

Comparative HPLC-Based Profiling of Quercetin in Wines Prepared from Chikoo (*Manilkara zapota*), Dragon Fruit (*Hylocereus undatus*), and Mandarin Orange (*Citrus reticulata*)

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Abstract

Quercetin is a bioactive flavonol distributed widely across fruits and vegetables. Despite extensive characterisation of quercetin in grape-derived wines, comparative data on its post-fermentation concentration in non-grape tropical fruit wines remain absent from the published literature. This study aimed to produce wines from chikoo (*Manilkara zapota*), dragon fruit (*Hylocereus undatus*), and mandarin orange (*Citrus reticulata*) and to quantify quercetin in each wine by reverse-phase HPLC (RP-HPLC). In addition, ethanol content and radical scavenging activity were determined in each wine. Fruit wines were produced using *Saccharomyces cerevisiae* at room temperature (25±2°C) and 37°C over 14 days. Quercetin was quantified by RP-HPLC on a C18 column against a 50 µg/mL external standard. Mandarin orange wine contained the highest post-fermentation quercetin concentration (44.76 mg/L), followed by chikoo wine (20.51 mg/L) and dragon fruit wine (20.50 mg/L). Mandarin orange wine also yielded the highest ethanol content (6.50% v/v) prepared at room temperature. Dragon fruit wine exhibited the greatest DPPH radical scavenging activity (41.86%), consistent with its beta l- and polyphenol content. All three fruit wines contained detectable quercetin concentrations within the range reported for non-grape berry wines. Mandarin orange wine emerged as the most promising candidate for further nutraceutical development. This study provides the first comparative RP-HPLC dataset for quercetin in chikoo, dragon fruit, and mandarin orange wines.

1. Introduction:

The concept of functional food has gained considerable interest over the past two decades as consumers and researchers have increasingly recognized the therapeutic

potential of diet in preventing chronic diseases. Functional foods are defined as those that, beyond meeting basic nutritional requirements, confer health beneficial effect attributed to specific biologically active compounds [1-2]. Among the most extensively

investigated categories of bioactive compounds are polyphenols—a structurally diverse group of plant-derived secondary metabolites unified by one or more hydroxyl groups on aromatic ring systems—classified broadly into flavonoids (flavonols, flavanones, anthocyanins, isoflavones, flavones, and flavan-3-ols) and non-flavonoid compounds (phenolic acids, stilbenes, and lignans) [1,3].

Dietary polyphenols exert their health beneficial effects primarily through antioxidant mechanisms—scavenging reactive oxygen species (ROS) and chelating transition metals—and through modulation of cell-signaling cascades involved in inflammation, apoptosis, and cellular differentiation [1,4,5].

Of the more than 8,000 identified flavonoids in the plant kingdom, quercetin (3,3',4',5,7-pentahydroxyflavone) is among the most abundant and pharmacologically characterized flavonoids [3,7]. Belonging to the flavonol subclass, it is distinguished by a pentahydroxy substitution pattern on the 2-phenylbenzopyran-4-one backbone, which confers potent electron-donating capacity and enables neutralization of a wide spectrum of free radicals [3]. Its anti-inflammatory activity is mediated through inhibition of NF- κ B signaling, suppression of pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6), and inhibition of cyclooxygenase and lipoxygenase enzymes [4,6]. Its cardioprotective effects encompass reduction of low-density lipoprotein (LDL) oxidation, upregulation of endothelial nitric oxide synthase (eNOS), improvement of endothelial function, and attenuation of platelet aggregation [4,9]. Estimated daily dietary intake ranges from 50 to 800 mg depending on population and dietary pattern [8].

Wine production has historically been synonymous with *Vitis vinifera*, but there is growing recognition that fermentation of non-grape fruits yields beverages of significant nutraceutical value [2,10]. Fermentation is not merely a preservation technique but a biochemical transformation that profoundly alters the phytochemical profile of the substrate. Yeast-secreted glycosidases, esterases, and phenolic acid decarboxylases hydrolyze glycosylated and esterified polyphenols into their aglycone forms, which generally exhibit superior bioavailability compared with their precursors [11-12]. The global non-grape fruit wine market has expanded substantially in recent years, driven by consumer interest in novel flavor profiles, perceived health benefits, and

the nutraceutical premiumization of tropical fruit-derived beverages [2,13].

Chikoo or Sapodilla (*Manilkara zapota* L., Sapotaceae) is cultivated extensively across South and Southeast Asia and is valued for its sweet, granular flesh and diverse polyphenol composition, including ellagitannins, gallotannins, phenolic acids, and flavonoids such as quercitrin, myricitrin, catechin, epicatechin, and gallic acid [14-16]. Red dragon fruit (*Hylocereus undatus*, Cactaceae) is notable for its exceptional betalain content—comprising betacyanins and betaxanthins—which confer potent antioxidant, anti-cancer, anti-lipidemic, and antimicrobial properties, alongside significant quantities of flavonoids, phenolic acids, and ascorbic acid [17-18]. Mandarin orange (*Citrus reticulata* Blanco) contains a rich repertoire of bioactive flavonoids, including the dominant flavanones hesperidin and narirutin, polymethoxylated flavones such as nobiletin and tangeretin in peel fractions, and quercetin together with its glycosidic derivatives, which are particularly concentrated in the flavedo [19-21].

Accurate quantification of individual flavonoids in complex food matrices requires analytical specificity that bulk spectrophotometric methods cannot provide. Reverse-phase HPLC coupled with diode array detection (RP-HPLC-DAD) enables simultaneous detection and quantification of multiple phenolic compounds in a single run, using both retention time and UV-visible spectral data for compound identity confirmation [22-25].

Despite extensive literature on quercetin in grape-derived wines, comparable data for non-grape tropical fruit wines remain absent. No published study has undertaken a comparative, HPLC-based evaluation of quercetin content across wines produced simultaneously from chikoo, dragon fruit, and mandarin orange under controlled conditions. In light of this, the present study was designed to produce wines from chikoo, dragon fruit, and mandarin orange under standardized laboratory conditions using *Saccharomyces cerevisiae* as the fermenting organism; to apply RP-HPLC for the quantification of quercetin in the three fruit wines; and to comparatively evaluate and contextualize post-fermentation quercetin concentrations across all three wine matrices. In addition, total reducing sugar, ethanol concentration, and antioxidant activity by the (2,2-diphenyl-1-picrylhydrazyl) DPPH scavenging method were also evaluated in the three different wines.

2. Materials and Methods:

2.1 Study Area and Sample Collection

All fruit samples were procured fresh from local retail markets in the Vasai-Virar region of Maharashtra, India. Chikoo (*Manilkara zapota* L.) was obtained from a marketplace in Virar; dragon fruit (*Hylocereus undatus*) was sourced from a market in Mira Road; and mandarin orange (*Citrus reticulata*) was purchased from Vasai. Fruits were selected at full commercial ripeness based on visual inspection and tactile assessment, transported in clean polyethylene bags, and processed within 24 hours of purchase to minimize post-harvest polyphenol degradation.

2.2 Pretreatment of Fruit Samples

Chikoo: Fruits were washed, manually peeled, and seeds were removed. The flesh was mashed to a homogeneous pulp, juice was extracted, and the pulp was diluted with distilled water (1:10 w/v), then gently heated to 60-65°C for 15 minutes to reduce microbial load.

Dragon fruit: The pink exterior rind was removed, and the white pulp was processed in a laboratory blender to produce a smooth, homogeneous paste, used without dilution to preserve the native polyphenol and betalain content.

Mandarin orange: fruits were washed, the outer peel was removed manually, seeds were extracted, and the flesh was blended and strained through double-layered muslin cloth, yielding a clarified juice, which was autoclaved at 121°C for 15 minutes at 15 psi.

2.3 Estimation of Reducing Sugars by the DNS Colorimetric Method

Reducing sugar concentrations were determined before and after fermentation using the 3,5-dinitrosalicylic acid (DNS) colorimetric method as described by Miller (1959), with minor modifications [26]. Briefly, 1 mL of appropriately diluted sample was combined with 3 mL of freshly prepared DNS reagent and incubated in a boiling water bath at 100°C for exactly 15 minutes. After rapid cooling, absorbance was measured at 530-540 nm using a digital colorimeter. A glucose standard calibration series was prepared at concentrations of 200, 400, 600, 800, and 1000 µg/mL. Results were expressed as µg glucose equivalents per mL (µg GE/mL). All measurements were performed in triplicate.

2.4 Wine Fermentation

Equal volumes (approximately 150 mL) of each pretreated fruit substrate were dispensed into two 250-mL Erlenmeyer flasks designated Flask 1 (room temperature fermentation) and Flask 2 (37°C fermentation). All flasks were autoclaved at 121°C for 15 minutes. Active dry *Saccharomyces cerevisiae* was rehydrated in sterile warm distilled water (35-40°C) for 15-20 minutes and inoculated into each flask at approximately 1-2% (v/v) of the substrate volume [19-20]. The pH of each flask was adjusted to 5.0-5.5. Flask 1 was incubated at 25 ± 2°C on a rotary shaker at 120 rpm; Flask 2 was incubated at 37°C in a thermostatically controlled incubator shaker at 120 rpm. Fermentation was proceeded for 14 days.

2.5 Determination of Ethanol Content by the Dichromate Oxidometric Method

Ethanol content was determined by potassium dichromate oxidation and back-titration of unreacted dichromate with Ferrous Ammonium Sulphate (FAS) [27-28]. A 2 mL wine sample was treated with an excess of standardized 0.1 N K₂Cr₂O₇ in 6 N H₂SO₄ and heated at 60°C for 20 minutes. After cooling, the unreacted dichromate was back-titrated against 0.1 N FAS using ferroin as a redox indicator. Ethanol content (% v/v) was estimated using the formula $7 - 7 [V_a / V_b]$, where V_a is the volume (mL) of FAS consumed for the sample and V_b is the volume (mL) consumed for the reagent blank.

2.6 Assessment of Antioxidant Activity by the DPPH Radical Scavenging Assay

Free radical scavenging activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay [29-30]. Three reaction mixtures were prepared: a blank (4 mL methanol), a reference (1 mL methanol + 3 mL of 0.1 mM DPPH in methanol), and a sample (1 mL of fruit wine + 3 mL of 0.1 mM DPPH in methanol). All tubes were covered with aluminum foil and incubated in darkness at room temperature for 30 minutes. Absorbance was recorded at 517 nm. Scavenging Activity (%) = $[(A_0 - A_s) / A_0] \times 100$, where A₀ denotes the absorbance of the unreacted DPPH reference and A_s denotes the absorbance of the reaction mixture after sample addition.

2.7 RP-HPLC Quantification of Quercetin

Wine samples exhibiting the highest ethanol content from each fruit type were selected for HPLC analysis. Each sample was diluted 1:10 (v/v) in HPLC-grade methanol, subjected to ultrasonication in three successive cycles of 15 minutes each, then centrifuged at

4,000 rpm for 15 minutes, and the clarified supernatant was filtered through a 0.22 µm nylon membrane syringe filtered into amber HPLC-compatible vials [24-25]. An analytical-grade quercetin reference standard was dissolved in HPLC-grade methanol to prepare a working standard of 50 µg/mL. Chromatographic separation was performed on a Jasco LC-2000 HPLC system on a C18 reverse-phase column (4.6 mm x 250 mm, 5 µm particle size). Mobile phase: HPLC-grade acetonitrile: water (95:5 v/v), delivered isocratically at 1.0 mL/min; detection wavelength: 280 nm; injection volume: 10 µL; acquisition time: 6.9 min per run. Quercetin concentration (µg/mL) = [AUC (sample) / AUC (standard)] x 50.

3. Result:

3.1 DNS Standard Calibration Curve

A standard calibration curve for glucose was constructed at five concentration points (200-1000 µg/mL) and absorbance was recorded at 530-540 nm (table 1). The relationship between glucose concentration and absorbance was linear across the working range ($R^2 > 0.98$), validating the assay for extrapolation of reducing sugar concentrations in all sample matrices.

Table 1. DNS Standard Calibration Data (Glucose, lambda = 530-540 nm)

Tube	Glucose Concentration (µg/mL)	Absorbance (530-540 nm)
Blank	0	0.000
1	200	0.07
2	400	0.15
3	600	0.21
4	800	0.32
5	1000	0.38

3.2 Reducing Sugar Analysis Before and After Fermentation

Reducing sugar concentrations in the raw fruit substrates and in post-fermentation wine samples from both incubation conditions are summarized in table 2. Active sugar consumption was confirmed in all three substrates, consistent with successful yeast-mediated

alcoholic fermentation. Chikoo demonstrated the highest raw reducing sugar content (950 µg/mL). Fermentation at RT reduced sugar by 310 µg/mL (32.6% utilization), while fermentation at 37°C resulted in a reduction of 445 µg/mL (46.8% utilization). Dragon fruit recorded a baseline reducing sugar content of 480 µg/mL, with sugar utilization of 33.3% at RT and 43.8% at 37°C. Mandarin orange showed a raw reducing sugar content of 800 µg/mL. Notably, the post-fermentation reducing sugar concentration in the mandarin orange 37°C flask was elevated to 1000 µg/mL, attributed to enzymatic hydrolysis of pectin, which liberates reducing monosaccharides at a rate exceeding concurrent yeast-mediated hexose consumption [11,31].

Table 2. Reducing Sugar Concentrations Before and After 14-Day Fermentation (DNS Colorimetric Method)

Sample	Raw (µg/mL)	Post-Ferm. RT (µg/mL)	Post-Ferm. 37°C (µg/mL)	Sugar Used RT	Sugar Used 37°C
Chikoo	950	640	505	310 (32.6%)	445 (46.8%)
Dragon Fruit	480	320	270	160 (33.3%)	210 (43.8%)
Mandarin Orange	800	600	1000*	-200 (net increase)	450 (apparent only)

Post-fermentation value at 37°C exceeds the raw value due to concurrent enzymatic hydrolysis of complex polysaccharides (pectin) liberating additional reducing monosaccharides. RT = Room Temperature (25 ± 2°C).

3.3 Ethanol Content by the Dichromate Oxidimetric Method:

Ethanol concentrations in the RT and 37°C fermented wines are presented in table 4. Mandarin Orange wine produced the highest ethanol content across both fermentation conditions (6.50% v/v at RT; 6.20% v/v at 37°C). Dragon Fruit wine yielded comparable ethanol at both temperatures (3.95-3.98% v/v). Chikoo wine produced marginally lower ethanol at 37°C (2.87%) than at RT (3.50%).

Table 3. Ethanol Content (% v/v) in Fruit Wines Determined by the Dichromate Oxidimetric Method

Sample	Ethanol % v/v (RT)	Ethanol % v/v (37°C)	Blank Titre (mL)
Chikoo Wine	3.50	2.87	46
Dragon Fruit Wine	3.95	3.98	46
Mandarin Orange Wine	6.50	6.20	48

RT = Room Temperature ($25 \pm 2^\circ\text{C}$). Ethanol % v/v = $7 - 7[V_a/V^b]$; V_a = FAS titre for sample; V^b = FAS titre for blank. Blank titre = volume (mL) of FAS consumed in the absence of wine.

3.4 Antioxidant Activity: DPPH Radical Scavenging Assay

DPPH radical scavenging activities of the three fruit wines are presented in Table 3. Dragon Fruit wine exhibited the highest scavenging activity (41.86%), followed by Mandarin Orange wine (27.27%) and Chikoo wine (17.12%), reflecting the distinct polyphenolic architectures of the three fruit substrates.

Table 4. DPPH Radical Scavenging Activity of Fruit Wines

Sample	A ₀ (Before DPPH)	A _s (After DPPH)	Scavenging Activity (%)
Chikoo Wine	1.46	1.21	17.12
Dragon Fruit Wine	1.72	1.00	41.86
Mandarin Orange Wine	1.03	0.78	27.27

A₀ = absorbance of unreacted DPPH reference control; A_s = absorbance of sample–DPPH reaction mixture. Scavenging Activity (%) = $[(A_0 - A_s)/A_0] \times 100$. Values represent the mean of three independent readings

3.5 RP-HPLC Quantification of Quercetin

Chromatographic separation (figure 1) resolved a distinct, symmetrical peak at t_R approximately 2.783 min for the quercetin external standard (50 µg/mL; AUC = 743,844 uV.sec). A commercial port wine positive control yielded an AUC of 1,263,328 uV.sec, corresponding to a

quercetin concentration of 84.91 mg/L. Among the experimental fruit wines, Mandarin Orange wine demonstrated the highest quercetin concentration (44.76 mg/L; AUC = 665,822 uV.sec), followed by Chikoo wine (20.51 mg/L; AUC = 305,176 uV.sec) and Dragon Fruit wine (20.50 mg/L; AUC = 305,835 uV.sec). Complete data is presented in table 5 and the chromatograms are shown in figures 1a to 1e.

Table 5. RP-HPLC Quercetin Quantification in Fruit Wines (External Standard: 50 µg/mL; t_R approx. 2.783 min; lambda = 280 nm)

Sample	AUC (uV.sec)	Quercetin Concentration (mg/L)
Quercetin Standard (50 µg/mL)	743,844	50.00 (reference)
Port Wine (positive control)	1,263,328	84.91
Chikoo Wine	305,176	20.51
Dragon Fruit Wine	305,835	20.50
Mandarin Orange Wine	665,822	44.76

3.6 Sensory Characterization

Organoleptic properties of the three fruit wines, assessed by a trained laboratory panel at the end of the 14-day fermentation period, are summarized in table 6

Table 6. Organoleptic Properties of the Three Fruit Wines After 14-Day Fermentation

Attribute	Chikoo Wine	Dragon Fruit Wine	Mandarin Orange Wine
Color	Dark maroon	White	Bright orange
Odor	Alcoholic	Pungent	Citrusy
Flavour	Distinct; woody notes	Unfavourable; requires improvement	Citrusy; pleasant; favourable
Texture	Thick; viscous	Thin; fluid	Smooth; velvety

Chikoo wine is presented as dark maroon with a pronounced alcoholic odor and markedly thick viscosity. Dragon fruit wine appeared as a clear white liquid consistent with the white-fleshed *H. undatus* variety used in this study. Mandarin Orange wine exhibited a bright orange colour.

anomalous net increase in reducing sugars in the mandarin orange must at 37°C (from 800 µg/mL raw to 1000 µg/mL post-fermentation) is attributed to the

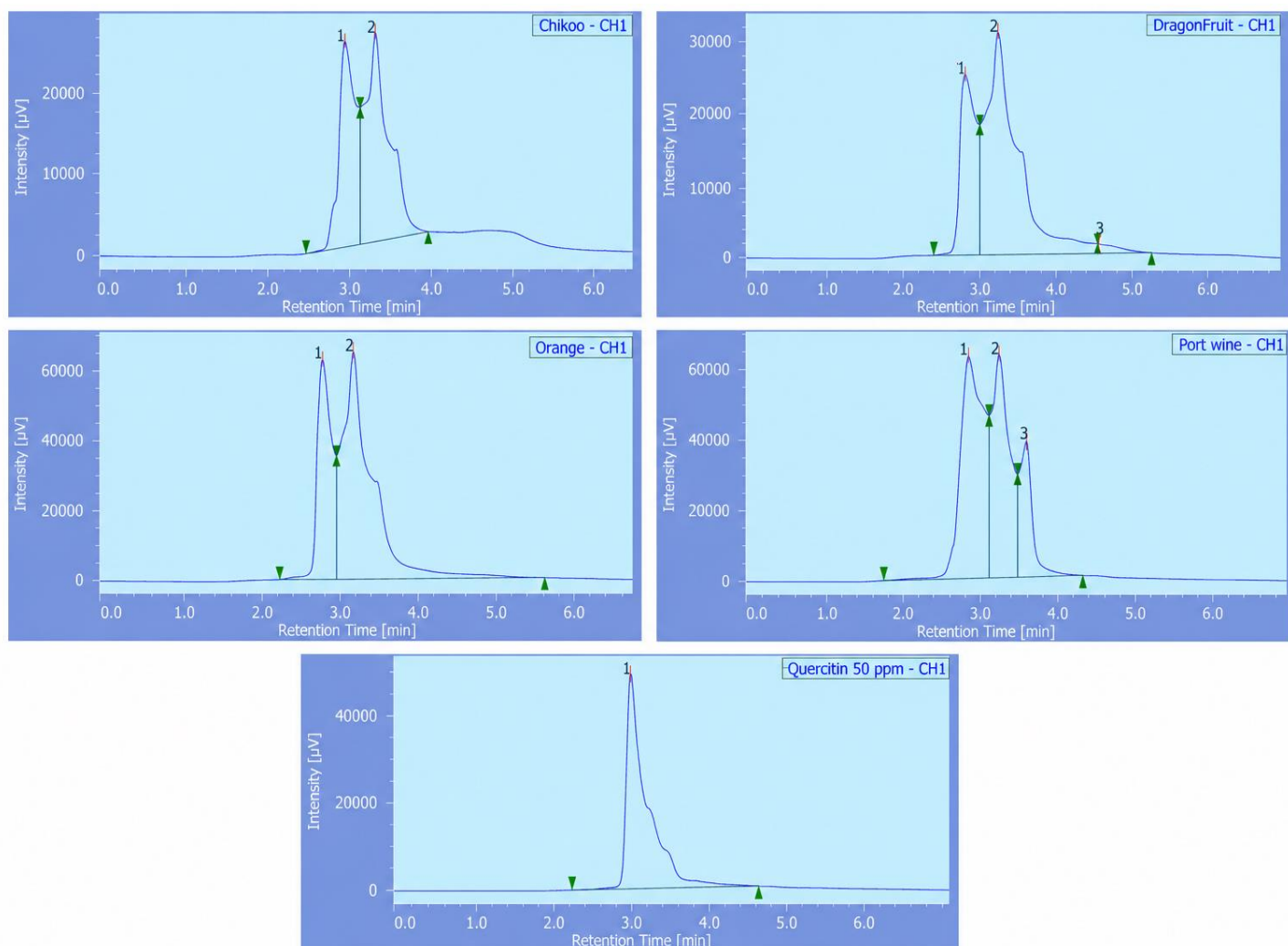


Figure 1. Chromatograms for estimation of quercetin in various samples top; (a) Chickoo (b) Dragon fruit, middle; (c) Orange (d) Port wine, bottom; (e) Quercetin (50 ppm)

4. Discussion:

The DNS reducing sugar data confirms that all three fruit substrates underwent active alcoholic fermentation during the 14-day incubation period. Sugar utilization was consistently greater at 37°C than at room temperature in the chikoo and dragon fruit substrates, consistent with the enhanced glycolytic activity of *Saccharomyces cerevisiae* at temperatures approaching its thermal optimum for fermentation [32-33]. The

combined activity of endogenous fruit pectin-methyl esterases and yeast-associated cell-wall-degrading enzymes, which release galacturonic acid monomers and other reducing sugars at a rate exceeding yeast-mediated hexose consumption [11,31].

The lower-than-expected ethanol yield from chikoo despite its high raw sugar content (950 µg/mL) is a notable finding. Chikoo pulp is characteristically rich in insoluble dietary fiber, pectin, and condensed tannins

that collectively increases substrate viscosity. High-viscosity fermentation media restrict yeast motility, impede substrate-enzyme contact, and limit CO₂ escape, all of which reduce overall fermentation efficiency [10,21].

The DPPH radical scavenging hierarchy observed—Dragon Fruit (41.86%) > Mandarin Orange (27.27%) > Chikoo (17.12%)—is consistent with the known antioxidant capacity of the source fruits yet diverges from the quercetin ranking (Mandarin Orange > Chikoo approximately equals Dragon Fruit). This deviation is expected as DPPH scavenging activity is a composite measure integrating the contributions of all antioxidant species present—betalains, phenolic acids, ascorbic acid, and flavonoids—rather than quercetin alone [17-18,35]. The superior antioxidant activity of dragon fruit wine may be attributed to the combined contribution of phenolic compounds, flavonoids, vitamin C, and naturally occurring betalains present in *Hylocereus undatus* [22-23]. Additionally, dragon fruit wine has high betalain content—particularly betacyanins and phenolic acids, which collectively provide multiple electron-donation pathways for DPPH quenching [17-18]. Minh and Trung (2024) similarly reported that fermentation of red dragon fruit enhanced antioxidant activity relative to the unfermented substrate [32]. The intermediate activity of Mandarin Orange wine is consistent with the flavanone and polyphenol content of the substrate [19, 36]. The comparatively lower DPPH activity of Chikoo wine may reflect partial oxidative degradation of its catechin and gallic acid constituents during the fermentation period [14].

The central finding of this study—that quercetin is detectable and quantifiable by RP-HPLC in all three fruit wines, with post-fermentation concentrations of 44.76 mg/L (mandarin orange), 20.51 mg/L (chikoo), and 20.50 mg/L (dragon fruit)—is novel and scientifically significant. Mandarin Orange wine's higher quercetin content is biologically plausible: *Citrus reticulata* accumulates quercetin and quercetin-3-glucoside predominantly in the flavedo at concentrations substantially exceeding those in the pulp or expressed juice [19-20]. The acidic must pH (5.0-5.5), combined with the 14-day fermentation period and yeast-produced beta-glucosidase activity, would have promoted progressive hydrolysis of quercetin glycosides to the free aglycone [11,37].

The port wine positive control yielded 84.91 mg/L, substantially exceeding all experimental fruit wines and confirming the expected superiority of prolonged maceration with polyphenol-dense *V. vinifera* skins [38-39]. Importantly, however, the quercetin concentrations in all three tropical fruit wines (20.50-44.76 mg/L) fall within or approach the range reported for several commercially produced red grape wines: Castillo-Munoz et al. (2007) reported quercetin concentrations of 1.2-19.4 mg/L in a range of *V. vinifera* red cultivar wines [38]. Mandarin Orange wine, at 44.76 mg/L, exceeds this range, representing a noteworthy quercetin yield for a non-grape tropical fruit wine.

Mandarin Orange wine presented the most favorable combination of organoleptic profile (bright orange color; characteristic citrus aroma; smooth, velvety texture) and quercetin content, making it the most promising candidate for further nutraceutical product development. Dragon Fruit wine's unfavorable flavor and pungent odor, attributed to higher alcohol accumulation at the fermentation temperatures applied, could be substantially improved through fermentation temperature reduction to 18-22°C and co-fermentation with aroma-positive non-*Saccharomyces* yeasts [34]. Prieto et al. (2021) identified fermentation temperature as a primary determinant of sensory quality in dragon fruit wines, with lower temperatures (25°C) producing more acceptable sensory profiles [34].

The demonstration that wines produced from three commercially tropical fruits contain detectable post-fermentation quercetin (20.50-44.76 mg/L) has meaningful implications for the functional beverage industry in South and Southeast Asia. Regular moderate consumption (150-300 mL/day) could contribute 3.1-13.4 mg quercetin daily, which is a meaningful increment to dietary intake—particularly in populations with low habitual polyphenol consumption [6,8].

5. Conclusion:

This study presents the first comparative RP-HPLC-based evaluation of quercetin content in wines produced from three non-conventional tropical fruits—chikoo (*Manilkara zapota*), dragon fruit (*Hylocereus undatus*), and mandarin orange (*Citrus reticulata*)—fermented under standardized laboratory conditions using *Saccharomyces cerevisiae*. RP-HPLC analysis confirmed the presence of quercetin in all three fruit wines, with concentrations within or exceed the range reported for several *Vitis vinifera* red cultivar wines. Thus, the study

brought out that tropical fruit wines can be competitive dietary quercetin sources. Organoleptic evaluation identified Mandarin Orange wine as the most commercially viable product, combining a favorable sensory profile with the highest quercetin content.

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References:

- Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev*. 2009;2(5):270-278.
- Vilela A. Strategies to improve the potential functionality of fruit-based fermented beverages. *Plants*. 2021;10(11):2263.
- Batiha GE, et al. The pharmacological activity, biochemical properties, and pharmacokinetics of the major natural polyphenolic flavonoid: quercetin. *Foods*. 2020;9(3):374.
- Ozorowski M, et al. The effects of quercetin on vascular endothelium, inflammation, cardiovascular disease and lipid metabolism—a review. *Nutrients*. 2025;17(9):1579.
- Almatroodi SA, et al. Potential therapeutic targets of quercetin and its role in cancer therapy. *Molecules*. 2021;26(5):1315.
- Aghababaei F, Hadidi M. Recent advances in potential health benefits of quercetin. *Pharmaceuticals (Basel)*. 2023;16(7):1020.
- Anand David AV, et al. Overviews of biological importance of quercetin: a bioactive flavonoid. *Pharmacogn Rev*. 2016;10(20):84-89.
- Dabeek WM, Marra MV. Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans. *Nutrients*. 2019;11(10):2288.
- Ferenczyova K, et al. Potential implications of quercetin and its derivatives in cardioprotection. *Int J Mol Sci*. 2020;21(5):1585.
- Zong L, et al. Recent advances of fermented fruits: a review on strains, fermentation strategies, and functional activities. *Food Chem X*. 2024; 22:101482.
- Lim SH, et al. Effects of fermentation on the bioactivity and composition of polyphenols in polyphenol-rich foods: a review. *Molecules*. 2023;28(17):6243.
- Xia EQ, et al. Biological activities of polyphenols from grapes. *Int J Mol Sci*. 2010;11(2):622-646.
- Koh WY, et al. Fermentation of fruit by-products as a tool for nutritional and environmental sustainability. *Nutrients*. 2025;17(9):1537.
- Gomez-Caravaca AM, et al. Phenolic compounds profile of *Manilkara zapota* L. fruit. *J Agric Food Chem*. 2013;61(16):3786-3793.
- Siddiqui AA, et al. Isolation of compounds from various parts of *Manilkara zapota*. *J Asian Nat Prod Res*. 2010;12(4):307-311.
- Kalra S, Bhardwaj R. A review on *Manilkara zapota* (Sapodilla). *Int J Pharm Clin Res*. 2016;8(7):584-586.
- Khoo HE, et al. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res*. 2017;61(1):1361779.
- Stintzing FC, Carle R. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci Technol*. 2004;15(1):19-38.
- Gattuso G, et al. Flavonoid composition of citrus juices. *Molecules*. 2007;12(8):1641-1673.
- Nogata Y, et al. Flavonoid composition of fruit tissues of citrus species. *Biosci Biotechnol Biochem*. 2006;70(1):178-192.
- Manthey JA, et al. Biological properties of citrus flavonoids pertaining to cancer and inflammation. *Curr Med Chem*. 2001;8(2):135-153.
- Mizzi L, et al. HPLC analysis of phenolic compounds and flavonoids with overlapping peaks. *Food Technol Biotechnol*. 2020;58(1):12-19.

23. Pereira V, et al. HPLC-DAD methodology for the quantification of organic acids, furans and polyphenols by direct injection of wine samples. *J Sep Sci.* 2010;33(9):1204-1215.
24. Careri M, et al. Direct HPLC analysis of quercetin and trans-resveratrol in red wine, grape, and winemaking by-products. *J Agric Food Chem.* 2004;52(25):7378-7384.
25. Pimentel-Moral S, et al. Chromatographic methods for quercetin quantification from natural sources: systematic review 2018–2022. *Molecules.* 2023;28(23):7714.
26. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem.* 1959;31(3):426-428.
27. Pilone GJ. Determination of ethanol in wine by titrimetric and spectrophotometric dichromate method. *J Assoc Off Anal Chem.* 1985;68(2):188-190.
28. Zhang P, et al. A high throughput method for total alcohol determination in fermentation broths. *BMC Biotechnol.* 2019;19(1):34.
29. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature.* 1958;181(4617):1199-1200.
30. Brand-Williams W, et al. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol.* 1995;28(1):25-30.
31. Shen J, et al. Co-fermentation of navel orange juice to improve phenolic content and aroma. *Front Microbiol.* 2024;15:1342847.
32. Minh TN, Trung NQ. Influences of fermentation conditions on the chemical composition of red dragon fruit (*Hylocereus undatus*) wine. *Beverages.* 2024;10(3):61.
33. Nguyen TH, et al. Characterization of red dragon fruit wine fermented with a newly identified yeast strain *Saccharomyces cerevisiae* M7. *Food Sci Nutr.* 2025;13(3):e70009.
34. Prieto MA, et al. Impacts of peel inclusion and fermentation temperature on the bioactive compounds, antioxidant activity, and sensory properties of dragon fruit wines. *Food Sci Biotechnol.* 2021;30(8):1013-1024.
35. Sadeer NB, et al. The versatility of antioxidant assays in food science and safety. *Antioxidants (Basel).* 2020;9(8):709
36. Xu G, et al. Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food Chem.* 2008;106(2):545-551.
37. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annu Rev Nutr.* 2002;22(1):19-34.
38. Castillo-Munoz N, et al. Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *J Agric Food Chem.* 2007;55(3):992-1002.
39. Corder R, et al. Oenology: red wine procyanidins and vascular health. *Nature.* 2006;444(7119):566.

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Appendices:

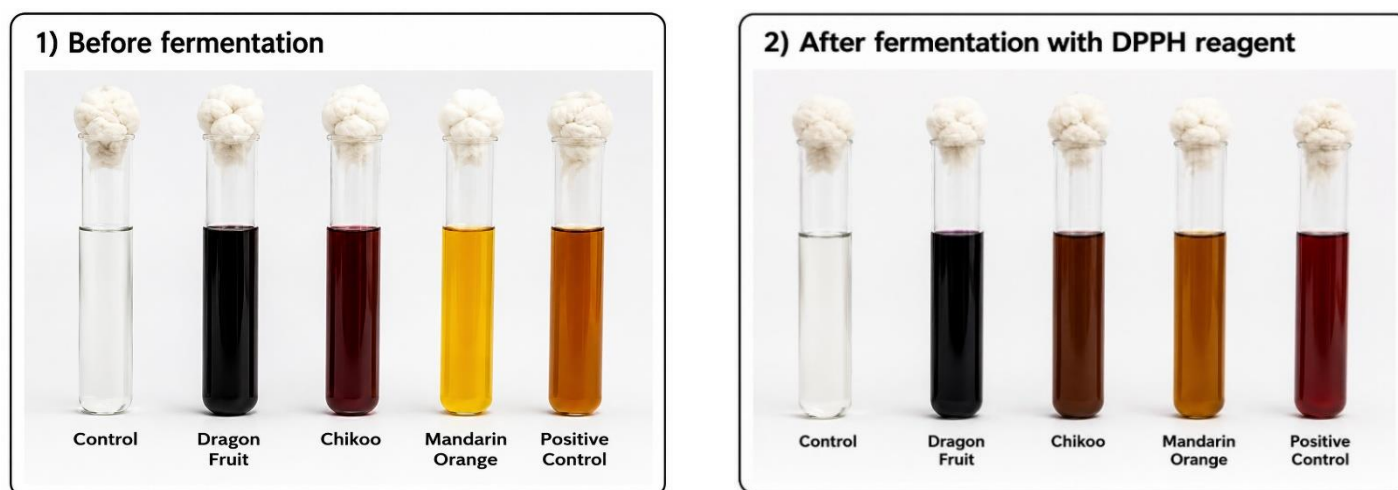


Figure 2: Comparative antioxidant activity of fruit juice samples before and after fermentation and ethanol estimation by the dichromate oxidimetric method. (1) DPPH radical scavenging assay of fruit juices before fermentation. (2) DPPH radical scavenging assay of fermented fruit wines.

Abbreviations:

HPLC- High Performance Liquid Chromatography

RP-HPLC- Reverse Phase High Performance Liquid Chromatography

RP-HPLC-DAD- Reverse Phase High Performance Liquid Chromatography Diode Array Detector

UV- Ultra Violet

RT- Room Temperature

µg/mL- Microgram/Milli Litre

mg/L- Milli Gram/Litre

% v/v- Percent Volume/Volume

% w/v- Percent Weight/Volume

GE- Glucose Equivalent

RPM- Revolution per Minute

N- Normality

K₂Cr₂O₇- Potassium dichromate

H₂SO₄- Sulphuric acid

mM- Milli Molar

AUC- Area Under Curve

Ferm.- Fermentation