Preparation, Quality Control Studies and Therapeutic Potential Estimation of Pomegranate Wine

Siddika Inamdar*, Vrunal More, Anil Landge, Mithun Maniyar, Vaibhav Gaikwad

SVERI's College of Pharmacy, Gopalpur, Pandharpur-413304, Maharashtra.

***Corresponding author:**

Ms. Siddika Altaf Inamdar

E-mail: siddikainamdar44@gmail.com

Abstract

The present investigation was conducted with the objective to explore the production of wine from pomegranate fruit (*Ganesha* variety) by manipulating the fermentation variables. When yeast *Saccharomyces cerevisiae* ATCC 204508 executes anaerobic fermentation, it transforms carbohydrates into alcohol and carbon dioxide, that ends in production of pomegranate wine. at 30^{o}C. The ability of yeast cells to produce wine using pomegranate juice with respect to sugar utilization, alcohol production, fermentation rate and final alcohol level was studied. Wine was chemically analysed and observed that there was no significant effect of inoculum level. Considering the chemical composition of wines, 5% inoculum level and fermentation temperature 30^{o}C were optimum conditions for the preparation of wine. Pomegranate wine has an appealing colour and a bench-mark level of alcohol content. This paper covers investigations and standardisation of the steps involved in wine manufacturing from pomegranates.

Keywords: Fermentation, *Saccharomyces cervisiae*, Pomegranate wine, Antioxidant, Phenolic content.

1. Introduction

India is the second largest producing country of pomegranates after Iran. Pomogranate botanically, *Punica granatum L.,* family: Punicaceae) is an ancient, beloved plant and fruit. It also known as grainy apple. Pomegranates can act as source of potent antioxidants. Pomegranate juice containing good amount of sugar rich in polyphenols, specifically ellagic acid and punicalagins[1]. Pomegranate wine is produced by fermentation growing yeast cells anaerobically where sugars are converted into carbon dioxide and alcohol[2]. The pomegranate wine containing the appreciable amount of alcohol. Different species of pomegranates cultivars are available like *Bhagwa, Ganesh, Ruby, Mridula and Arakta*[3]. These cultivars have been selected based on consumer preference for high sugar to high acid ratios, and dark red arils. *Bhagwa* has dark red arils which gives pleasant appearance to final wine and *Ganesha* has good sugar content which is beneficial for fermentation process in wine production[4].

Saccharomyces cerevisiae has been used for thousands of years as yeast in the fermentation processes for human household interest, especially to produce wine, brewer's yeast, and doughs. yeasts are responsible for the conversion of sugar into ethanol, carbon dioxide and hundreds of secondary products that collectively contribute to the quality of the product[5].

The soluble polyphenolic presence in pomegranate juice (0.2 to 1.0%) includes catechins, anthocyanins, gallic acids, ellagic acids and tannins[6]. To get the benefits better, consumption of wine should be limited to a glass or two a day, as yeast converts certain amount of sugar during fermentation into alcohol.

Fermentation is more often used as a process to preserve juices. Earlier researchers describe their conservation techniques, such as the storage in hermetically sealed vessels, the

installation of special fruit-rooms called *pomeriums*, the external fruit treatment with plaster, wax, or clay to prevent dehydration, and the short treatment in hot salty water to make the pericarp harder. Since then it has been well known that the fruit processing could add value to products, extending the shelf life and allowing the movement of the foods over long distances[7].

Now a days, wine consumers are willing to try new sensorial experiences: this is demonstrated by the current tendency towards wines with more complex organoleptic properties. It ultimately leads to the establishment of diversified fermentation processes *viz.* use of sequential or simultaneous co-inoculation with non-*Saccharomyces* yeasts and actual *Saccharomyces* species to enrich the flavoured production of spontaneously fermented wines with unique but non-reproducible characteristics (even if this might affect the microbiological stability of the product). While on other hand, novel fermented beverages are being created from different fruits and traditional beverages via fermentation were re-discovered[8].

Armenia and Israel are the major countries known for the production and large consumption of pomegranate wine as a part of their food tradition. In Italy, and specifically in Sicily, there are records of "pomegranate wine" beverages called "*Sciadde*", a tradition that has been lost. Thus, its too difficult to find scientific literature on this topic. Instead, a consistent literature exists on lactic acid bacteria fermentation of pomegranate juice, clearly indicating its potential as a fermented beverage[9].

2. Material and Methods

2.1 Extraction of juice: Ten kg of *Bhagwa* pomegranate destemmed manually were washed with KMS solution (0.01% w/v). By use of a knife, the part of the pomegranate 'crown' was removed. The pomegranate was scored into sections. The pomegranates into sections. The pomegranate arils were removed manually and pulsed it. The pomegranate arils were

poured into a blender until all have been crushed. The juice was poured through a strainer. This juice was supplemented with KMS, 0.01% and ammonium hydrogen phosphate about 0.025% (w/v). The TSS of the juice was measured as a Brix (B) with a hydrometer. In the present study T.S.S. content of known volume of juice was adjusted to required Brix by addition of 5% of sucrose[10]. The acidity was adjusted to 0.9 per cent by addition of citric acid so that the pH would be around 3.5 which is required for better fermentation and better quality of wine[11].

2.2 Alcoholic fermentation

2.2.1 Yeast inoculum

The yeast, *Saccharomyces cerevisiae* ATCC 204508 strain was used @ 5% (v/v), which was prepared in 500 ml of grape juice, 24h before preparation of juice as a standard practice. The inoculated juice was incubated at a temperature of 28-30°C for 24 h at 100 rpm.

2.2.2 Fermentation

The fermentation of pomegranate juice was carried out to determine the Brix values. The yeast inoculum prepared above was added to the juice in the 10L fermentation flask and incubated at 26±2°C. The fermentation was complete in 3 days when bubbling ceased, and the sediment settled at the bottom of the fermentation flask with final Brix falling to 0°B.

2.2.3 Post-fermentation treatment

The wine was kept undisturbed in a refrigerator for two days and decanted. The same was kept further at 15°C to settle the dead yeast and other sediments. This process of racking was repeated 3-4 times involving a settling time of at least two weeks in between or till there was no sediment. Finally, the clarified wine was bottled in 200 ml capacity bottles. The capped

bottles were then, pasteurized at 70°C for 10 min. and stored at room temperature. The bottles were cooled in a refrigerator before sensory evaluation.

2.2.4 Analyses

The ethanol concentration in the fermenting need to be analysed by official method. Besides, wines were also analysed for titrable acidity, pH (digital pH meter) and total phenolics. The sensory analysis was carried out by a panel of 7 judges on an 80-point modified hedonic scale[12] (Superior, 68- 80; Standard, 52-68; Below standard, 36-52 and Unacceptable/ Spoiled, 4-36).

2.2.5 Determination of alcohol content

2.2.5.1 Pharmacopeial method

Determined the alcohol content in wine by specific gravity method using the following procedure. To the distillation flask, 25 ml of preparation was added, and its temperature was noted. It was diluted with equal volume of water. Afterward, it was distilled and distillate about 2 ml less than the total volume was collected. Water was added to measure the same volume of original test liquid and adjusted to temperature which was already noted before. Specific gravity of this liquid was determined, and alcohol content was analysed using relative density table, which is prescribed by in official Pharmacopoeia[13].

2.2.5.2 Specific gravity method

About 25 ml of preparation was mixed separately with about 100 ml of water and saturated with sodium chloride. Then, 100 ml of hexane was added, and the mixtures were vigorously shaken for 2–3 min and were allowed to stand for 15–20 min. Then, the lower layers were run into the distillation flask, the hexane layer was washed by shaking vigorously with about 25 ml of sodium chloride solution, allowed to separate and washed liquors were run into the first saline solution. Mixed solutions were made alkaline with 1 M sodium hydroxide using solid phenolphthalein as indicator. Little pumice powder and 100 mL of

water were added. Mixture was then distilled and not less than 90 ml were collected and made up to 100 ml of distilled water, specific gravities of both mixtures were calculated[14].

2.2.6 Quality parameters of wines

2.2.6.1 Cold stability test[15]

2.2.6.1.1 Conductivity

The test is performed at -4° C for 4 hours and reports a percentage change in conductivity. If the drop is less than 5% a wine is considered stable for up to 6 days at -4° C and may not benefit from electrodialysis.

2.2.6.1.2 Refrigeration/brine

Filtered sample of the wine is held at -4^0 C for three days and then checked for any crystalline deposit.

2.2.6.1.3 Freeze/thaw

Around 8 cycles of freezing-thawing were conducted for each 4 h.

2.2.6.2 Heat stability test[16]

The sample of filtered wine was placed in a 10 ml of tube on 80°C pre-heated water bath. It is ensured that the entire volume of wine in tube is immersed in the water bath, but not the top of the tube. Simultaneously, other tube (the control sample) was kept at 25°C. The sample was heated at 80°C for two hours. After heating, the heated tube was removed from the water bath and left it to return to room temperature for 3 hours. After the cooling period, the heated sample was investigated for any haze by holding it against the strong light source and then compare it against the unheated control.

2.2.6.3 Methanol presence test in wine[17]

Methanol gets converted into formic acid during its metabolism and this formic acid causes toxicity that leads to liver failure, nerve damage (leading to blindness) and kidney/renal failure. Generally, 20-50ml of methanol is considered fatal. About 0.1 ml of potassium dichromate reagent was added to 1 ml of sample and allowed to stand at room temperature for 5 minutes. A violet colour at the junction of the two liquid layers depicts the presence of methanol in sample.

2.2.6.4 Determination of antioxidant activity of pomegranate wine

The antioxidant activity was analyzed according to the DPPH (2,2 diphenyl-1-picrylhydrazyl) method reported[18]. The antioxidant activity was calculated as Trolox equivalents (TE) per 100 mL of beverage (Eq. 1).

$$TE\left(\frac{mg}{100mL}\right) = \left(\frac{A-b}{m}\right) * DF * 100 \quad (1)$$

where *A* is the absorbance of the sample, *b* is the intercept, *m* is the slope of the standard curve, and *DF* is the dilution factor of the sample. In case of pomegranate juice, 5 µL sample was diluted with 1995 µL of absoulute ethanol with addition of 2.0 mL of DPPH solution, mixed thoroughly and analyzed at 517 nm by spectrophotometer.

2.2.6.5 Total phenolic compounds

The investigation of total phenolic compounds were carried out using the Folin–Ciocalteu method with some modifications[19]. The total phenolic compounds content was calculated as Gallic acid (GA) per 100 mL of juice (Eq. 2).

$$GA\left(\frac{mg}{100mL}\right) = \left(\frac{A-b}{m}\right) * DF * 100$$

where *A* is the absorbance of the sample, *b* is the intercept, *m* is the slope of the standard curve and *DF* is the dilution factor of the sample. For pomegranate, 10 µL of beverages,

3990 µL of water, 250 µL of Folin–Ciocalteu regent, 750 µL of 20% NA_2CO_3 were mixed, left for 2 h, and analyzed By Spectrophotometry At 765 Nm.

3. Results and Discussion

3.1 Alcoholic fermentation

The alcoholic fermentation carried out with *S. cerevisae* strain revealed the consumption of sugar (decrease in Brix) with time which was accompanied with an increase in ethanol content. It is a typical trend of ethanolic fermentations[20].

It was evident that blending increases the content of TSS, TRS and acid content alcohol content also increased. TRS estimation was undertaken at initial level of sucrose utilization by ethanolic yeasts to its complete hydrolysis into glucose and fructose by an extracellular enzyme invertase[21]. In sugarcane samples complete glucose consumption on one hand and on the other fructose in pomegranate arils produced during fermentation by sucrose hydrolysis accumulated in the medium possibly due to the impairment of fructose transport system, hence more fructose was analytically detected at the end of fermentation was reported[21]. A similar result was published for ethanolic fermentation of sweet sorghum juice[22]. Thus, maximum alcohol production (10.5%-11.3%) was observed. Pasteurization of juice results in less alcohol per cent in comparison of non-pasteurized juice which might be due to the presence of wild microorganisms in the fermenting substrate which enhances the utilization of sugar and increasing in ethanol production[23]. The overall pasteurized samples are higher in TSS, TRS and acidity but less in alcohol % comparative to non-pasteurized samples. As one may expect, there would be high alcohol content in wines, but TSS contents may lower. At the end part of fermentation, least change was detected in sugar concentration but after 3 months of storage it showed a slight decrease in TSS, reducing sugar but increase in the alcohol % and acidity. These variations in values might be due to further utilisation of

the remaining sugar by the fermenting yeast left and converting the sugar to CO2 and ethanol[24].

The bottled clarified/ racked wine was stored for 3 months and evaluated for chemical and sensory characteristics. As 70% of the total amount of alcohol was produced during primary fermentation which last up to (3-7) days and the remaining 30% was produced by secondary fermentation that last up to two weeks. The TRS (total reducing sugar) content was also found decreased likewise to TSS while alcohol content of wine was found increased after preparation up to 3 months storage. The typical quality test results are shown in Table-1

Table 1. Quality control tests on pomegranate wine after fermentation and after 3-month storage

	Wine after fermentation	**After 3-month storage**
pH (F)	3.81±0.12	3.97±0.15
TSS (F)	7.26±0.10	6.7±0.14
Alcohol %	7.45±0.04	7.75±0.05
TA %	0.21±0.02	0.26±0.04
TRS %	1.45±0.04	1.23±0.04
Total phenolics (mg/L)	2358±23.8	2567.3±28.9
Sensory score (80)	60.7±2.44	68.3±2.03
Rate of Brix (ºB) utilization	6.1 ± 0.56	
Rate of Ethanol production per day (%)	3.29 ± 0.24	
Fermentation efficiency (%)	90.4	
Recovery (%, v/v)	62.4	

3.2 Physico-chemical characteristics

3.2.1 Sensory evaluation

The parameters such as colour (Tan Brown), odour (Fruity), taste (Fruity) and clarity (Opaque) were evaluated having results satisfactory.

3.2.2 Determination of relative density

The relative density of wine was found to be 0.988. The density of wine juice quite obviously higher than water, it is thicker. The typical density or specific gravity of the must (the term we give to wine before we add or pitch the yeast) is generally between 1.080 and 1.090. This essentially means your wine is 8-9% denser than water.

3.2.3 Quality parameters of wines

Cold stability test involves holding a sample of filtered wine at -4°C for three days and then inspecting for any sign of crystalline tartrate precipitation. The wine is generally interpreted as being cold stable if crystalline deposits are observed to redissolve upon warming to ambient temperature however the presence of any persistent crystalline particles indicate that the wine is cold unstable[25]. The results are expressed as a 'pass' since there was no permanent crystalline deposit after refrigeration, which were also detected by warming at ambient temperature.

Heat instability and freeze-thaw cycle test for wine is generally conducted to investigate precipitation of unstable proteins. Most wineries expel visible and unstable proteins from wine before bottling by fining with adsorbent bentonite. The bentonite adsorbs and minimizes the precipitated protein if any to lowest levels; however, several factors can affect this stability and it is important to test heat stability prior to bottling[26].

The change in conductivity of sample can be observed by addition of potassium bitartrate seed crystals (1 g/L). The conductivity of sample was observed stable and satisfactory before and after addition of potassium bitartrate (KHT). If the change in conductivity of the sample is 5% or more (due to precipitation of KHT and a resulting decrease in conductivity), the sample is unstable with respect to KHT[15]. The critical quality attributes are shown in Table-2.

Table 2. Critical quality tests on pomegranate wine after production

Test	Observation
Cold stability test	No settlings were seen
Heat stability test	No settlings were seen
Methanol presence test	No violet ring was appeared hence methanol is absent
Conductivity (Before KHT Addition)	0.393±0.002 S/m at 30℃
Conductivity (After KHT Addition)	0.333±0.004 S/m at 30℃

3.2.4 Antioxidant activity

The antioxidant activity of pomegranate fresh juice (PFJ) was observed as 432.18 ± 0.22 mg TE/100 mL. According to earlier reports[27] the antioxidant activity of commercial pomegranate juice was higher than that in red wine (354.89 mg TE/100 mL) and green tea (276.02 mg TE/100 mL). On the other hand, the antioxidant activity of pomegranate wine (PW) was observed to be 262.12 ± 0.12 mg TE/100 mL according to earlier research[28].

Table 3. Determination of antioxidant activity and total phenolic content to estimate therapeutic potential

	(PFJ)	(PW)
Antioxidant activity (mg TE/100 mL)	432.18 ± 0.22	281.65 ± 0.11b
Total phenolics (mg GA/100 mL)	382.78 ± 0.13	232.28 ± 0.13

3.2.5 Total phenolic compounds

Pomegranate is rich in phenolic compounds such as gallic and ellagic acid, tannins, anthocyanins *p*-coumaric acid, and catechin. The total phenolic compounds content in this study (PFJ) was observed as 382.78 ± 0.13 mg GA/100 mL and in case of pomegranate wine (PW) was observed to be 232.28 ± 0.13 mg GA/100 mL as ellagic acid, punicalagin and gallic acid could be the major phenolic compounds in pomegranate fruit[6]. The estimation of antioxidant activity and total phenolic content (Table 3) indirectly suggests therapeutic importance of pomegranate wine.

4. Conclusion

It is possible to achieve a complete fermentation of pomegranate juice with varied experimental settings. This work could be the base perfectioning the fermentation process with consideration of quality parameters, thus opening new exploited market opportunities. The combination of different cultivars and yeasts could be a powerful leverage to tailor pomegranate wines with desired chemical profiles and, consequently, sensory properties, including the organic acid profile, as well as colour and volatile pattern. Considering the current interest in pomegranate beverages caused due to their well-known beneficial properties, pomegranate wine is a promising candidate as a new commercial standard product for large consumption around the globe. Further studies will be necessary to investigate the perceptions of new potential consumers for these products in different countries, from both the organoleptic and commercial points of view. As this method is cheap, safe, and easily applicable with cost effective approach.

5. References

1. Zarfeshany A, Asgary S, Javanmard SH. Potent health effects of pomegranate. Adv Biomed Res. 2014 Mar 25;3:100. doi: 10.4103/2277-9175.129371. PMID: 24800189; PMCID: PMC4007340.

2. Dżugan M, Wesołowska M, Zaguła G, Puchalski C. The comparison of the physicochemical parameters and antioxidant activity of homemade and commercial pomegranate juices. Acta Sci Pol Technol Aliment. 2018 Jan-Mar;17(1):59-68. doi: 10.17306/J.AFS.0529.

3. Hasnaoui, Nejib & Mars, Messaoud & Ghaffari, Sana & Trifi, Mokhtar & Melgarejo, Pablo & Hernandez, Francisca. (2011). Seed and juice characterization of pomegranate fruits grown in Tunisia: Comparison between sour and sweet cultivars revealed interesting properties for prospective industrial applications. Industrial Crops and Products. 33. 374-381. 10.1016/j.indcrop.2010.11.006.

4. Cardinale M, Trinchera R, Natrella G, Difonzo G, De Benedittis C, D'amato I, Mascellani M, Paradiso VM, Rustioni L. Dynamics of the Fermentation Process and Chemical Profiling of Pomegranate (*Punica granatum* L.) Wines Obtained by Different Cultivar×Yeast Combinations. Foods. 2021 Aug 18;10(8):1913. doi: 10.3390/foods10081913. PMID: 34441690; PMCID: PMC8392717.

5. Maicas S. The Role of Yeasts in Fermentation Processes. Microorganisms. 2020 Jul 28;8(8):1142. doi: 10.3390/microorganisms8081142. PMID: 32731589; PMCID: PMC7466055.

6. Munteanu IG, Apetrei C. Analytical Methods Used in Determining Antioxidant Activity: A Review. Int J Mol Sci. 2021 Mar 25;22(7):3380. doi: 10.3390/ijms22073380. PMID: 33806141; PMCID: PMC8037236.

7. Cardinale M, Trinchera R, Natrella G, Difonzo G, De Benedittis C, D'amato I, Mascellani M, Paradiso VM, Rustioni L. Dynamics of the Fermentation Process and Chemical Profiling of Pomegranate (*Punica granatum* L.) Wines Obtained by Different Cultivar×Yeast Combinations. Foods. 2021 Aug 18;10(8):1913.

doi: 10.3390/foods10081913. PMID: 34441690; PMCID: PMC8392717.

8. Xu A, Xiao Y, He Z, Liu J, Wang Y, Gao B, Chang J, Zhu D. Use of Non-*Saccharomyces* Yeast Co-Fermentation with *Saccharomyces cerevisiae* to Improve the Polyphenol and Volatile Aroma Compound Contents in Nanfeng Tangerine Wines. J Fungi (Basel). 2022 Jan 27;8(2):128. doi: 10.3390/jof8020128. PMID: 35205881; PMCID: PMC8875693.

9. Cardinale, Massimiliano & Trinchera, Roberto & Natrella, Giuseppe & Difonzo, Graziana & Benedittis, Carlo & D'amato, Ilario & Mascellani, Marco & Paradiso, Vito & Rustioni, Laura. (2021). Dynamics of the Fermentation Process and Chemical Profiling of Pomegranate (Punica granatum L.) Wines Obtained by Different Cultivar×Yeast Combinations. Foods. 10. 1913. 10.3390/foods10081913.

10. Zangué Steve Carly, D., & Emmanuel Jong, N. (2019). Sorghum Coffee–Lactose Stout Production and Its Physico-Chemical Characterization. Beverages, 5(1), 20.

11. Resende Oliveira É, Caliari M, Soares Soares Júnior M, Ribeiro Oliveira A, Cristina Marques Duarte R, Valério de Barros Vilas Boas E. Assessment of chemical and sensory quality of sugarcane alcoholic fermented beverage. J Food Sci Technol. 2018 Jan;55(1):72-81. doi: 10.1007/s13197-017-2792-4. Epub 2017 Nov 4. PMID: 29358797; PMCID: PMC5756184.

12. Bianchi A, Taglieri I, Venturi F, Sanmartin C, Ferroni G, Macaluso M, Palla F, Flamini G, Zinnai A. Technological Improvements on FML in the Chianti Classico Wine Production: Co-Inoculation or Sequential Inoculation? Foods. 2022 Mar 30;11(7):1011. doi: 10.3390/foods11071011. PMID: 35407098; PMCID: PMC8997436.

13. Government of India, Ministry of Health. Pharmacopoeia of India : (the Indian Pharmacopoeia). Delhi: Manager of Publications, 1955.

14. A standardization Of Methods for Determination of The Alcohol Content of

Beverages and Distilled Potable Spirits. Pure Appl Chem. 1968;17(2):273-312. doi: 10.1351/pac196817020273. PMID: 5720568.

15. Haze and Sediments. Understanding Cold Stability. 12June2023 https://www.etslabs.com/publications/publication/16

16. Haze and Sediments Interpretating Heat Stability. 12June2023 https://www.etslabs.com/publications/publication/20

17. Methanol Poison Testing. 12June2023. https://www.arishtam.com/methanol-poison-testing/troubleshooting

18. Clarke G, Ting KN, Wiart C, Fry J. High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen for Antioxidant Activity of Extracts of Plants from the Malaysian Rainforest. Antioxidants (Basel). 2013 Jan 4;2(1):1-10. doi: 10.3390/antiox2010001. PMID: 26787618; PMCID: PMC4665400.

19. Singleton L, Rudolf O, Rosa M. Lamuela-Raventós. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology, 1999,299, Pages 152-178, https://doi.org/10.1016/S0076-6879(99)99017-1.

20. Soni SK, Bansal N and Soni R. 2009. Standardization of conditions for fermentation and maturation of wine from Amla (Emblica officinalis Gaertn.). Natural Product Radiance, 8: 436-444.

21. Guillaume C, Delobel P, Sablayrolles JM, Blondin B. Molecular basis of fructose utilization by the wine yeast Saccharomyces cerevisiae: a mutated HXT3 allele enhances fructose fermentation. Appl Environ Microbiol. 2007 Apr;73(8):2432-9. doi: 10.1128/AEM.02269-06. Epub 2007 Feb 16. PMID: 17308189; PMCID: PMC1855598.

22. Liang Y, Sarkany N, Cui Y, Yesuf J, Trushenski J, Blackburn JW. Use of sweet sorghum juice for lipid production by Schizochytrium limacinum SR21. Bioresour Technol. 2010 May;101(10):3623-7. doi:

10.1016/j.biortech.2009.12.087. Epub 2010 Jan 15. PMID: 20079633.

23. Ferreira RM, Costa AM, Pinto CA, Silva AMS, Saraiva JA, Cardoso SM. Impact of Fermentation and Pasteurization on the Physico-Chemical and Phytochemical Composition of *Opuntia ficus-indica* Juices. *Foods*. 2023; 12(11):2096. https://doi.org/10.3390/foods12112096

24. Dashko S, Zhou N, Compagno C, Piškur J. Why, when, and how did yeast evolve alcoholic fermentation? FEMS Yeast Res. 2014 Sep;14(6):826-32. doi: 10.1111/1567-1364.12161. Epub 2014 Jun 9. PMID: 24824836; PMCID: PMC4262006.

25. Xia N, Cheng H, Yao X, Pan Q, Meng N, Yu Q. Effect of Cold Stabilization Duration on Organic Acids and Aroma Compounds during *Vitis vinifera* L. cv. Riesling Wine Bottle Storage. Foods. 2022 Apr 19;11(9):1179. doi: 10.3390/foods11091179. PMID: 35563903; PMCID: PMC9103779.

26. Cosme F, Fernandes C, Ribeiro T, Filipe-Ribeiro L, Nunes FM. White Wine Protein Instability: Mechanism, Quality Control and Technological Alternatives for Wine Stabilisation—An Overview. *Beverages*. 2020; 6(1):19. https://doi.org/10.3390/beverages6010019

27. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J Agric Food Chem.* 2000;48(10):4581–4589. doi: 10.1021/jf000404a.

28. Çam M, Hışıl Y, Durmaz G. Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food Chem.* 2009;112(3):721–726. doi: 10.1016/j.foodchem.2008.06.009.