Research Article

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Processing of alcohol pomelo beverage (*Citrus grandis* (L.) Osbeck) using *saccharomyces* yeast: Optimization, physicochemical quality, and sensory characteristics

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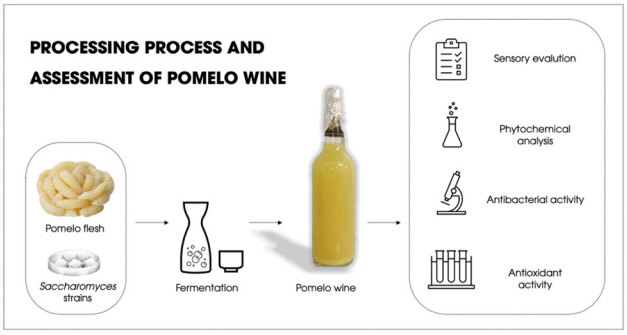
Abstract: In the harvest season, besides good quality pomelos, many unqualified pomelos exist for commercial purposes. However, these products still have much potential to be exploited to optimize profits for producers. Therefore, this study was conducted with the aim of developing a alcohol pomelo beverage (APB) process from pomelo to create added value for this fruit. In this study, pomelo fruit, a tropical fruit with high nutritional values, was used as the primary substrate for a process of alcoholic fermentation using *Saccharomyces* strains. The indicators included yeast with a density of 10^3, 10^5, and 10^7 CFU/mL, initial total soluble solid (TSS) was 19, 23, and 27, and the fermentation time was 0, 6, 8, 10, 12, and 14 days. The result shows that the optimal fermentation process could be carried out at the initial TSS of 27%, yeast density of 10^3 CFU/mL, and fermentation time of 12 days to attain the final product with the alcohol content of 10.35% (v/v). In addition, the final product was found to show the presence of phytochemicals such as phenols, tannins, flavonoids, alkaloids, coumarins, quinones, saponins, terpenoids, and steroids. The total polyphenol content in beverage was 271.3 mg GAE/mL, highly correlated to its antibacterial capacity. Besides, the antioxidant capacity of APB was also recorded through the DPPH free radical scavenging ability of 11,599 µg/mL and H_2O_2 of 14.33 µg/mL, respectively. Sensory evaluation results recorded positive feedback on odor (4.2/5) and acceptability (4/5). In summary, APB products have nutritional value and organoleptic characteristics that are suitable for the consideration of large-scale production expansion in the future.



Graphical abstract

Keywords: alcohol pomelo beverage, pomelo (*Citrus grandis* (L.) Osbeck), fermentation, antioxidant, quality

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1 Introduction

Pomelo (*Citrus grandis* (L.) Osbeck.) is a fruit of the family Rutaceae. It is the first citrus fruit employed for production, consumption, and international trade [1]. This citrus fruit is considered to be more prominent than the others due to its unique sweetness and sour taste [2]. In Vietnam, this fruit has been widely grown in alluvial soils such as the Red River Delta and the Mekong River Delta. There are

different varieties of pomelos in Vietnam, including Da Xanh, Doan Hung, Dien, and Nam Roi. Among them, Da Xanh and Nam Roi pomelos are mostly consumed due to their juiciness, sweetness, and delicious taste. In general, pomelos are pear-shaped or spherical, and the peel is bright with a medium thickness. The pulp is easy to peel, and more juicy, with no or few seeds.

Pomelo has been shown to be a rich source of phytochemical compounds such as flavonoids, carotenoids, limonoids, organic acids, pectin, and folate [3]. Among them, narirutin, naringin, hesperidin, neohesperidin, didymin, and poncirin are the main flavonoids that have been identified in pomelo [4,5]. Among them, naringenin-7-*O*-neohesperidoside (a flavonoid glucoside) is thought to contribute significantly to the bitterness of pomelo fruit. In addition, pomelo contains a large amount of citric acid and ascorbic acid, which play an important role in the antioxidant capacity of pomelo [4,6]. Optimal intake of antioxidants is positively correlated with health benefits such as the prevention of certain cancers and cardiovascular diseases [7]. Accordingly, these phytochemical compounds have been shown to be effective in protecting human health in both *in vitro* and *in vivo* studies such as anti-inflammatory, anti-aging, anti-obesity, anti-cell proliferation, anti-cancer, antibacterial, neuroprotective, and other positive effects [8–10]. Therefore, a specific dietary phytochemical supplement plays a vital role in maintaining an ideal state of health for humans.

Wine is traditionally derived from grape juice. Till date, many innovative ideas have been created by developing wine from different fruit sources. Therefore, a new concept of "fruit wine" has been currently defined. Fruit wine is a fermented fruit juice that uses yeast (*Saccharomyces*) to convert sugar in fruit juice into ethanol. Besides, fruit wine also shows the presence of other constituents such as unfermented sugar, tannins, minerals, proteins, organic acids, volatile compounds, and phenolic compounds that contribute to the distinct flavor and taste of each fruit wine [11]. The phenolic components in wine have been recorded to determine the organoleptic characteristics such as color, bitterness, astringency, flavor, and stability of the final product [12,13]. Fruit wine undergoes only the fermentation process, so it is virtually free of toxins but has the same nutritional value as juice. Many studies have documented the benefits of wine against neurological problems, cancer, diabetes, and cardiovascular diseases [13,14].

The specific flavor of each wine will be mainly dependent on the characteristics of fruit substrates, applied technologies, and yeast strains, including *Saccharomyces uvarum*, *Saccharomyces bayanus*, *Saccharomyces oviformis*, *Saccharomyces carlbergensis*, *Saccharomyces logo*, and *Saccharomyces oxydans* [15]. These yeasts have also been noted to be involved in developing blue and red pigments, supporting the release of natural antioxidant compounds (glutathione) and bioactive phenolic compounds in raw materials [16,17]. Therefore, it is considered a versatile yeast and plays an important role in wine production.

Currently, pomelo is increasingly asserting its position in a daily meal because of its favorable sensory and nutritional values. In Vietnam, the economic value from the exploitation of pomelo has increased, so the planting area, as well as the output of pomelo, is also continuously increasing (950,000 tons in 2022). This means that a great number of unqualified pomelo fruits for commercialization (small size, bad appearance) are released and considered a by-product that should be addressed. Through a brief assessment in Vietnam, there are currently a number of wine products from grapefruit on the market, but these products are all handcrafted and are not appreciated for their nutritional quality and sensory value. Besides, to the best of our knowledge, there are currently not many publications on wine production made from pomelo in Vietnam. Based on these concerns, the use of this by-product as a main substrate to produce pomelo fruit wine may be an ideal option to address this issue. Therefore, the aim of this study is to develop fruit wine from unqualified pomelo fruits from Vietnam. The influences of factors such as total soluble solid (TSS), yeast density, and fermentation time on the fermentation process were investigated. Besides, assessing the presence of phytochemicals, determination of polyphenols content, and antioxidant and antibacterial activities of the final product were also evaluated. The results of the study will contribute to the diversification of fruit-based wine products.

2 Materials and methods

2.1 Materials

The pomelo variety, namely, Nam Roi, was collected from pomelo orchards in the Phu Thuan area, Tan Phu ward, Cai Rang district, Can Tho city, Vietnam. Selected pomelo fruits were characterized as fully ripe with yellow flavedo. The pomelos were washed with tap water, and then removed their flavedo and albedo to collect pomelo pulp. The pomelo pulp was pressed to extract the pomelo juice and stored in the freezer (Sanyo, Japan) to be prepared for further steps. The pomelo juice was pasteurized using a microwave system (88–91°C for 15 s) before subjecting it to the fermentation process.

2.2 Starter culture preparation

The yeast strain *Saccharomyces* sp. was isolated from cana fruit (*Canarium album*) and stored at the Food Biotechnology Laboratory, Biotechnology Research and Development Institute, Can Tho University. This yeast strain was grown in Petri dishes for about 24–48 h at 30°C (Incucell 111, Germany). Then, it was stored at 4°C in a slanted agar containing yeast peptone dextrose adenine (YPDA) medium. To prepare the starter inoculum, yeast strains were subcultured in a 250 mL culture flask containing 100 mL of YPDA medium at 30°C for 24–48 h at 150 rpm. The inoculum density was adjusted to 10^3–10^7 CFU/mL and prepared for the fermentation process.

2.3 Wine preparation process

The pasteurized pomelo juice (99 mL) was put into each conical flask with the addition of 1 mL of starter culture at varying inoculum densities (10^3, 10^5, and 10^7 CFU/mL). The TSS (°Brix) content was adjusted to 19, 23, and 27% by adding sugar. pH value of the mixture was controlled by the addition of citric acid or Na_2CO_3 to obtain pH 4.0. The mixture was fermented for 6, 8, 10, 12, and14 days at room temperature (30 ± 2°C). After fermentation, the fermented juice was settled at 20°C and filtered. The filtrate was then pasteurized to obtain the final pomelo fruit wine product. Figure 1 illustrate the principal alcohol pomelo beverage (APB) production process.

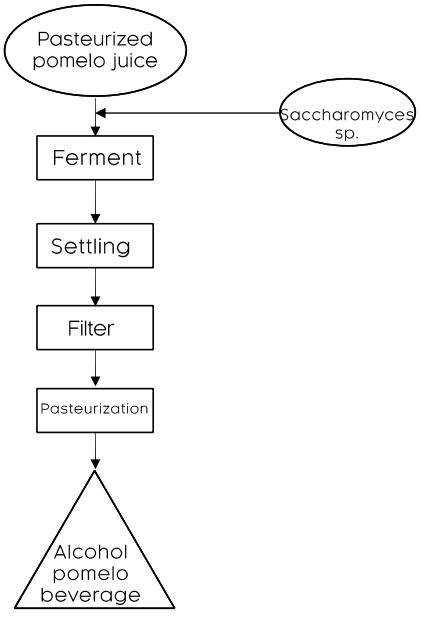

Figure 1: APB production process.

2.4 Phytochemical analyses

2.4.1 Determination of some phytochemical compounds

The qualitative experiment was carried out according to the model of Tiwari [18]. The process is summarized and described in Table 1.

2.4.2 Total polyphenols content (TPC) determination

The TPC was determined according to the description of Ribiero with corrections [19]. A volume (0.5 mL) of pomelo juice solution (or APB) and 1.25 mL 10% Folin–Ciocalteu solution were added to the test tube and allowed to react for 5 min. Then, 1 mL of 2% Na_2CO_3 was included in the mixture, followed by an incubation step in the dark for 45 min. The absorbance was then recorded at 765 nm. The polyphenol content (mg GAE/mL sample solution) was calculated using the equation of the gallic acid standard curve.

2.4.3 Determination of DPPH free radical scavenging capacity

The DPPH free radical scavenging ability of pomelo juice and APB after fermentation was performed as described by Pham with modifications [20]. A series of pomelo juice and APB solutions, including 2, 4, 6, 8, 10, and 12 µg/mL, were primarily prepared. An aliquot (1 mL) of pomelo juice and pomelo wine at each diluted concentration and 2 mL of 0.1 mM DPPH solution were mixed and incubated in the dark at room temperature (30 ± 2°C) for 30 min. Then, the absorbance was measured at the wavelength of 517 nm. The blank sample did not contain a DPPH solution. The capacity to scavenge DPPH radicals is calculated according to the following formula:

$$\mathrm{IC} = \frac{\mathrm{AC} - \mathrm{AS}}{\mathrm{AC}} \times 100,$$

(where IC is the percentage inhibition of DPPH, AC is the absorbance of the control sample, and AS is the absorbance of the sample).

By constructing a calibration curve $y = ax + b$, which is a function of percent inhibition of DPPH vs different concentrations, the concentration that inhibited 50% of DPPH radicals (IC_{50}) was interpolated. Vitamin C served as a positive control.

2.4.4 Antibacterial activity measurement

E. coli strains were cultured on nutrient agar (NA) medium and incubated at 37°C after 24 h to create a suspension with

Table 1: Methods for qualitative phytochemical compounds

Compounds	Sampling preparation	Reaction observation
Phenolic, tannin	Sample + $FeCl_3$	Blue-black precipitate
Flavonoid	Sample + $Pb(CH_3COO)_2$	Gold precipitation
Alkaloid	Sample + Wagner reagents	Red-brown precipitate
Quinone	Sample + HCl	Green
Coumarin	Sample + 1.5 mL NaOH 10%	Yellow
Saponin	Sample + 1 mL distilled water and a few drops of olive oil. Heating to 90°C	The solution turns into a milky emulsion
Terpenoid	Sample + 1 mL chloroform and a few drops of concentrated sulfuric acid	Red-brown or green
Steroids	Sample + 1 mL $CHCl_3$ + 1 mL CH_3COOH	Red-brown

a density of 10^6 CFU/mL. The bacterial suspension (100 µL) was aspirated and spread on the NA medium. Five wells (5 mm in diameter) were prepared by using a sterile cork borer on the NA agar. Then, 20 µL of pomelo juice, APB, and control sample, respectively, was poured into each of the specified wells on a Petri dish. Each Petri dish was then incubated at 37°C for 24 h. The negative control is sterile distilled water, and the positive control is Ampicillin (5 mg/mL) antibiotic. The results were determined by measuring the diameter of clear zone inhibition [21]. The antibacterial activity was evaluated by the diameter of the sterile ring (DSR) with the formula:

$$\text{DSR} = D - d(\text{mm}),$$

where D is the diameter of the sterile ring and d is the diameter of the agar well (5 mm).

2.4.5 Sensory evaluation

Thirty participants in the consumer preference research completed a scale of 0 to 5 (5 being very much liked, 4 being moderately liked, 3 being slightly liked, 2 being neither liked nor disliked, 1 being highly disliked, and 0 being very much disliked).

The Vietnamese standard TCVN 8007:2009 on alcohol – Preparation of test samples and sensory inspection issued by the Ministry of Science and Technology of Vietnam, including a sensory assessment of 30 people – was used to evaluate the finished product. The product was evaluated based on its color, taste, scent, and condition. The degree of disability of each sensory indicator is measured on a scale from 0 to 5, where 0 represents extremely low, poor, and 5 represents very high, good.

The committee's average of the evaluation findings for that criterion determines the average score of a sensory indication. The significant factor demonstrates the significance of every sensory requirement. The average score for each metric multiplied by significant factors yields the weighted scores. The final sensory score is the sum of all the weighted scores.

2.4.6 Data processing

Experimental data were processed by Excel 2013 software and statistically analyzed by MiniTab 16.0 software, analyzed by ANOVA, and the mean values were compared by Tukey's test at the significance level of 5%.

3 Results and discussion

3.1 Influence of TSS content and yeast density on APB fermentation

The influences of TSS and yeast density on the fermentation performance of APB after 7 days are shown in Table 2.

The remaining TSS showed a significant variation when varying the initial TSS (19, 23, and 27%) and starter culture density (10^3, 10^5, and 10^7 CFU/mL). In general, with the same inoculum density, the higher the initial TSS, the greater the TSS difference is achieved. In the same initial TSS (19 and 23%), the TSS difference was insignificantly different when varying inoculum density. Whereas, at the initial TSS of 27%, the increase in starter density was found to reduce the TSS difference. The fermentation process at the inoculum density of 10^5 CFU/mL and initial TSS of 19%, and the treatment with inoculum density of 10^7 CFU/mL and initial TSS of 27% showed the lowest TSS difference between initial TSS and remaining TSS. Meanwhile, the treatment with yeast density of 10^3 CFU/mL and initial TSS of 27% had the largest TSS difference of 14,67 and showed a statistically significant difference compared with the others. Thus, it can be seen that the pomelo juice medium with the initial TSS of 27 and the starter inoculum density of 10^3 CFU/

Table 2: Results of alcohol and TSS obtained after fermentation

Starter culture density (CFU/mL)	Original TSS (%)	Remaining TSS	TSS difference	Alcohol concentration (% v/v)
	19	8.33	10.67^{bc}	5.09^{d}
10^3	23	12.33	10.67^{bc}	6.40^{bc}
	27	12.33	14.67^{a}	8.27^{a}
	19	9.00	10.00^{c}	5.47^{cd}
10^5	23	12.16	10.83^{bc}	5.36^{cd}
	27	14.66	12.33^{b}	6.75^{b}
	19	8.16	10.83^{bc}	4.98^{d}
10^7	23	12.00	11.00^{bc}	5.91^{bcd}
	27	17.00	10.00^{c}	4.88^{d}
CV (%)			0.55	0.38

Note: the figures in the table are the average of three replicates. In the same column, values with the same letter are not significantly different at 99% confidence level through the Tukey test ($P < 0.01$).

mL facilitated the fermentation process with high alcohol production. A similarity in the fermentative capacity of yeast in the medium with high initial TSS to that with low initial TSS was shown in the report of Singh et al. [22].

Alcohol is the most easily found volatile compound in wine, and it is the factor that improves the organoleptic properties and the acceptability of wine [22]. The alcohol content of wine largely depends on the initial TSS of juice medium from raw material [23]. The results of alcohol content after the fermentation process significantly differed from each treatment ($p < 0.05$). The alcohol content of fermented pomelo juice was the result of the interaction between the two factors: initial TSS and yeast density, shown in Figure 2. In general, at the same starter culture, the increase in the initial TSS from 19 to 27% was observed with an increase in the level of alcohol content. However, at the yeast density of 10^3 CFU/mL, when the TSS level increased, the alcohol difference was not statistically significant. In the same value of TSS (19 and 23%), as the density increased (10^3, 10^5, and 10^7 CFU/mL), the alcohol concentration was not statistically significant. Meanwhile, at the TSS of 27%, the higher the density, the lower the alcohol obtained after fermentation. The alcohol content was 8.27% v/v when the yeast density was 10^3 CFU/mL, 6.75 and 4.88% of alcohol content obtained corresponding to the yeast density of 10^5 and 10^7 CFU/mL, respectively. The results showed that there was an interaction between initial TSS and yeast density that significantly affected the alcohol level during fermentation.

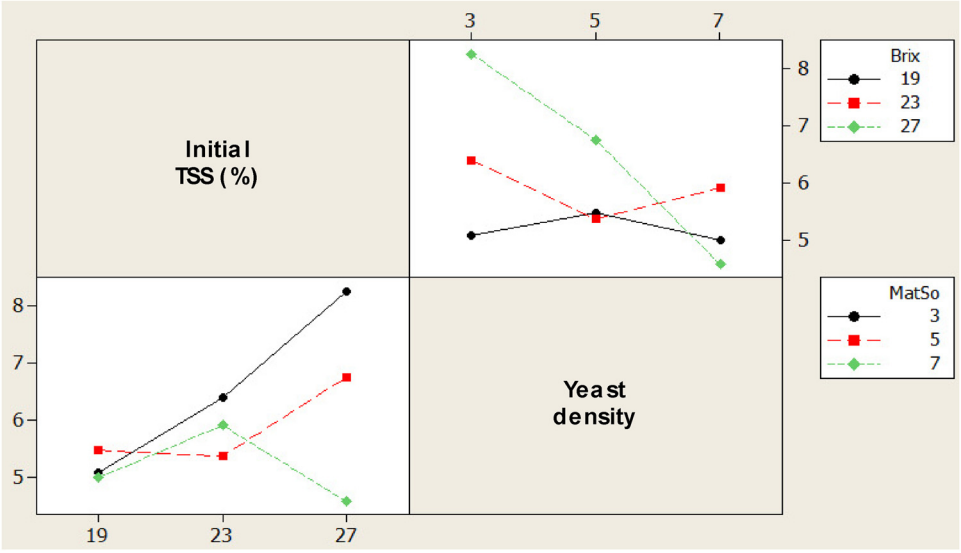

Figure 2: Survey on fermentation time of pomelo juice and APB.

During fermentation, about 95% of the sugar turns into alcohol and CO_2, the rest are other products and residual sugar. The lower the residual sugar remained, the higher the alcohol content was achieved. If the initial TSS is too high, it will cause yeast inhibition due to protozoal shrinkage. Therefore, the treatment with the TSS of 27 and yeast density of 10^3 cells/mL was the most optimal treatment for fermenting pomelo juice according to the statistical results of the difference in TSS before and after fermentation and alcohol level. In addition, Udeagha et al. indicated that the alcohol content of pineapple wine was 10.66%, and it was considered a low-alcohol wine with 6% v/v yeast concentration and 25°Brix TSSs [24]. Accordingly, the highest alcohol content (8.27%) in this study could be accepted as a low-alcohol wine. Many recommendations suggest that consuming a small amount of low-alcohol wine once or twice a day will help reduce the risk of stroke and diabetes. On the contrary, excessive use has been noted to increase the risk of cardiovascular problems, stroke, and cancer [25,26].

3.2 Survey on fermentation time of pomelo juice and APB

The results from Table 3 are the average statistical values of three replicates of remaining TSS and produced alcohol content after the fermentation process. The result showed that the remaining TSS in the APB and produced alcohol levels were significantly different ($p < 0.05$). At the same time, the TSS was found to continuously reduce during the fermentation period, indicating the continuous use of sugar to convert into alcohol and many other by-products by yeast.

The TSS of fermented pomelo juice at days 6, 10, 12, and 14 showed no statistically significant difference. In particular, the fermentation process at day 12 has the lowest remaining TSS, showing that the yeast effectively consumed sugar in the pomelo juice. Extending fermentation up to 14 days was not found to increase alcohol production. The lowest alcohol level was at day 6 of fermentation (7.01% v/v), and the highest alcohol content was 10.35% v/v after 12 days of fermentation. The alcohol content obtained on days 12 and 14 was not statistically significant. Thus, the fermentation time of 12 days was selected as it is considered a time-saving and cost-effective option in the production of APB.

Table 3: Survey results on fermentation time of pomelo juice and APB

No.	Time (days)	Remaining TSS after fermentation	Alcohol concentration (% v/v)
1	6	19.17^{b}	7.01^{c}
2	8	19.17^{b}	8.54^{b}
3	10	18.33^{ab}	9.07^{ab}
4	12	16.50^{a}	10.35^{a}
5	14	18.50^{ab}	9.45^{ab}
CV (%)		0.39	0.56

Note: the figures in the table are the average of three replicates. In the same column, values with the same letter are not significantly different at the 99% confidence level through the Tukey test ($P < 0.01$).

3.3 Evaluation of biological activities of pomelo juice and APB

The qualitative results of phytochemical compounds correspond to the degree of color expression and the characteristic response of the biochemical experiments. The strong positive reaction indicates a great number of phytochemicals in the APB. The result in Table 4 showed that pomelo juice and APB both showed the presence of phytochemical compounds, including phenols, tannins, flavonoids, alkaloids, coumarins, terpenoids, quinones, steroids, and saponins.

In the phenol and tannin test, both pomelo juice and APB showed positive results with a blue-black precipitate. Phenol and tannin contents in pomelo juice (++) were relatively higher than those in APB (+). Phenolic is the most abundant group of substances in all parts of the plant. Plants produce this compound to suppress oxidative free radicals produced during photosynthesis. These phenolic compounds are characterized by their antioxidant

Table 4: Phytochemical compounds in pomelo juice and APB

Compounds	Pomelo juice	APB
Phenol and tannin	++	+
Flavonoid	++	+++
Alkaloid	++	+++
Quinone	++	+++
Coumarin	++	+++
Saponin	+	+
Terpenoid	+++	+++
Steroid	+++	+++

Based on the degree of reaction and the color expression of the solution after the reaction.
(−): There is no such compound in the sample.
(+): A group of compounds that exists in low quantity in the sample.
(++): A group of compounds that exists in the sample.
(+++): A group of compounds that exists in high quantity in the sample.

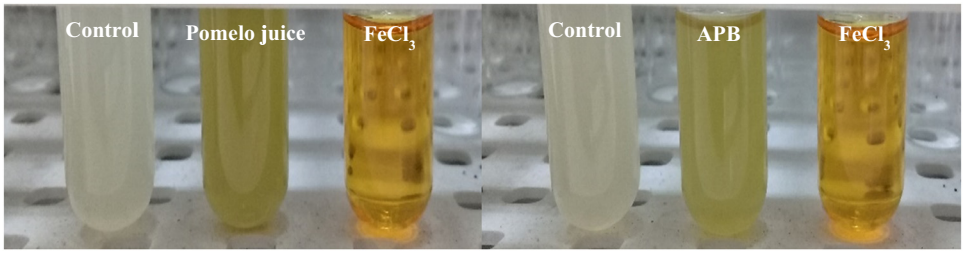

Figure 3: Positive reaction (blue-black precipitate) of pomelo juice and APB treatments in phenol and tannin test.

capacity and are of great interest to researchers [8,20] (Figure 3).

For flavonoid test results, pomelo juice and APB both showed the presence of flavonoids, indicated by the appearance of a yellow precipitate as a result of reacting with 10% $Pb(CH_3COO)_2$. This qualitative result was consistent with the result of Caengprasath et al. [27]. In particular, the APB (+++) showed a stronger reaction than the pomelo juice (++), showing that the APB contained more flavonoid compounds than the pomelo juice. The results also showed that during alcoholic fermentation, there was no loss of flavonoids. Instead, it was found to increase, possibly due to the fermentation process (Figure 4).

Besides, in the alkaloid test, both pomelo juice and APB had a reddish-brown precipitate, indicating the presence of alkaloids in the sample. Qualitative results of alkaloid compounds showed that APB (+++) contained a large amount of alkaloid pomelo juice (++). Alkaloids produced in the roots are transported to the aboveground part of the plant, and they are accumulated in leaves, fruits, or seeds after performing a secondary transformation. Alkaloids are usually solid at room temperature, stable, insoluble at boiling point, and insoluble in water. Therefore, alkaloids were reported to be abundant in pomelo juice and did not show their loss after the fermentation process [28] (Figures 5–10).

A common point observed in the three compounds, including alkaloids, quinones, and coumarins, was that their presence after the fermentation of pomelo juice was reported to increase significantly. For the others, such as saponins, terpenoids, and steroids, no difference was observed in pomelo juice and APB.

3.4 TPC in pomelo juice and APB

The TPC was determined based on the Folin–Ciocalteu method and evaluated by mg GAE/mL sample solution. The standard curve using gallic acid as a standard solution

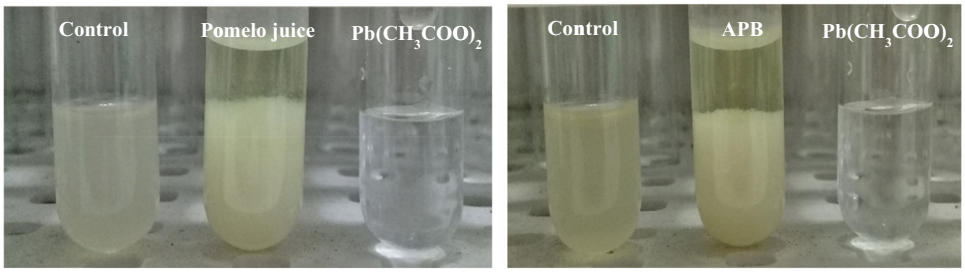

Figure 4: Positive reaction (yellow precipitate) of pomelo juice and APB treatments in the flavonoid test.

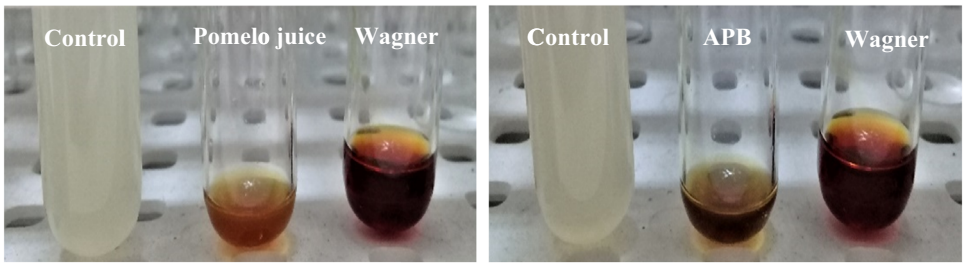

Figure 5: Positive reaction (reddish-brown precipitate) of pomelo juice and APB treatments in the alkaloid test.

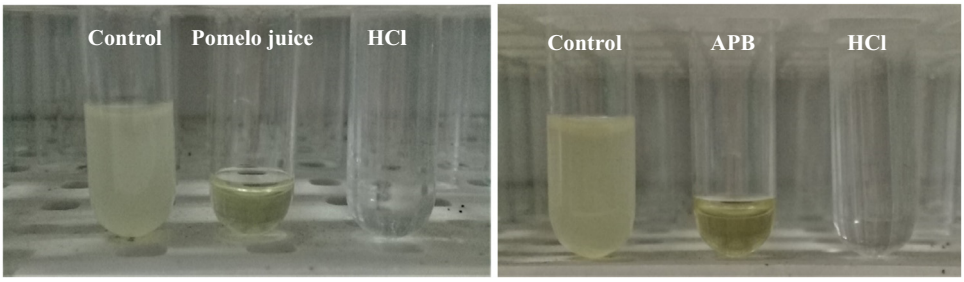

Figure 6: Positive reaction (green precipitate) of pomelo juice and APB treatments in the quinone test.

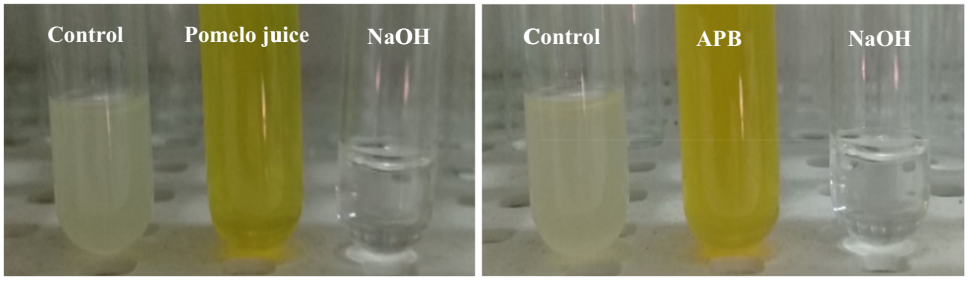

Figure 7: Positive reaction (orange yellow) of pomelo juice and APB wine in the coumarin test.

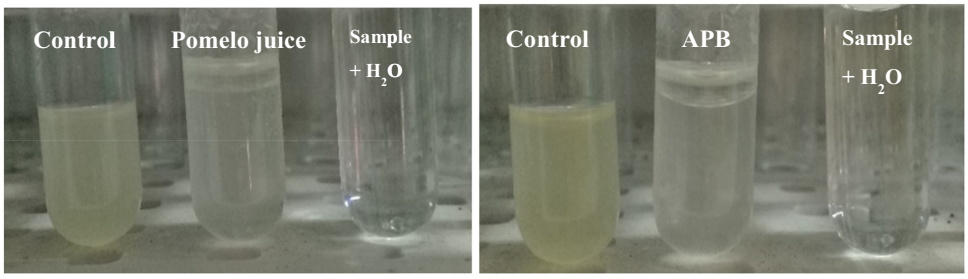

Figure 8: Positive reaction (milky emulsion) of pomelo juice and APB treatments in the saponin test.

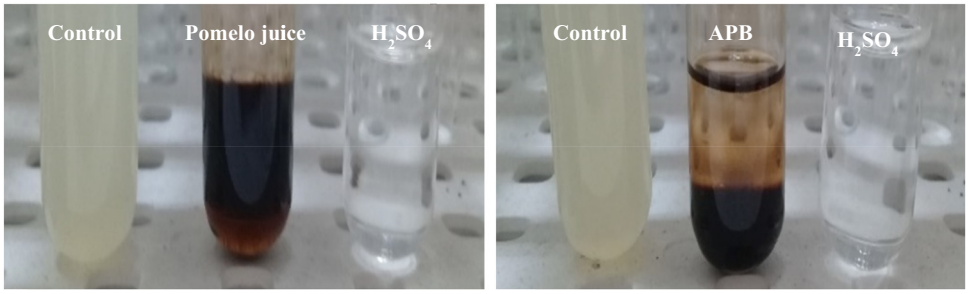

Figure 9: Positive reaction (reddish brown or green) of pomelo juice and APB wine in the terpenoid treatment.

was constructed. The obtained result was the equivalent linear regression equation $y = 0.0207x + 0.1384$. The TPC of pomelo juice and APB was 235.53 ± 7.27 mg GAE/mL and 271.3 ± 5.74 mg GAE/mL (shown in Figure 11), respectively. The TPC in the APB was 1.2 fold higher than that in the pomelo juice, indicating the generation of polyphenols during the fermentation process (Figure 12).

Polyphenol compounds are among the largest and most widespread groups of secondary plant metabolites. Polyphenols possess antioxidant, anti-cancer, anti-inflammatory,

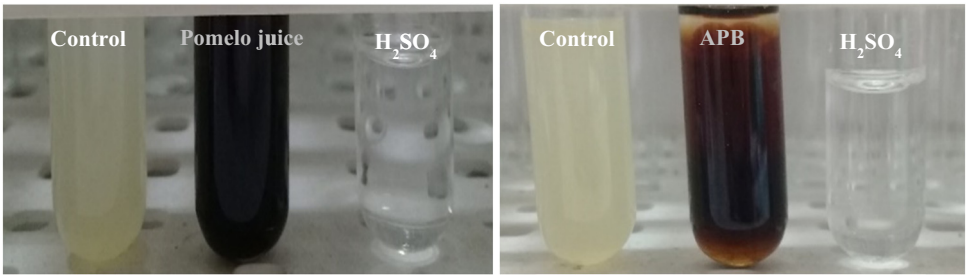

Figure 10: Positive reaction (reddish brown) of pomelo juice and APB wine in steroid test.

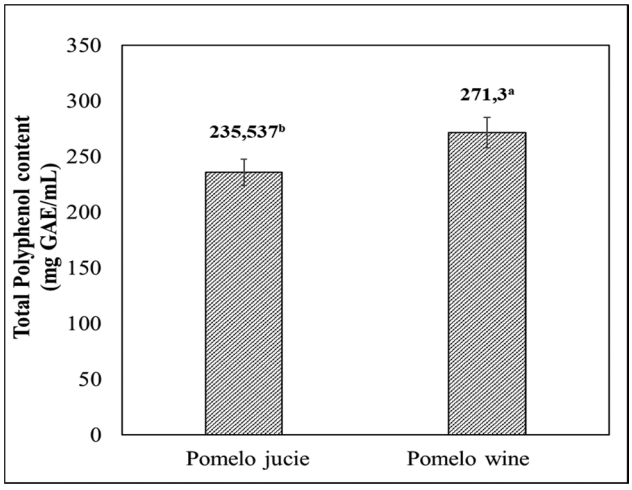

Figure 11: TPC (mg GAE/mL) of pomelo juice and APB.

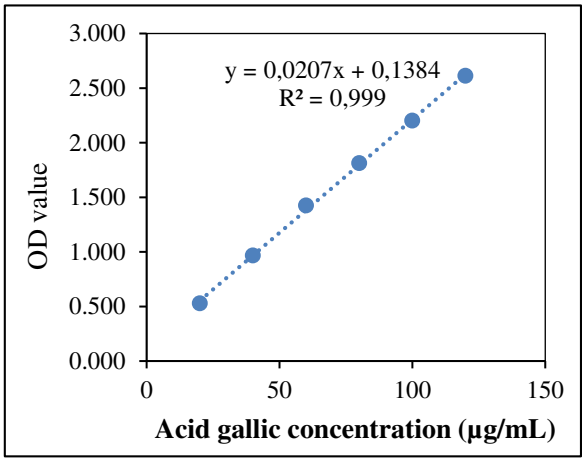

Figure 12: Equation of the gallic acid standard curve.

and cardioprotective properties and the ability to inhibit cell proliferation activities. Natural antioxidants are mainly in the form of phenolic compounds such as flavonoids, phenolic acids, and tocopherol. The TPC in pomelo juice in this study was 1,500 times higher than that in pomelo in Italy (ranged from 0.15–0.17 mg GAE/mL) reported by Sicari et al. [6]. However, the TPC of APB was about seven times lower when compared with the results of Padilha et al., who assessed commercial wines in Brazil with values ranging from 1.99 to 2.65 mg/mL [29]. This difference might be attributed to the origin of materials and the incubation time of each product.

3.5 Antioxidant activity by scavenging H_2O_2 radicals

The inhibition percentage of vitamin C and sample solution increased with increasing concentration. The concentration used for vitamin C was from 40 to 140 µg/mL with an inhibition percentage of 16.71–50.68% while they varied from 6 to 14 µL/mL with an inhibition percentage from 34.84–50.021% to 28.18–47.67% for pomelo juice and APB, respectively. The antioxidant activity by scavenging peroxide radicals can be expressed in terms of IC_{50} values, as shown in Table 5.

From the results, the IC_{50} value of pomelo juice was 13.66 ± 0.09 µL/mL, which was lower than the IC_{50} value of APB (14.34 ± 0.1 µL/mL). However, the difference was not found to be statistically significant. When compared with the IC_{50} value of vitamin C (133.09 ± 1.26 µg/mL), the results showed that a small amount of pomelo juice (13.66 µL) or

Table 5: IC_{50} value of vitamin C, pomelo juice, and APB

Sample	IC_{50} (H_2O_2)	IC_{50} (DPPH)
Vitamin C	133.09^{b} ± 1.26	3.86^{a} ± 0.08
Pomelo juice	13.66^{a} ± 0.09	5.05^{b} ± 0.28
APB	14.34^{a} ± 0.10	11.60^{c} ± 0.14
CV (%)	0.73	0.19

Note: the figures in the table are the average of three replicates. In the same column, values with the same letter are not significantly different at the 99% confidence level through the Tukey test ($P < 0.01$).

APB (14.34 µL) exhibited the same 50% scavenging capacity. This proved that pomelo juice and APB showed a very strong ability to scavenge peroxide radicals.

Good antioxidant capacity is due to the presence of plant compounds such as phenols and tannins, flavonoids, alkaloids, glycosides, and saponins [30]. Polyphenols are constituents that help eliminate free radicals via the hydroxyl group. Therefore, as the polyphenol content increases, the antioxidant activity also increases [31]. This was in agreement with a report done by Khan et al., peroxide radicals scavenging capacity was closely related to the polyphenol content [32]. Besides, flavonoids are also well known for their high antioxidant capacity, especially kaempferol compounds which are constructed by the C_2–C_3 bonds combined with oxy group at C-4 and hydroxyl group (–OH) at C_3, C_4, and C_5, mainly involving in antioxidant activity. Kaempferol was found to possess the ability to reduce superoxide radicals (O^{2-}), and hydroxyl (OH^-) at low concentrations. The IC_{50} value of kaempferol in H_2O_2 free radical scavenging was 0.5 µM [33].

Vitamin C is a strong antioxidant and inactivates free radicals by donating a hydrogen electron (H^+) to neutralize free radicals. Therefore, vitamin C is often used as a positive control in experiments investigating antioxidant activity by reducing DPPH radicals. DPPH is a carrier of free radicals, free radicals of DPPH carry an odd electron, so its solution appears with characteristic purple color.

In general, the inhibition percentage of vitamin C and pomelo samples increased with increasing concentrations. Vitamin C used a concentration range from 2 to 10 µg/mL with an inhibition percentage of 40.21–82.88%. Meanwhile, the pomelo juice and APB was applied for a concentration range of 2–10 µL/mL with an inhibitory percentage of 40.41–66.42% and 36.90–48.01%, respectively. The DPPH free radical scavenging ability of pomelo juice and APB is shown through the IC_{50} value (Table 6).

Table 6: Effective inhibition of *E. coli* bacteria of pomelo juice and APB for 24 h

Sample	Diameter of the sterile ring (mm)
Pomelo juice	9.67^c ± 0.58
APB	16.67^b ± 2.08
Ampicillin	27.33^a ± 1.16
CV (%)	1.41

Note: Sterile ring diameter data are mean value of three replicates. In the same column, values with the same letter are not significantly different at the 99% confidence level through the Tukey test ($P < 0.01$). Negative control – sterile distilled water for a sterile ring diameter of 0, CV% indicates the percentage variation in the dependent variable in the experiment.

From the results, both pomelo juice and APB possessed the ability to eradicate DPPH free radicals, and the difference was statistically significant at 1%. The APB had an IC_{50} value of 11.60 ± 0.14 µL/mL, which was 2.3 folds higher than that of the pomelo juice (5.052 ± 0.284 µL/mL). This means that the DPPH scavenging capacity of pomelo juice was 2.3 folds stronger than that of APB. The IC_{50} values of APB and pomelo juice were comparable with that of Vitamin C, indicating that they possessed very strong antioxidant activities.

In many fruits, antioxidant activity is dependent on the polyphenol content. From the experimental results on antioxidant capacity by scavenging DPPH free radicals, the antioxidant capacities of pomelo juice and APB were not found to be dependent on the TPC. Pomelo juice had a lower polyphenol content (235.54 mg GAE/mL) than APB (271.30 mg GAE/mL), but pomelo juice exhibited a higher antioxidant capacity (IC_{50} = 5.05 µL/mL) than APB (11.60 µL/mL). This could be explained as due to that the pomelo juice might possess many other active substances that exhibit antioxidant activity, such as non-phenolic compounds: Vitamin C, Vitamin E, and L-ergothioneine [34]. In pomelo, the percentage of vitamin C accounts for 95% (mg/100 g). Vitamin C is one of many substances involved in the body's antioxidant defense system. Antioxidants (vitamin E, β-carotene) can convert oxidizing agents into harmless substances. Vitamin C attaches many forms of free radicals and "scavenges" them out of the body, helping restore vitamin E back to its antioxidant form. Thus, the antioxidant capacity of pomelo juice and APB may not be highly correlated with TPC.

3.6 Antibacterial activity of pomelo juice and APB

The antibacterial activities of pomelo juice and APB were investigated by a good diffusion method. The larger the diameter of the sterile ring, the stronger the antibacterial activity of the sample. As a result, both pomelo juice and APB showed a bactericidal effect against *E. coli*, as shown in Table 6 and Figure 13. The inhibition diameter ranged from 9.67 ± 0.58 to 16.67 ± 2.08 (mm). The APB showed an inhibition diameter of 16.67 ± 2.082 (mm) which was 1.7 folds larger than that of pomelo juice (9.67 ± 0.58 (mm).This indicated that the antibacterial activity of APB was stronger than that of pomelo juice. The antibiotic ampicillin at the concentration of 5 mg/mL created a sterile ring of 27.33 ± 1.16 (mm), which was 2.8 times higher than pomelo juice and 1.6 times higher than APB.

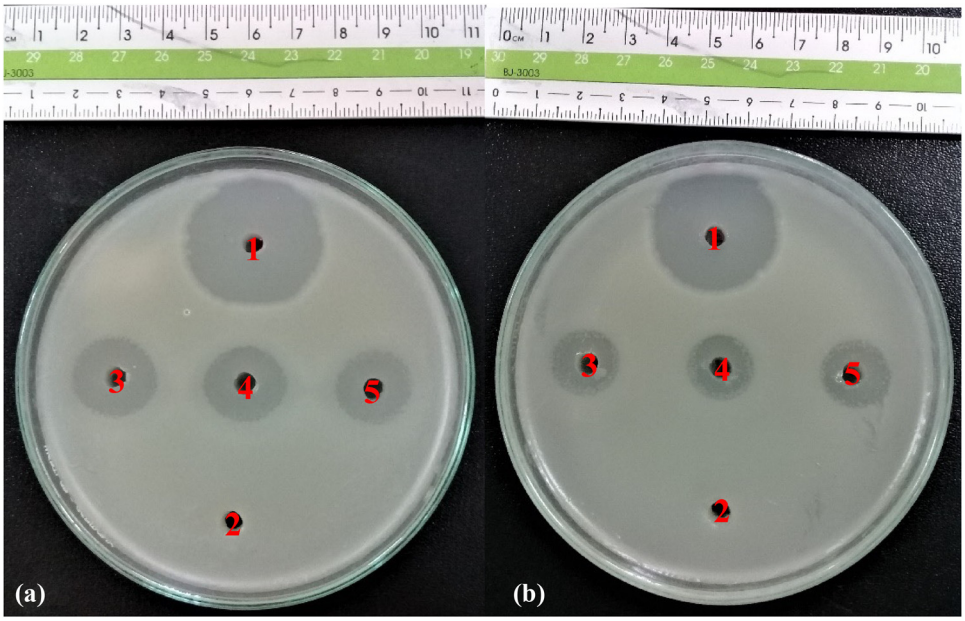

Figure 13: The inhibitory effect of *E. coli* bacteria of (a) APB and (b) Pomelo juice. (1) Ampicillin; (2) distilled water; and (3, 4, and 5) sample.

Polyphenols are naturally active ingredients that have many effects, such as antioxidant, anti-inflammatory, antibacterial, anti-allergic, and anti-aging. The mechanism action of antibacterial activity differed from the types of polyphenols. Terpenoids are thought to cause cell membrane disruption, and quinones bind to outer membrane surface components. Meanwhile, flavonoids affect the activity of enzymes forming peptidoglycans and alkaloids intercalate into nucleic acid structures [18]. Therefore, the TPC in pomelo juice and APB can be highly correlated to antibacterial activity. Besides, other components, such as limonene, ɢ-terpinene, pinene, etc., also contribute to the inhibitory effect against pathogens, especially Gram-negative. APB has antibacterial properties, proving that plant compounds in this product have antibacterial effects that are not lost during fermentation, such as phenolic compounds, tannins, flavonoids, alkaloids, quinones, coumarins, terpenoids, steroids, and saponins. The higher bactericidal effect in APB could be ascribed to the presence of ethanol in wine which is considered an antibacterial agent as it can penetrate the cell membrane, causing protein coagulation and cell death.

E. coli is one of the most common food-poisoning microorganisms. Therefore, the inhibition of E. *coli* by natural ingredients such as pomelo juice and APB elucidated their potential as an anti-agent. The results indicated that pomelo juice and APB could be potential sources of antioxidant and antibacterial compounds due to them containing many natural compounds such as phenols and tannins, flavonoids, alkaloids, quinones, coumarins, saponins, terpenoids, and steroids. Pomelo juice and APB contained very high TPC, according to quantitative results. There was a fairly close linear correlation between polyphenol content and antibacterial activity. Through the biological activity survey, pomelo juice showed little or no change in the content of plant compounds after alcoholic fermentation.

3.7 Sensory evaluation of APB products

The APB (with an alcohol content of 9.05% v/v, pH = 4.02, TSS = 18.67%, 12 days) used for the sensory test was fermented from the pomelo juice with TSS = 27%, yeast density of 10^3 CFU/mL, and pH = 4.0. The sensory panelist was established with ten members who were experts in sensory evaluation. The results of the sensory evaluation of APB according to TCVN 8007:2009 standard are shown in Table 7.

APB samples were evaluated with good organoleptic (Table 6). Clarity and color achieved was 68% (3.4 points), odor reached 84% (4.2 points), taste reached 70% (3.5 points), and acceptability reached 80% (4 points). It can be concluded that this product was moderately favorable by the panelist. However, there is also an opinion that APB has a bitter taste. This was due to the presence of naringin, a flavanone glycoside, naringin which showed strong pharmacological effects such as antioxidant activity, lowering blood lipids, and anti-cancer, so the bitter taste of APB could be acceptable [6]. With the above sensory evaluation results, Nam Roi APB should be further adjusted in terms of clarity in order to increase its sensory value.

Table 7: Results of sensory evaluation of APB

Targets	Results of sensory evaluation of APB		Quality requirements (TCVN 3217-79)
	Medium score	Comment	
Clarity and color	3.4	The liquid has a completely specific color to the product but is still not transparent	Clear liquid, free of turbidity and foreign objects. The color is completely specific to the product
Odor	4.2	Harmonious, aromatic, completely specific to the product	Harmonious, fragrant, and completely unique to the product
Taste	3.5	Harmonious, completely specific to the product, the aftertaste has a characteristic sour taste of the juice	The harmonious, mellow, good aftertaste is completely specific to the product
Acceptability	4.0	Like it is just right	Like so much

4 Conclusion

This study has successfully proposed a APB production process with highly acceptable organoleptic and quality parameters. Besides, most of the valuable phytochemicals were retained in pomelo juice and wine, such as phenols and tannins, flavonoids, alkaloids, coumarins, quinones, steroids, terpenoids, and saponins. Research showed that the polyphenol content in APB was higher than in pomelo juice and showed a linear correlation with the antibacterial activity of APB and pomelo juice. The product of this study showed a potential to be exploited in improving the use value of pomelo fruit.

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Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

[1] Deng W, Liu K, Cao S, Sun J, Zhong B, Chun J. Chemical composition, antimicrobial, antioxidant, and antiproliferative properties of grapefruit essential oil prepared by molecular distillation. Molecules. 2020;25(1):217. doi: 10.3390/molecules25010217.

[2] Gupta AK, Dhua S, Sahu PP, Abate G, Mishra P, Mastinu A. Variation in phytochemical, antioxidant and volatile composition of pomelo fruit (Citrus grandis (l.) Osbeck) during seasonal growth and development. Plants. 2021;10(9):1941. doi: 10.3390/plants10091941.

[3] Khalil MNA, Farghal HH, Farag MA. Outgoing and potential trends of composition, health benefits, juice production and waste management of the multi-faceted grapefruit citrus X paradisi: A comprehensive review for maximizing its value. Crit Rev Food Sci Nutr. 2022;62(4):935–56. doi: 10.1080/10408398.2020.1830364.

[4] Uckoo RM, Jayaprakasha GK, Balasubramaniam VM, Patil BS. Grapefruit (Citrus paradisi Macfad) phytochemicals composition is modulated by household processing techniques. J Food Sci. 2012 Sep;77(9):C921–6. doi: 10.1111/j.1750-3841.2012.02865.x.

[5] Toh JJ, Khoo HE, Azrina A. Comparison of antioxidant properties of pomelo [Citrus Grandis (L) Osbeck] varieties. Int Food Res J. 2013;20(4):1661–8.

[6] Sicari V, Pellicanò TM, Giuffrè AM, Zappia C, Capocasale M, Poiana M. Physical chemical properties and antioxidant capacities of grapefruit juice (Citrus paradisi) extracted from two different varieties. Int Food Res J. 2018;25(5):1978–84.

[7] Kim K, Park KI. A review of antiplatelet activity of traditional medicinal herbs on integrative medicine studies. Evidence-Based Complementary Altern Med. 2019;2019:1–18. doi: 10.1155/2019/7125162.

[8] Gupta AK, Yumnam M, Medhi M, Koch P, Chakraborty S, Mishra P. Isolation and characterization of naringinase enzyme and its application in debittering of Pomelo juice (citrus grandis): A comparative study with macroporous resin. J Food Process Preserv. 2021;45(5). doi: 10.1111/jfpp.15380.

[9] Nhut PT, An TNT, Minh LV, Truc TT, Anh NHT. Phytochemical screening of Allium Tuberosum Rottler. ex Spreng as food spice. IOP Conf Series: Mater Sci Eng. 2020;991(1):012021. doi: 10.1088/1757-899X/991/1/012021.

[10] Ortuño A, Díaz L, Alvarez N, Porras I, García-Lidón A, Del Río JA. Comparative study of flavonoid and scoparone accumulation in different Citrus species and their susceptibility to Penicillium digitatum. Food Chem. 2011;125(1):232–9. doi: 10.1016/j.foodchem.2010.09.012.
[11] Yang H, Cai G, Lu J, Gómez Plaza E. The production and application of enzymes related to the quality of fruit wine. Crit Rev Food Sci Nutr. 2021;61(10):1605–15. doi: 10.1080/10408398.2020.1763251.
[12] Avizcuri JM, Sáenz-Navajas MP, Echávarri JF, Ferreira V, Fernández-Zurbano P. Evaluation of the impact of initial red wine composition on changes in color and anthocyanin content during bottle storage. Food Chem. 2016;213:123–34. doi: 10.1016/j.foodchem.2016.06.050.
[13] Gutiérrez-Escobar R, Aliaño-González MJ, Cantos-Villar E. Wine polyphenol content and its influence on wine quality and properties: a review. Molecules. 2021;26(3):718. doi: 10.3390/molecules26030718.
[14] Vecchio R, Decordi G, Grésillon L, Gugenberger C, Mahéo M, Jourjon F. European consumers' perception of moderate wine consumption on health. Wine Econ Policy. 2017;6(1):14–22. doi: 10.1016/j.wep.2017.04.001.
[15] Puškaš VS, Miljić UD, Djuran JJ, Vučurović VM. The aptitude of commercial yeast strains for lowering the ethanol content of wine. Food Sci Nutr. 2020;8(3):1489–98. doi: 10.1002/fsn3.1433.
[16] Loira I, Vejarano R, Morata A, Ricardo-da-Silva JM, Laureano O, González MC, et al. Effect of Saccharomyces strains on the quality of red wines aged on lees. Food Chem. 2013;139(1–4):1044–51. doi: 10.1016/j.foodchem.2013.01.020.
[17] Morata A, Escott C, Loira I, Del Fresno JM, González C, Suárez-Lepe JA. Influence of saccharomyces and non-saccharomyces yeasts in the formation of pyranoanthocyanins and polymeric pigments during red wine making. Molecules. 2019;24(24):4490. doi: 10.3390/molecules24244490.
[18] Tiwari P, Kumar B, Kaur M, Kaur GK. Phytochemical screening and extraction: A review. Internationale pharmaceutica sciencia. 2011;1(1):98–106. doi: 10.1002/hep.29375.
[19] Ribiero D, Freitas R, Sena E, dos Santos R. Fermentation of Cocoa Juice (Theobroma cacao L.) and Roselle (Hibiscus sabdariffa L.) extracts into a wine-like alcoholic drink. Fruit, Veg Cereal Sci Biotechnol. 2009;2003:57–63.
[20] Pham TN, Nguyen XT, Phan TD, Le TD, Nguyen TBT, Hoang TPL, et al. Anti-arthritic activity and phytochemical composition of 'Cao Khai' (Aqueous extracts of Coptosapelta flavescens Korth.). Heliyon. 2022;8(2):e08933. doi: 10.1016/j.heliyon.2022.e08933.
[21] Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71–9. doi: 10.1016/j.jpha.2015.11.005.
[22] Singh S, Tripathi AD, Chauhan AK, Gupta AK. Production of beetroot (Beta vulgaris L.) wine using different Saccharomyces strains and study of physicochemical and sensorial characteristics. J Food Sci Technol. 2021;58(11):4442–9. doi: 10.1007/s13197-021-05136-y.
[23] Ejikeme U, Egbuna S. Optimization of process conditions for alcoholic wine production from pineapple using RSM. Int J Multidiscip Sci Eng. 2015;6(3):23–30.
[24] Udeagha EC, Ishiwu CN, Obiora CU, Iwouno JO. Effects of yeast concentration and total soluble solids on the quality of wine produced from pineapple. Curr J Appl Sci Technol. 2020;39:28–42. doi: 10.9734/cjast/2020/v39i3030968.
[25] Jin M, Cai S, Guo J, Zhu Y, Li M, Yu Y, et al. Alcohol drinking and all cancer mortality: a meta-analysis. Ann Oncol. 2013;24(3):807–16. doi: 10.1093/annonc/mds508.
[26] O'Keefe JH, Bhatti SK, Bajwa A, DiNicolantonio JJ, Lavie CJ. Alcohol and cardiovascular health: the dose makes the poison...or the remedy. Mayo Clin Proc. 2014;89(3):382–93. doi: 10.1016/j.mayocp.2013.11.005.
[27] Caengprasath N, Ngamukote S, Mäkynen K, Adisakwattana S. The protective effects of Pomelo extract (Citrus grandis L. Osbeck) against fructose-mediated protein oxidation and glycation. EXCLI J. 2013;12:491–502.
[28] Nhut PT, Quyen NTN, Truc TT, Minh LV, An TNT, Anh NHT. Preliminary study on phytochemical, phenolic content, flavonoids and antioxidant activity of Coriandrum Sativum l. originating in Vietnam. IOP Conf Series: Mater Sci Eng. 2020;991(1):012022. doi: 10.1088/1757-899X/991/1/012022.
[29] Padilha CVDS, Camarão Telles Biasoto A, Corrêa LC, Dos Santos Lima M, Pereira GE. Phenolic compounds profile and antioxidant activity of commercial tropical red wines (vitis vinifera l.) from São Francisco Valley, Brazil: PADILHA et al. J Food Biochem. 2017;41(3):e12346. doi: 10.1111/jfbc.12346.
[30] Babu D, Gurumurthy P, Borra SK, Cherian KM. Antioxidant and free radicals scavenging activity of triphala determinded by using different in vitro models. J Med Plant Res. 2013;7(39):2898–905. doi: 10.5897/JMPR2013. 5124.
[31] Saha S, Verma RJ. Antioxidant activity of polyphenolic extract of Terminalia chebula Retzius fruits. J Taibah Univ Sci. 2016;10(6):805–12. doi: 10.1016/j.jtusci.2014.09.003.
[32] Khan RA, Khan MR, Sahreen S, Ahmed M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchus asper (L.) Hill. Chem Cent J. 2012;6(1):12. doi: 10.1186/1752-153X-6-12.
[33] Calderón-Montaño JM, Burgos-Morón E, Pérez-Guerrero C, López-Lázaro M. A review on the dietary flavonoid kaempferol. Mini Rev Med Chem. 2011;11(4):298–344. doi: 10.2174/138955711795305335.
[34] Oboh G, Shodehinde S Distribution of nutrients, polyphenols and antioxidant activities in the pilei and stipes of some commonly consumed edible mushrooms in Nigeria. Bull Chem Soc Ethiopia. 2009;23(3):391–8. doi: 10.4314/bcse.v23i3.47663.