**This study proposes the winemaking of ivy gourd and investigates the influence of microwave, thermovinification, and enzymatic pretreatments on the total flavonoid content (TFC), total phenolic content (TPC), and antioxidant capacity of the wines. The results showed that the pretreatments had no negative effects on the chemical composition of the products. Instead, there were positive changes in antioxidant content and activities when different processing techniques were employed. The wines pre-treated with enzymatic treatment contained much higher TPC (up to 39.37 µg GAE/ml), TFC (18.61 µg CE/ml), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (28.98 µg AAE/ml) than the untreated wines. Microwave and thermovinification also exhibited positive effects but to lesser extents. A fermentation process helped increase the TPC and antioxidant capacity of the wine samples while reducing their TFC values as compared to those of the musts.**

***Keywords:*** **antioxidant, chemical composition, fermentation, flavonoid content, ivy gourd fruits, phenolic content.**

***Classification numbers:*** **2.2, 3.5**

## Introduction

Reported to contain numerous beneficial health compounds, ivy gourd (*Coccinia grandis*) can potentially be utilised in the food processing industry. The plant has been known to provide a variety of alkaloids, flavonoids, tannins, steroids, glycosides, saponins, ellagic acid, and triterpenoids [1]. Along with phytonutrients, the fruit also contains a certain amount of pectin that is considered as important as soluble fiber, which control lipid concentrations in human body. Due to its prominent nutritional content and properties, ivy gourd fruit has been used and studied as medicine for the treatment of diabetes [2, 3]. Though beneficial for human health and readily available as a raw ingredient, the fruit has not been made use of and thus remains unfamiliar to most consumers. In order to promote and enhance its usage, this research aims to study the wine fermentation of ivy gourd fruit and assess its antioxidant content and capacity.

One of the most concerning matters in fruit wine processing is nutritional quality. Some studies have shown that the nutritional benefits of wines not only depend on types of fruits but are also strongly affected by winemaking techniques [4]. Some winemaking techniques have been reported to increase the phenolic concentration such as high-temperature fermentation, thermovinification, must freezing, microwave treatments, enzymatic treatments, and extended maceration. Thermovinification can be used for red wines to increase the contents of some phenolics and volatile compounds during fermentation. For wines that are to be consumed young, and when the colour intensity is a problem, thermovinification may help to enhance the colour [5]. Microwave treatment is a novel red wine pre-treatment process that has shown potential results in improving phenolic extraction of the finished wines [6]. Microwave has been demonstrated to be efficient in terms of both rate and effectiveness of extraction for a range of plant compounds. By conducting the pre-fermentation maceration with microwave radiation, the heated water within the cells of grapes are transformed into vapours, which increases the pressure inside and hence the content of intracellular constituents were released. The use of commercial enzymes as a pre-treatment in winemaking is also a common and well-known practice. Pectinases obtained from *Aspergillus* sp. are commonly used in red winemaking to enhance the extraction of free-run juice

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during maceration, to aid in clarification and filtration, and to facilitate the processes [7]. Some reports on the effect of pectinases demonstrated an increase in extraction of phenolic compounds, especially anthocyanins that are the red pigments of grapes, and thus wine colour intensity is enhanced. These enzyme preparations degrade the structural polysaccharides of grape skin cell walls, which facilitates the release of phenolic compounds.

Therefore, the purpose of this research was to conduct wine fermentation of ivy gourd fruit to promote its usage. Effects of pretreatments including thermovinification, microwave, and enzymes on the nutritional values and health benefits of the fruit wine were also studied to compare among these methods and find out the most suitable treatment technique for the production of ivy gourd fruit wine.

## Materials and methods

### *Materials and chemicals*

Ripe ivy gourd fruits (*Coccinia grandis*) with bright red colour were harvested from Tay Ninh province, Vietnam. They were transported to the laboratory within a day for processing. Commercial yeast *Saccharomyces cerevisiae* RV100 was purchased from Angel Yeast Co., Ltd, China. The enzyme used was Pectinex Ultra SP-L supplied by Novozyme, Denmark. Chemicals required were of analytical grade including diammonium phosphate ($(NH_4)_2HPO_4$), potassium metabisulfite ($K_2S_2O_5$), sodium hydroxide, Folin-Ciocalteau's reagent, anhydrous sodium carbonate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, ethanol, aluminium chloride, and potassium acetate obtained from various suppliers. Standards including gallic acid, catechin, and L-ascorbic acid were purchased from Sigma Aldrich, Singapore.

### *Sample preparation*

After being collected, ivy gourd fruits were washed and crushed by a mechanical grinder (Philips HR2118, Japan). The mixture was then mixed with water at 1:1 (w/w) ratio. The final fruit musts were stored frozen at -20$^o$C until further processing. At this temperature, deterioration reactions as well as enzymatic activities of fruit was reported to be negligible, and thus, the fruit's nutritional values were hardly altered [8].

### *Thermovinification*

Thermovinification process was performed according to M. Atanacković, et al. (2012) [5] with minor modifications. The ivy gourd fruit musts (200 ml per sample) were heated to 85$^o$C in a water bath (WiseCircu, Germany) for 2 min then cooled down to 40$^o$C in an ice bath. After that, the musts were held at 40$^o$C for 12 h in a sterilised incubator (Memmert, Denmark) before being cooled down to 27$^o$C for fermentation.

### *Microwave treatment*

Microwave treatment was done following the method of A.L. Carew, et al. (2014) [6]. Each 200 ml of ivy gourd fruit musts was microwave-macerated using an 800 W microwave oven (Sharp R-209VN, China) at the maximum power level for three periods of time including 2 min, 1 min, and 15-40 s, respectively (final heating time was varied according to temperature reading). The stop time between two treatment periods was 5 min to stir and evaluate the temperature using a thermometer. The temperature for all treated samples would be in the range of 70-71$^o$C and held there for 10 min. After that, the musts were cooled down in an ice bath until reaching 27$^o$C for fermentation.

### *Enzymatic treatment*

Enzymatic treatment was prepared according to M.A. Zaker, et al. (2014) [7]. The commercial enzyme Pectinex Ultra SP-L was added to 200 ml of the musts at a concentration of 0.03% (w/w) before the incubation for 90 min at 40$^o$C in a shaking water bath (WiseBath, Germany). Enzyme was deactivated at 90$^o$C for 5 min. After maceration, the musts were cooled down in ice bath to 27$^o$C for fermentation.

### *Wine fermentation*

The wine fermentation process was performed according to the method of [9] with modifications. The ivy gourd fruit musts (200 ml each) were adjusted to pH 4.0 by adding acetic acid 10% and adjusted to 22$^o$Brix by adding sucrose. The musts were also added with 1 g/l diammonium phosphate for enhancement of yeast growth as well as with 0.1 g/l sodium metabisulfite as preservation additive. The fermentation was then proceeded with pre-activated *Saccharomyces cerevisiae* at 8% (v/v), which was approximately $8x10^6$ cells/ml, with the expectation for the final products to reach 10% (v/v) in alcohol content. Fermentation was performed anaerobically in a closed bottle at 25$^o$C for 10 days in a dark incubator (Memmert, Denmark). The cap of the bottle was slightly loosened every day for the first 3 days to release gases. The ivy gourd fruit wines produced were filtered by using cheese cotton cloth and then centrifuged (Hettich Zentrifugen Universal 320 R, Germany) at 2500g and 4$^o$C for 20 min and stored at -20°C for analyses within one month. The storage at freezing conditions minimises the nutritional changes because deterioration reactions as well as enzymatic activities of the fruit are reported to be negligible at this temperature [8]. All experiments were carried out in triplicate.

***Analytical methods***

Total soluble solid (TSS) content was determined by measuring the Brix value using a refractometer. The pH was measured using a pH meter. Ethanol content was measured by using the distillation method. Briefly, 20 ml of wine samples were put in a rotary evaporator (Steroglass 300, Italy) for 15 min at 40$^o$C. The obtained distillates were made up to 20 ml with distilled water and then measured using an alcohol refractometer.

Titratable acidity (TA) was measured using the titration method, where 5 ml of ivy gourd fruit wine was mixed 45 ml of distilled water before the titration with 0.1 N NaOH until a pH of 8.2 (endpoint) was reached. TA was expressed as g malic acid equivalent (MAE) per litre.

The TPC was determined by the Folin-Ciocalteu method and expressed as micrograms of gallic acid equivalents per ml (µg GAE/ml) [9]. The TFC was determined using the colorimetric method described by L. Wang, et al. (2015) [10] and expressed as micrograms of catechin equivalents per ml (µg CE/ml). The antioxidant capacity was evaluated through 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay modified from the method of [10] and expressed as micrograms of L-ascorbic acid equivalent per ml (µg AAE/ml).

***Data analysis***

All measurements were done in triplicate and results were expressed as the average value ± standard deviation. Statistical analyses were determined using one-way ANOVA by the SPSS software version 22.0 with a 95% confident interval. The differences among samples were assessed by Post-hoc's multiple test. Sample letters in value indicated no significant differences.

## Results and discussion

***Physicochemical properties of the ivy gourd fruit musts and wines***

Table 1 shows that three studied pretreatment methods did not significantly influence the TSS content (mainly sugar) of the musts ($p>0.05$). After fermentation, the initially adjusted TSS values of approximate 22$^o$Brix for all types of treatments decreased to 6.14-7.17$^o$Brix. This was due to the conversion of sugar into ethanol by yeast activity. The ethanol contents of all wine samples after fermentation were not significantly different ($p>0.05$), which varied from 10.87 to 11.52 (% v/v). The insignificant variation in ethanol concentration for all ivy gourd wines was due to the same fermentation condition of temperature and pH among the tested samples. These two parameters were reported to govern the yeast growth and fermentation capacity and thus no differences in these parameters led to no significant changes in ethanol content [11].

The pH value and TA content of the wines noticeably changed in comparison with those of the musts (Table 1). Particularly, the pH values of the wines decreased significantly with corresponding increases in TA content. These values are approximate to those reported by J. Foong, et al. (2012) [12]. The reduction in pH was caused by the alternation of buffer capacity in wine, adsorption of bases, and excretion of organic acids during fermentation [13]. Consequently, TA values increased with the releases of more organic acids into the fermented samples. Meanwhile, the wines with pretreatments had slightly lower pH values and higher TA contents than the control wine. These results were consistent with their slightly higher ethanol contents observed, which imply that pretreatments may slightly increase wine fermentability. Beside the main substrates, i.e., sugar, yeasts also need minor nutrients for their growth and fermentation such as certain vitamins (e.g., thiamine and pantothenic acid) [13] or nitrogen-carrying compounds (e.g., amino acids) [14]. Therefore, although pretreatments did exhibit the additional extraction of soluble solids, they might induce the release of certain minor nutrients that promote the fermentation of yeasts.

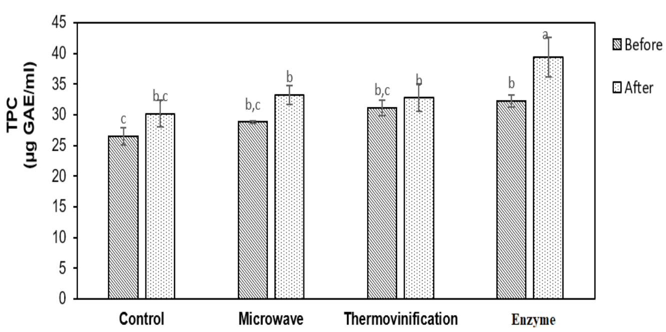
**Table 1. Physicochemical properties of ivy gourd fruit musts and wines with different pretreatments.**

| Pretreatment | Fermentation | TSS ($^o$Brix) | Ethanol content (% v/v) | pH | TA (g MAE/ 100 ml) |
|---|---|---|---|---|---|
| Control | Before | 1.66±0.01$^c$ | - | 4.51±0.08$^a$ | 0.23±0.01$^c$ |
| | After | 6.73±0.12$^{ab}$ | 10.87±0.66$^a$ | 3.96±0.03$^b$ | 0.49±0.02$^b$ |
| Microwave | Before | 1.70±0.02$^c$ | - | 4.38±0.02$^a$ | 0.23±0.01$^c$ |
| | After | 6.14±0.33$^b$ | 11.37±0.83$^a$ | 3.88±0.01$^{bc}$ | 0.53±0.01$^{ab}$ |
| Thermo-vinification | Before | 1.69±0.03$^c$ | - | 4.53±0.13$^a$ | 0.23±0.01$^c$ |
| | After | 6.81±0.62$^{ab}$ | 11.17±0.62$^a$ | 3.80±0.05$^{bc}$ | 0.55±0.02$^a$ |
| Enzyme | Before | 1.69±0.02$^c$ | - | 4.48±0.09$^a$ | 0.24±0.02$^c$ |
| | After | 7.17±0.10$^a$ | 11.52±0.68$^a$ | 3.71±0.03$^c$ | 0.54±0.02$^a$ |

Values are expressed as mean ± SD (n=3).
Same letters in the same row express that values are not significantly different ($p>0.05$).

***Antioxidant contents and capacity of the ivy gourd fruit musts and wines***

*TPC:* it is shown in Fig. 1 that the TPC of the control must was less affected by microwave and thermovinification methods while the enzymatic treatment displayed a major enhancement of the compounds ($p<0.05$). This trend is similar to the results obtained in a report for black currant juice [15]. The reason is that the pectinase family in the Pectinex Ultra SP-L could damage the skin cell membranes releasing phenolic substances into the musts. The wine samples after fermentation had TPC ranging from 30.15 to 39.37 µg GAE/ml. There was an increase in TPC after fermentation ($p<0.05$) for the enzyme-treated sample while there were no significant differences for others ($p>0.05$). One of reasons of the observed increase could be due to their greater solubility in ethanol (in wine) than in purely aqueous solutions (in juice) [16]. As a result, the phenolic compounds in fruit mash were continuously extracted into wine during fermentation. Another reason may be attributed to the fact that less oxidation of polyphenols occurred during the fermentation process [17]. Phenolic compounds can act as antioxidants and also can easily be oxidized leading to its loss. As reported by J.M. Salmon (2006) [18], the yeast *Saccharomyces cerevisiae* used in the alcoholic fermentation requires oxygen for lipid synthesis and can consume much oxygen with no detrimental effect on the fermentation process. As yeasts have much higher affinities for oxygen than phenolic compounds, their further extracted amount during fermentation could be protected from oxidation leading to their high content in wine.



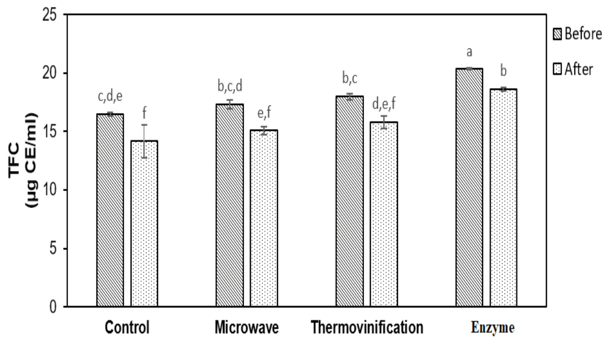

Values are expressed as mean ± SD (n=3).
Same letters in the same row express that values are not significantly different ($p>0.05$).

**Fig. 1. TPC of ivy gourd fruit musts before and after wine fermentation.**

Amongst all wine samples, the highest value of TPC was found in the wines with enzymatic pretreatment. Although the values of the wine pretreated with microwave and thermovinification were not statistically different ($p>0.05$) from that of the control wine, there existed obvious differences between them and the value of the control musts (while the control wine was not), which indicates the efficiency of these pretreatment techniques in winemaking. During the thermovinification process, heat could damage the hypodermal cell membranes and denature polyphenol oxidases [16]. The first effect facilitated the release of phenolic compounds and the second prevented their loss, especially during the long duration of fermentation. The positive effect of thermovinification in winemaking was also reported by M. Atanacković, et al. (2012) [5] for grape wines. On the other hand, microwaves are considered similar to thermal treatment but with shortened duration. Enhanced extraction of phenolic compounds during fermentation by pretreating the must with microwaves was also reported by other authors [6] for grape wines. The application of enzymatic treatment in juice, however, still produced more phenolic compounds in wine than microwave treatment and thermovinification techniques in this study ($p<0.05$). Various studies have pointed out that pectolytic enzymes have been used to slow fermentation and increase phenolic extraction [19] because they could break down skin cell walls for substance release [16].

*TFC:* similar to the results for TPC, enzymatic treatment enhanced the TFC of ivy gourd musts the most while microwave and thermovinification methods only showed a minor but statistically insignificant ($p>0.05$) (Fig. 2) increase. After fermentation, the TFC of the wine samples decreased substantially in all four samples. Flavonoids are certain polyphenolic compounds known for their high antioxidant properties and free radical scavenging ability [20]. It has been reported that during fermentation, polyphenolic compounds of wine change significantly due to the combinative effects of their adsorption on solids, proteins, or even yeasts; their extraction from the mash into the wine; their polymerisation, degradation or oxidation; and their release from bound polyphenolic complexes with other substances induced by micro-organisms [21].

Depending on which reactions dominate, an increasing or decreasing trend could be observed. Among three studied pretreatments, enzymatic treatment again exhibited the best efficiency.

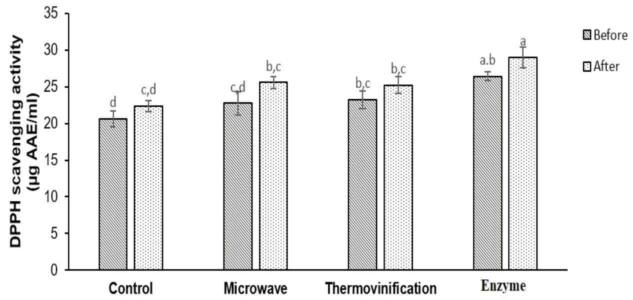



Values are expressed as mean ± SD (n=3).
Same letters in the same row express that values are not significantly different (p>0.05).

**Fig. 2. TFC of ivy gourd fruit musts before and after wine fermentation.**

### *Antioxidant capacity by the DPPH assay*

As shown in Fig. 3, the differences in antioxidant capacity of the thermovinification-treated and enzymatically-treated musts were significant compared to that of the control. In addition, the antioxidant capacity of the wines after fermentation was slightly higher than the initial musts, however the deviations were not large enough to produce a statistically significant difference (p>0.05). Further interpretation reveals that although there were no significant differences in DPPH scavenging activity among the wines pre-treated with microwave and thermovinification compared to the control wine (p>0.05), the former did create a significance with the control must while the control wine did not. This again verifies the possibly positive effects of microwave and thermovinification in winemaking. In fact, the trend of DPPH scavenging activity followed that of TPC, which confirms that the antioxidant activity of wine depended mainly on phenolic compounds. Some previous studies also demonstrated a positive correlation between the TPC and the antioxidant activities of wines [22]. On the other hand, the content of total flavonoids, another group of antioxidants did not exhibit the correlation with DPPH scavenging activity in this study. Similar negative correlation between total flavonoids and DPPH scavenging activity were also reported by A. Meda, et al. (2005) [23].




Values are expressed as mean ± SD (n=3).
Same letters in the same row express that values are not significantly different (p>0.05).

**Fig. 3. Total antioxidant capacity of ivy gourd fruit musts before and after wine fermentation.**

## Conclusions

The total flavonoid, total phenolic, and antioxidant contents contribute to the health benefits of wines. The development of procedures and techniques that contribute to these characteristics is important for improvement of their quality. In this study, the physicochemical and antioxidant properties of the ivy gourd fruit wines under three different pretreatment winemaking techniques including microwave, thermovinification, and enzymatic treatment were evaluated. Results showed that fermentation of ivy gourd fruit musts increased the content of certain antioxidants and the antioxidant capacity in wines. Enzymatic treatment was shown to be the best pretreatment amongst the three applied methods. With these primary data, further investigation is needed to determine the optimal conditions of each technique.

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## COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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