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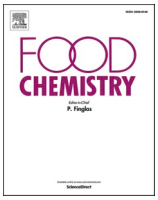

Kiwi (*Actinidia deliciosa*) juice as a natural inhibitor of the enzymatic activity of sugarcane juice, insights from experimental assessment and molecular docking analysis

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ABSTRACT

The present work evaluated kiwi juice addition alongside pasteurization (at 85 °C for 5 min) or microwave treatment (for 3 min) on the quality improvement of sugarcane juice. The juice was treated in the presence of kiwi juice (0–8%), and its physicochemical properties and microbial load were compared with raw juice. The study also highlighted the key enzymes causing sugarcane juice discoloration, peroxidase (POD) and polyphenol oxidase (PPO), by quantifying kiwi juice constituents using GC–MS and monitoring their effects by molecular docking. Kiwi addition considerably raised ($p < 0.05$) acidity, ascorbic acid (54.28%), and phenolic compounds (32%), and decreased the POD and PPO activity of raw cane juice. Pasteurization in the presence of kiwi, rather than microwave treatment, has significantly ($p < 0.05$) increased the phenolic compounds and reduced POD and PPO activities until barley was detected. Molecular docking revealed that heptacosane, oleic acid, and melezitose are the primary kiwi components responsible for enzyme inactivation.

1. Introduction

Consumers are increasingly seeking natural, inexpensive, and safe foods, beverages, and drinks with higher nutritional value (Dhansu et al., 2023). Sugarcane (*Saccharum officinarum* Linn.) juice is the most popular drink in the main producing countries, which include India (Kaavya et al., 2019), Brazil, and China (Silva et al., 2019). Its fresh greenish juice is high in natural sugars, vitamins, minerals, phenolic compounds, and amino and organic acids with a pH of >5.0 (Nishad et al., 2017; Panigrahi et al., 2021). It cannot be kept fresh for longer than 6 h because of its nutritional value and is rapidly attacked by fungi, lactic acid bacteria, and other spoilage-pathogenic microflora (Arif et al., 2019). Furthermore, it is extracted fresh using an unsanitary mechanical crusher and sold to the public by street vendors; as a result, it is processed and distributed in unhealthy conditions and informal marketplaces (Dhansu et al., 2023). The lack of hygienic practices during processing increases its microbial load, whereas browning caused by peroxidase and polyphenol oxidase activity, as well as sedimentation during storage, reduces its sensory acceptability (Panigrahi et al., 2020).

It is critical to deliver raw juice to non-sugarcane-growing areas or countries and to make it conveniently available everywhere, therefore, sugarcane juice preservation is necessary. In addition, it is important to develop appropriate preservation aids in order to extend its shelf life. Several studies have looked into various preservation methods, including chemicals (such as sodium benzoate, potassium meta-bisulphite, citric acid, and ascorbic acid) (Dhansu et al., 2023; Mao et al., 2007; Paraluppi & Ceccato-Antonini, 2019), to inhibit PPO activity. Thermal treatments (such as pasteurization, microwave, or steam treatment) (Adulvitayakorn et al., 2020; Fatima et al., 2023) and non-thermal methods (such as UV, high-pressure processes, or ozonation) (Mukhtar et al., 2022; Panigrahi et al., 2020; Sreedevi & Rao, 2018) were also assessed. Each technique has advantages and limitations, and some are already in use to produce packed cane juice.

For the health-conscious consumer market, food producers are now looking for natural, environmentally friendly, and safe food preservatives that are cheaper and more nutritional. Natural preservatives are in high demand because they are easy to obtain and do not pose any health hazards (Dhansu et al., 2023). Various natural plant extract

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preservatives were evaluated in sugarcane juice, and they demonstrated a wide range of PPO inactivation and antibacterial properties. Lemon juice, for example, was used earlier to reduce pH, red color, and microbial growth (Khare et al., 2012), whereas ginger extract, *Zingiber officinale*, acted against microbial deterioration due to its linalool and eugenol content (Ramachandran et al., 2017). In addition, 1.05% lemon grass essential oil +6.84% lemon juice resulted in a 10.3% rise in total phenolics, 49.3% PPO inactivation, and a 2-log cycle bacterial reduction in the raw juice (Bag et al., 2022). The combination of thermal treatment with natural preservatives also resulted in juice with greater acceptability. For example, Khare et al. (2012) stated that adding flavor enhancers [ginger extract (0.6%) + lemon juice (3.0 ml)] and citric acid with heat pasteurization at 75 °C for 10 min improved the shelf life and inhibited microorganisms' population. According to Verma et al.'s (2016) study, combining ascorbic acid (40 ppm) and lemon juice (pH maintained at 4.2) with a 10-min heat treatment at 85 °C increased the quality of sugarcane juice for up to 45 days, but no reports have included kiwi juice addition.

This work aimed to test the effect of kiwi juice addition on the physicochemical properties and enzyme activities of sugarcane juice before proceeding with conventional heating or microwave-assistant treatment. In addition, the impact of each kiwi constituent was assessed as a potential inhibitor of POD and PPO by molecular docking analysis.

2. Materials and methods

2.1. Plant materials and chemical reagents

Fresh canes (*Saccharum officinarum* var. G 2003-47) were collected from the nearby local market. Mold- and insect-infected sugar canes were removed, while excellent canes were scrubbed and rinsed with a strong stream of water to eliminate dirt and germs. Sugarcane juice was extracted by a stainless-steel crusher with an extraction efficiency of 70% (Dhansu et al., 2023). Undamaged fresh kiwi fruits (*Actinidia deliciosa* cultivar Hayward), on the other hand, were acquired from a local market, rinsed in distilled water, and peeled using a clean knife. The flesh was chopped into 1-cm cubes and mixed using a hand blender (Braun, MQ725) with no water. To remove debris, seeds, and insoluble materials, the slurry was filtered using muslin fabric (Khiralla & Ali, 2020). The used reagents, such as 3, 5-dinitrosalicylic acid, ethanol, catechol, guiacol, and Folin-Ciocalteu reagent, were obtained from Sigma-Aldrich (St. Louis, Mo., USA), and the others were of analytical grade.

2.2. Treatments of sugarcane juice

Fresh cane juice was packed into 100-ml glass bottles, and then filtered fresh kiwi juice was added immediately in different concentrations (0, 1, 2, 4, 6, and 8%). For sugarcane juice treatment, the closed bottles were divided into three batches: the first was left untreated, the second was conventionally treated in a heated water bath at 85 °C for 5 min, and the third was treated by microwave (TM-25 MS Tornado, B-tech Company, Egypt) for 3 min, followed by quick cooling to room temperature (25 °C). All treatments were performed in triplicate (Dhansu et al., 2023).

2.3. Sugarcane juice analysis

The bottles of the two treated sugarcane juice samples compared with the raw samples were subjected to the following analyses: physicochemical properties, enzymatic activity determination, and microbial load. The microbiological, some physicochemical (i.e., pH, phenolic compounds, and ascorbic acid content), and enzymatic activities of all the samples were determined immediately, whereas the other physicochemical parameters were determined in samples held at −20 °C.

2.3.1. Physicochemical examination

At ambient temperature (25 °C), the pH of all samples (raw, pasteurized, and microwave-treated cane juice with varying kiwi juice concentrations besides kiwi juice) was measured using an Adwa AD1030 pH meter (Romania) calibrated with two different pH buffers (pH 4 and 9) (Eggleston, 2002). After titration with NaOH 0.1 M until pH 8.0, the total titratable acidity was estimated as citric acid (%, *w/v*). A handheld refractometer (Reichert, New York, USA, model AR 200) was used to determine the soluble solid content (°Brix). The total phenolic content of the prepared samples was evaluated using the Folin-Ciocalteu reagent, as modified by Quan et al. (2018). 2.5 ml of the reagent were mixed with 200-μl sample/distilled water (blank), and after 5 min, 2.0 ml of sodium carbonate (7.5%) was added. After 15 min in the dark, absorbance at 760 nm was recorded using a spectrophotometer (Unico-UV2000, USA). The results were represented in mg gallic acid/ml cane juice. The yellow reagent, 3, 5-dinitrosalicylic acid, was used to detect and quantify the reducing sugar content at 540 nm, according to Miller (1959), by the same spectrophotometer. The ascorbic acid content was determined by reducing the dye 2,6-dichlorophenol-indophenol with ascorbic acid. Two milliliters of the sample were homogenized with 60 ml (meta-phosphoric acid, 3%, and acetic acid, 8%). After titration with the dye (0.25%) containing 0.25% sodium carbonate until rose, ascorbic acid was expressed as mg vitamin C per 100 ml of juice (Gullo et al., 2016). The color of sugarcane samples was measured three times using a chromameter (CR-410, Konica Minolta, Tokyo, Japan). The values were extracted as lightness L^*, redness a^*, and yellowness b^* readings (Lima Gomes et al., 2020). Finally, the antioxidant activity of sugar cane juice samples was assessed using the DPPH free radicals test (Zhang et al., 2023). Briefly, the sample was diluted 1:10 with distilled water before being combined with a methanolic DPPH solution (0.4 mM) in a 1:1 ratio. After incubation in the dark for 30 min, the absorbance (*A*517) was measured using the aforementioned spectrophotometer, with methanol as a control. The DPPH scavenging activity was calculated as follows:

$$\%\text{DPPH scavenging activity} = \frac{Acontrol - Asample}{Acontrol} \times 100$$

2.3.2. Enzymatic activity examination

Sugarcane is recognized for its high POD and PPO content (Hithamani et al., 2018). To determine POD activity, a juice sample (raw or treated), 1 ml, 1.5 ml phosphate buffer (pH 6.5, 50 mM), 0.2 hydrogen peroxide (1%), and 0.5 ml of an alcoholic solution of guaiacol (1.5%) were combined and held at 35 °C for 3 min, and absorbance at 470 was recorded every minute. For PPO activity determination, a sample of juice (0.5 ml) was combined with 2 ml of phosphate buffer (pH 6.5, 50 mM), and 1 ml of catechol (0.2 M) was added immediately before recording the absorbance at 420 nm every minute at 25 °C for the same time (Queiroz et al., 2011). The activity of both enzymes was expressed as U/ml/min, where U is equivalent to a fluctuation of 0.001 Abs/min (De Bomdepacho, Silva, Lapa-Guimarães, Ditchfield, & Petrus, 2018). Reactivation of POD activity was assayed by keeping the samples at 25 °C for 5 h, and then the activity was assayed as described previously (Lopez & Burgos, 1995).

2.3.3. Microbial examination

The bottles of sugarcane juice after each treatment were immediately tested for their microflora count, including a standard plate count (SPC) as well as yeast and mold (YM). Designated decimal dilutions (10^{-1}–10^{-5}) were performed using NaCl solution (0.85%). SPC was enumerated using total plate count agar after incubation at 37 °C/24–48 h, while Sabouraud dextrose agar at 25 °C/72 h was used to enumerate YM. Log CFU/ml was used to express the various counts (Dhansu et al., 2023).

Table 1
Physiochemical changes of raw, pasteurized, and microwave-treated cane juice containing kiwi juice at concentrations of 0–8%.

Treatment	Kiwi conc.	TSS	pH	Acidity	Vitamin C content	Reducing sugar content	Phenolic compound content	Antioxidant activity power
	(%)	°Brix		% (w/v)	mg/100 ml	g Glucose/100 ml	mg Gallic acid/ml	%
	Fresh kiwi	**14.3 ± 0.1**	**3.23 ± 0.01**	**10.08 ± 0.67**	**15.0 ± 0.54**	**1.79 ± 0.09**	**189.18 ± 9.42**	**801.82 ± 5.10**
Raw	0	*16.0^{e} ± 0.0	5.3^{b} ± 0.00	0.53^{g} ± 0.06	3.5^{g} ± 0.00	0.37^{j} ± 0.01	127.64^{f} ± 10.87	1077.25^{a} ± 6.10
	1	16.7^{de} ± 0.7	4.9^{d} ± 0.00	0.67^{g} ± 0.13	3.8^{fg} ± 0.00	0.59^{gh} ± 0.00	136.36^{ef} ± 9.42	884.36^{c} ± 7.46
	2	17.1^{cd} ± 0.1	4.6^{e} ± 0.00	0.72^{fg} ± 0.06	4.1^{ef} ± 0.01	0.67^{g} ± 0.04	139.95^{ef} ± 5.07	569.10^{e} ± 4.07
	4	17.3^{cd} ± 0.4	4.3^{h} ± 0.00	0.77^{efg} ± 0.13	4.8^{bc} ± 0.27	0.82^{ef} ± 0.02	147.38^{def} ± 2.53	520.634^{f} ± 8.82
	6	17.6^{cd} ± 0.0	4.2^{i} ± 0.02	1.01^{de} ± 0.06	5.1^{ab} ± 0.02	1.09^{cd} ± 0.02	165.85^{cde} ± 3.98	502.40^{f} ± 7.46
	8	17.6^{cd} ± 0.0	4.0^{jk} ± 0.00	1.25^{cd} ± 0.00	5.4^{a} ± 0.54	1.15^{bc} ± 0.03	168.67^{cde} ± 10.15	347.89^{j} ± 2.03
Pasteurization	0	17.0^{cd} ± 0.0	5.5^{a} ± 0.03	0.96^{ef} ± 0.00	3.5^{g} ± 0.00	0.43^{ij} ± 0.00	231.23^{a} ± 17.40	921.30^{b} ± 5.42
	1	17.2^{cd} ± 0.2	5.1^{c} ± 0.00	1.01^{de} ± 0.06	4.0^{ef} ± 0.27	0.63^{g} ± 0.02	209.95^{ab} ± 14.55	424.18^{h} ± 13.57
	2	17.3^{cd} ± 0.4	4.5^{ef} ± 0.01	1.25^{cd} ± 0.00	4.1^{ef} ± 0.10	0.83^{ef} ± 0.05	180.72^{bcd} ± 28.64	390.60^{i} ± 19.00
	4	17.2^{cd} ± 0.0	4.4^{g} ± 0.00	1.58^{ab} ± 0.20	4.2^{def} ± 0.00	0.91^{e} ± 0.02	156.36^{cdef} ± 15.22	357.97^{j} ± 4.07
	6	17.5^{cd} ± 0.4	4.2^{i} ± 0.00	1.66^{ab} ± 0.17	4.4^{cde} ± 0.27	1.13^{c} ± 0.00	144.56^{ef} ± 10.15	239.44^{l} ± 18.32
	8	17.8^{cd} ± 0.0	4.1^{jk} ± 0.07	1.83^{a} ± 0.03	4.6^{cd} ± 0.00	1.14^{bc} ± 0.00	138.00^{ef} ± 0.01	161.71^{n} ± 10.17
Microwave	0	16.9^{cd} ± 0.1	5.2^{c} ± 0.09	0.96^{ef} ± 0.00	4.2^{def} ± 0.00	0.50^{hi} ± 0.08	127.38^{f} ± 1.81	938.58^{b} ± 4.07
	1	17.4^{cd} ± 0.0	4.6^{e} ± 0.04	1.25^{cd} ± 0.13	4.4^{cde} ± 0.27	0.77^{f} ± 0.04	128.92^{f} ± 6.16	685.70^{d} ± 6.10
	2	17.6^{cd} ± 0.0	4.5^{f} ± 0.02	1.39^{bc} ± 0.20	4.4^{cde} ± 0.00	0.85^{ef} ± 0.00	136.62^{ef} ± 3.60	472.65^{g} ± 8.82
	4	17.8^{c} ± 0.0	4.3^{h} ± 0.00	1.58^{ab} ± 0.06	4.6^{cd} ± 0.00	1.03^{d} ± 0.09	142.51^{ef} ± 7.25	357.97^{j} ± 4.06
	6	18.9^{b} ± 1.4	4.1^{j} ± 0.07	1.58^{ab} ± 0.20	4.7^{bc} ± 0.00	1.23^{b} ± 0.02	182.25^{bcd} ± 7.61	287.91^{k} ± 6.78
	8	21.2^{a} ± 0.3	4.0^{k} ± 0.01	1.83^{a} ± 0.13	4.8^{bc} ± 0.27	1.87^{a} ± 0.04	186.87^{bc} ± 9.79	209.21^{m} ± 13.57

* All values were expressed as the mean ± SD of three determinations. Different letters between rows indicate statistically significant differences between the samples (p-value <0.05).

2.4. Gas chromatography-mass spectrometry (GC–MS) analysis

The chemical compounds of the kiwi juice and cane juice samples were detected using Trace GC 1310-ISQ-MS (Thermo Scientific, TX, USA) and a capillary column TG-5MS (30 m × 0.25 mm with a film thickness of 0.25 μm). The column oven temperature was initially held at 50 °C, then increased by 5 °C/min to 230 °C for 2 min, then increased by 30 °C/min to the final temperature of 290 °C for another 2 min. The temperatures of the MS transfer line and the injector were held at 250 °C and 260 °C, respectively. At a stable flow rate of 1 ml/min, helium was utilized as a carrier gas. A diluted sample of 1-μl was injected by an Autosampler (AS1300), while the solvent pause time was 3 min. The following MS conditions were applied: 200 °C ion source temperature, ionization voltages of 70 eV, and a complete scan range of m/z 40–1000. The mass of components was recognized by referring to the NIST 11 (Gaithersburg, MD) and WILEY 09 databases (Salem et al., 2016).

2.5. Molecular docking analysis

The CDOCKER algorithm in Discovery Studio 4.5 (Accelrys Software, Inc., San Diego, CA, USA) was used to evaluate the probable molecular binding ways of the identified compounds in kiwi juice with POD and PPO. Crystal structures of protein targets 1BEM (peroxidase) and 3WKY (polyphenol oxidase) were obtained using the Protein Data Bank (http://www.rcsb.org/pdb/). After the exclusion of the water molecules from the protein, it was refined. The binding of the co-crystalized ligand inhibitors 2-(N-morpholino)-ethanesulfonic acid (MES) and 2-acetamido-2-deoxy-beta-D-glucopyranose (NAG) to the target enzymes 1BEM and 3WKY, respectively, served as the basis for identifying the binding site for each tested enzyme (Bayrakdar et al., 2023).

The co-crystallized ligand was removed from each enzyme, and then rule-based docking was applied. Each identified compound is docked, along with the specific ligand for each enzyme, to the protein binding site. The interaction energy was calculated in kcal/mol to investigate how the ligand and receptors interacted. The best ligand-binding poses were determined by sorting the top ten ligand-binding poses for each ligand based on their CDOCKER interaction energies and examining the predicted binding interactions.

2.6. Statistical analysis

A one-way analysis of variance (ANOVA) was performed using CoStat statistical software on the data from the three treatments with three replicates. Duncan's test (Duncan, 1955) revealed statistical differences at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Physicochemical characteristics

Table 1 compares the physicochemical properties of several treated cane juice samples to untreated samples with a gradual addition of kiwi juice (0–8%).

Total soluble solids (TSS) in untreated cane juice samples were significantly affected by kiwi addition until 2%, as compared to a no-kiwi-added sample, after which no significant difference was noticed. Kiwi juice contains fewer total soluble solids than sugarcane juice (14.3 vs. 16° Brix), which may explain this. Additionally, kiwi had no significant effect ($p \geq 0.05$) on °Brix at levels ranging from 1 to 6% in all treated samples, with only higher concentrations (≥6%) showing significance in microwave-treated samples. It may be due to the formation of some aggregates after this treatment. Adulvitayakorn et al. (2020) also observed an increase in the soluble solids in cane juice after microwave treatments at three levels: 120 W, 400 W, and 700 W. They attributed it to evaporation during the treatment, which was not the case in our investigation because the juice was treated in closed bottles. The pH value of untreated cane juice (0% kiwi), on the other side, was 5.3 ± 0.00, which is within the range of De Bomdepacho, Silva, Lapa-Guimarães, Ditchfield, & Petrus, 2018 (5.09 to 5.2), while kiwi is more basic (3.23 ± 0.01). By increasing the amount of kiwi juice in all samples, the pH value dropped considerably ($p < 0.05$). Because kiwi juice contains ascorbic and citric acids (Doymaz, 2020), this is to be expected. The same pattern was observed in the acidity percentage.

Regarding ascorbic acid (Vit. C) content, untreated free cane juice contains 3.5 mg/100 ml, which is comparable to the 3.39 mg/100 ml found by Sankhla et al. (2012). In contrast, kiwi juice contains nearly three times the amount of vitamin C. By increasing kiwi concentrations in all cane juice samples, it increased considerably ($p < 0.05$) to reach the maximum of 5.4 mg/100 ml in untreated cane juice at 8% concentration. This may be due to the high vitamin C level of kiwi juice (Khiralla & Ali, 2020). On the contrary, this vitamin was considerably ($p < 0.05$) lower in pasteurized juice and microwave-treated samples than in untreated juice, particularly at kiwi concentrations >2%. For example, at 4, 6, and 8% kiwi levels, it was reduced by pasteurization by 12.5, 13.73, and 14.81%, respectively, whereas the decline was 4.17, 7.84, and 11.11%, respectively, by microwave treatment. Its content was lower in pasteurized samples than in microwave-treated samples, with no statistical difference ($p \geq 0.05$) at the same kiwi concentration (except at 8%). That is, pasteurization destroyed some of this essential vitamin, but microwave treatment may preserve it. These findings suggested that sugarcane juice with kiwi might be regarded as a good source of this key antioxidant vitamin. Similarly, Khiralla and Ali (2020) observed a 42% drop in ascorbic acid in kiwi juice after pasteurization at 65 °C for 30 min.

Considering the reducing sugars of cane juice, their content increased significantly ($p < 0.05$) by increasing the kiwi juice concentration in untreated and pasteurized samples. The high reducing sugar content of kiwi fruit (1.79 ± 0.09 mg/100 ml) compared to the sugarcane juice could explain this increase. Microwave treatment in the presence of kiwi greatly increased the reducing sugars to the maximum reported amount in cane juice, with 8% kiwi (3.74-fold compared to 0% kiwi). It was noticed that at ≥4 kiwi level, reducing sugars in microwave-treated samples were considerably greater than in pasteurized samples, and both were higher than those that were untreated. This could be related to the heat effect in the presence of acidity, which promotes acid sucrose inversion (Eggleston, 2002); as the pH of microwave samples was lower than that of pasteurized samples at the same kiwi level. In agreement, Siguemoto et al. (2019) noticed a rise in reducing sugars by 39% (glucose) in pasteurized apple juice, while Chauhan et al. (2002) observed just a 4% and 2% upsurge in reducing sugar content of pasteurized cane juice in the occurrence of citric acid and ascorbic acid, respectively.

The most frequent secondary plant metabolites identified in the bulk of fruits, vegetables, and teas are phenolic and polyphenol compounds (Zhu et al., 2019). These bioactive substances reduce the risk of several diseases, including arthritis, arteriosclerosis, cancer, cognitive dysfunction, inflammation, and cardiovascular disease (Rana et al., 2022). In the current study, the phenolic content of untreated free juice was 127.64 mg gallic acid/ml, whereas kiwi juice contained 189.18 mg gallic acid/ml, indicating that both juices would be important sources of these vital chemicals in our diet, with kiwi being the higher. The total phenolic content of sugarcane juice quantified by Duarte-Almeida et al. (2007) was relatively lower, 0.16 mg chlorogenic acid/ml. Due to changes in cultivars and analytical protocols; it may differ from that determined in our study. The phenolic compounds were considerably increased ($p < 0.05$) by increasing the kiwi concentration in untreated and microwave-treated juice, particularly at concentrations >4%, as compared to the 0% kiwi sample. Among fruits, kiwi (*Actinidia deliciosa*) is a vital source of naturally active constituents such as vitamin C, phenolic, and flavonoid compounds that follow orange juice (Park et al., 2015; Quan et al., 2018). According to these findings, adding kiwi juice (8%) to cane juice raised the bioactive component concentration by 32.14% and 46.7% in untreated and microwave-treated samples, respectively. Pasteurized cane juice samples containing ≤2% kiwi had a higher phenolic compound concentration (e.g., 209.95 mg gallic acid/ml at 1% kiwi level) than both raw and microwave-treated samples, but at >4% it was lower than both. Siguemoto et al. (2019) connected the rise in these chemicals to the deactivation of PPO and POD after the thermal treatment of apple juice, implying that these enzymes would not influence phenolic compounds. That means the pasteurization of sugarcane juice with high concentrations of kiwi juice degraded the phenolic compounds (40.4% degradation at 8% kiwi level vs. 0%). This could be due to the reactions that took place during heating under highly acidic conditions. These findings are consistent with those of Khiralla and Ali (2020), who discovered a 24.7% drop in the phenolic content of kiwi juice after pasteurization at 65 °C for 30 min. Rodrigues et al. (2021), on the other hand, maintained the phenolic content of sugarcane juice comparable to fresh juice even after ultrasound or ohmic heating treatments related to conventional heating systems at 80 °C for 20 min.

According to the data (Table 1), the antioxidant capacity of raw free sugarcane juice was 1077.25, whereas that of kiwi juice was 801.82%. It is well known that kiwi fruit has more antioxidant capacity than apple, pear, and grapefruit, but less than raspberry, strawberry, plum, and orange (Miguel, 2011). The addition of kiwi juice dramatically reduced the antioxidant capacity of raw, pasteurized, and microwave-treated cane juices. However, this addition increased ascorbic acid and the phenolic compound levels in raw juice, which are primarily responsible for the anti-oxidative action. This could be attributed to the fact that sugarcane juice and kiwi juice contain distinct phenolic chemicals at different concentrations (Rodrigues et al., 2021; Vila et al., 2008). Researchers suggest that phenolic mixtures can interact antagonistically or synergistically to neutralize free radicals when compared to pure phenolic compounds. The best explanation for the reduction in antioxidant activity is that phenolic compounds interact with peroxy radicals (ROO) based on the availability of their hydroxyl groups (OH). This suggests that phenolics with a higher OH content have the highest antioxidant activity. Freshly squeezed sugarcane juice contains schaftoside (10-OH), tricin-7-*O*-rhamnosylglucuronide (7-OH), and diosmetin-6-*C*-hexosyl-7-*O*-methyl (7-OH) (Rodrigues et al., 2021), as well as isoschaftoside (10-OH), vitexin (7-OH), and 4′,5′-Di-*O*-methyl-luteolin-8-*C*-glucoside (10-OH) (Vila et al., 2008). Conversely, kiwi juice contains protocatechuic acid (3-OH), chlorogenic acid (6-OH), and *p*-coumaric acid (2-OH) (Dawes & Keene, 1999). This means that raising kiwi levels reduces phenolics with high hydroxyl groups rather than low hydroxyl groups, resulting in lower antioxidant power. Furthermore, both pasteurization and microwave treatments caused an additional drop in antioxidant power, and microwave-treated samples had higher power. Many studies have reported this effect of heat on the oxidation of

Table 2
Enzymatic activity of polyphenol oxidase (PPO) and peroxidase (POD) and its recovery (after 5 h) of raw, pasteurized, and microwave-treated cane juice containing kiwi juice at concentrations of 0–8%.

Treatment	Kiwi conc. (%)	PPO activity	POD activity	POD activity after 5 h
		U/ml/min	U/ml/min	U/ml/min
	Fresh kiwi	**58.0 ± 3.50**	**0.06 ± 0.01**	
Raw	0	*400.0^{b} ± 16.97	1.50^{a} ± 0.70	1.50^{a} ± 0.00
	1	470.0^{a} ± 31.11	1.10^{ab} ± 0.15	1.22^{b} ± 0.02
	2	460.0^{a} ± 14.50	0.90^{bc} ± 0.14	0.65^{d} ± 0.07
	4	354.0^{c} ± 2.82	0.80^{bcd} ± 0.05	0.33^{e} ± 0.03
	6	320.0^{d} ± 11.31	0.70^{cd} ± 0.09	0.16^{f} ± 0.01
	8	254.0^{e} ± 8.48	0.50^{efg} ± 0.00	0.03^{ij} ± 0.00
Pasteurization	0	82.0^{j} ± 4.14	0.65^{cde} ± 0.08	0.21^{f} ± 0.01
	1	86.0^{ij} ± 4.84	0.55^{ef} ± 0.11	0.15^{fg} ± 0.02
	2	34.0^{k} ± 2.82	0.40^{f} ± 0.14	0.10^{gh} ± 0.05
	4	26.0^{kl} ± 2.82	0.20^{g} ± 0.08	0.05^{hij} ± 0.00
	6	4.6^{lm} ± 0.84	0.15^{fg} ± 0.07	0.01^{j} ± 0.00
	8	1.0^{m} ± 0.41	0.10^{g} ± 0.07	0.00^{j} ± 0.00
Microwave	0	148.0^{f} ± 5.65	0.90^{bc} ± 0.14	0.75^{c} ± 0.07
	1	152.0^{f} ± 0.00	0.84^{bcd} ± 0.02	0.64^{d} ± 0.01
	2	136.0^{fg} ± 5.65	0.79^{bcd} ± 0.01	0.33^{e} ± 0.00
	4	118.0^{gh} ± 8.48	0.60^{cde} ± 0.00	0.17^{f} ± 0.02
	6	108.0^{hi} ± 0.00	0.55^{ef} ± 0.14	0.08^{hi} ± 0.03
	8	90.0^{ij} ± 2.28	0.40^{f} ± 0.12	0.02^{ij} ± 0.00

* All values were expressed as the mean ± SD of three determinations. Different letters between rows indicate statistically significant differences between the samples (p-value <0.05).

phenolic compounds, as both treatments are thermal (Saikia et al., 2015).

3.2. Enzymatic activity

The fundamental concern with sugarcane juice is POD and PPO activities. When sugarcane juice is extracted from stalks, PPO alters its organoleptic properties and appearance by producing dark-colored polymers in the presence of O_2 (Mao et al., 2007; Panigrahi et al., 2021), whereas POD is an enzyme capable of oxidizing phenolics to quinones in the occurrence of H_2O_2 (Hithamani et al., 2018). The activity of these naturally occurring enzymes causes undesired changes in sugarcane juice's color, flavor, aroma, and nutritional composition (Brochier et al., 2016). Table 2 illustrates the activity of both enzymes in fresh kiwi juice, which contains 0.06 U/ml POD and 58.0 U/ml PPO. It possesses low POD activity, comparable to PPO activity. Yi et al. (2016) detected the same POD activity of 0.012 U/g. Furthermore, in untreated cane juice, increasing kiwi juice significantly inhibited POD activity. Both treatments exhibited the same pattern, and pasteurization accelerated this inhibition to reach the lowest detected activity (0.1 U/min/ml). Interestingly, in untreated, pasteurized, and microwave-treated samples, kiwi at 8% reduced POD by 66.60%, 84.61%, and 55.6%, respectively. However, no treatment completely stopped it. Regarding PPO, its activity was 400 U/ml, which was raised to 470.0 U/ml when 1% kiwi was added, which may contain a considerable amount of PPO (9200 U/ml) (Park & Luh, 1985). PPO was then dramatically reduced ($p < 0.05$) by increasing kiwi concentration by >2%, demonstrating the influence of kiwi on PPO inhibition in cane juice. This could be explained by kiwi juice's effect on pH and acidity, in addition to its chemical contents (e.g., ascorbic and citric acids). PPO was 82.0 U/ml/min in a pasteurized kiwi-free sample, which is 79.5% lower than the untreated sample at the same kiwi level. This was primarily due to denaturation during heating, as PPO can be destroyed at 80 °C (Mao et al., 2007). When compared to microwave treatment, pasteurization with kiwi juice had the greatest effect on PPO activity, which was barely detectable at 6 and 8% levels. Even in the presence of kiwi, microwave treatment of cane juice was unable to completely block PPO. Enzyme inactivation is beneficial in most preservation techniques (Brochier et al., 2016), which implies kiwi juice might be considered a novel natural PPO and POD inhibitor to avoid sugarcane juice discoloration, and pasteurization at 85 °C can aid in this regard. According to Dhansu et al. (2023), acidification (pH 4.8–4.25) or steam treatment (5–15 min) drastically lowered PPO activity from 0.57 to 0.1 U/ml.

To inhibit both enzymes in sugarcane juice, various additions and treatments were investigated. PPO activity was totally suppressed by ascorbic acid in the study of Hithamani et al. (2018) in cane juice samples below pH 4.3, whereas POD activity was completely inhibited only below pH 3.9. Even though the lowest pH of the samples in the

Table 3
Color attributes (L*, a* and b*) of raw, pasteurized, and microwave-treated cane juice containing kiwi juice at concentrations of 0–8%.

Treatment	Kiwi conc. (%)	L*	a*	b*
Raw	0	†46.60^{j} ± 1.40	3.89^{defg} ± 0.03	18.10^{j} ± 0.20
	1	47.11^{j} ± 1.45	4.27^{cdefg} ± 0.55	19.16^{j} ± 0.82
	2	53.57^{h} ± 0.79	4.57^{cde} ± 1.08	25.26^{ghi} ± 2.35
	4	58.69^{f} ± 1.47	4.48^{cdef} ± 0.93	28.99^{cde} ± 3.10
	6	64.90^{c} ± 0.70	4.78^{bcd} ± 0.19	32.26^{ab} ± 1.33
	8	69.13^{a} ± 0.45	5.07^{abc} ± 0.15	34.20^{a} ± 0.36
Pasteurization	0	47.44^{j} ± 1.40	2.37^{j} ± 0.26	14.25^{k} ± 0.38
	1	50.84^{i} ± 0.25	3.44^{ghi} ± 0.15	14.32^{k} ± 0.49
	2	56.79^{g} ± 0.56	3.66^{efgh} ± 0.10	23.53^{hi} ± 0.45
	4	59.33^{f} ± 0.36	3.37^{ghi} ± 0.43	25.54^{fgh} ± 1.44
	6	62.96^{d} ± 0.64	3.50^{ghi} ± 0.30	27.70^{defg} ± 0.51
	8	65.57^{c} ± 0.80	3.67^{efgh} ± 0.15	29.86^{bcd} ± 0.41
Microwave	0	48.20^{j} ± 1.04	5.66^{a} ± 0.41	22.77^{i} ± 1.46
	1	54.13^{h} ± 0.97	5.56^{ab} ± 0.80	26.35^{efg} ± 2.66
	2	56.98^{g} ± 0.39	4.21^{cdefg} ± 0.55	27.53^{defg} ± 2.16
	4	60.92^{e} ± 0.38	3.63^{fgh} ± 0.40	28.15^{def} ± 1.87
	6	64.17^{cd} ± 0.40	2.93^{hij} ± 0.35	29.36^{cd} ± 1.20
	8	67.03^{b} ± 0.41	2.66^{ij} ± 0.20	30.93^{bc} ± 0.98

† All values were expressed as the mean ± SD of three determinations. Different letters between rows indicate statistically significant differences between the samples (p-value <0.05).

current study was 4.0, there was still POD and PPO activity in the microwave-treated samples. This suggests that not only an acidic pH but also heating is required to inactivate these enzymes. Bucheli and Robinson (1994) confirmed that POD is more heat-stable than PPO, the main enzyme involved in color changes. This is consistent with the current study, in which the proportion of POD after pasteurization or microwave treatment was higher than PPO.

Concerning POD reactivation, data shows that after 5 h at 25 °C, POD activity remained consistent in raw sugarcane juice with and without 1% kiwi. However, increasing the kiwi content in the untreated juice lowered POD activity. This demonstrates the inhibitory effect of kiwi components on POD. Both thermal treatments inactivate POD in sugarcane juice, with decreased activity found after 5 h compared to untreated no-kiwi-added cane juice. Increasing kiwi also drastically reduced activity in treated samples after 5 h, until barley was detected. This demonstrated that adding fresh kiwi juice to sugarcane juice prevents POD reactivation. The lower the activity detected after both treatments, the slower the recovery of activity after 5 h. For example, pasteurization and microwave treatment at 4% kiwi lowered POD activity by 86.7% and 60%, respectively, when compared to free untreated cane juice. Aside from kiwi constituents, this could be attributed to increased glucose content (Table 1). Lopez and Burgos (1995) demonstrated that increasing the concentrations of sucrose or glucose resulted in higher inactivation of horseradish POD. This impact was linked to the interaction of reducing groups with protein amino acids.

3.3. Color

Color is the most important feature that influences consumer approval or rejection. Fresh sugarcane juice looked olive-green and displayed obvious signs of de-greening throughout processing or storage (Mao et al., 2007). Therefore, kiwi juice was chosen in this study for its green color and excellent nutritional value (Doymaz, 2020). The color characteristics L*, a*, and b* of untreated, pasteurized, and microwave-treated samples in the presence of kiwi juice (0 to 8%) are shown in Table 3. There were no statistically noteworthy differences ($p \geq 0.05$) in L* values across all the free cane juice samples. However, increasing kiwi concentrations considerably increased the L* value in all treatments, implying that the samples are lighter. Adding kiwi juice to raw sugarcane juice did not result in a significant change in a* value (−green to +red). Although kiwi juice (*Actinidia deliciosa* var. Hayward) is high in chlorophyll (Liu et al., 2017) and causes an increase in phenolic compounds (Table 1), this may be owing to the low amount of kiwi juice in the samples (≤8%). In addition, there are no significant variances ($p \geq 0.05$) in a* values between untreated and pasteurized samples by increasing the kiwi juice level by ≥1%, but it significantly declined in the samples of microwave-treated samples (from 5.66 to 2.66). That means microwave-treated samples tend to be greener. Furthermore, at 0% and 1% kiwi levels, a* values were considerably lower in pasteurized samples than in microwave-treated samples, indicating that microwave samples were likely to be red at these kiwi levels. For b* values, the addition of kiwi juice in all treatments caused a considerable increase in their values, especially at concentrations of >1%, indicating that the green hue of the samples inclines to be yellow. Browning is caused mostly by the activity of the PPO enzyme on phenolic chemicals, which produces dark-colored polymers when sugarcane is crushed to release its juice. As a result, we may trace these color changes to PPO and POD activities (Table 2), both of which were greatly hindered by pasteurization and reduced by microwave treatment. Mao et al. (2007) also discovered that adding 0.1% ascorbic acid to cane juice inhibited de-greening significantly more than samples without ascorbic acid. Furthermore, the lightness L* of Lima Gomes et al.'s (2020) free cane juice samples increased significantly after pasteurization at 80 °C and 85 °C for 40 and 30 s, respectively, although this was not found in the current investigation, possibly due to the extended treatment time. According to Brochier et al. (2018), color variations in sugarcane juice may be influenced by a variety of process conditions, including Maillard reaction, thermal/electrical degradation of chemicals, POD, and PPO activity, among others.

Table 4
Microbial content (Log CFU/ml) of raw, pasteurized, and microwave-treated cane juice containing kiwi juice at concentrations of 0–8%.

Treatment	Kiwi conc. (%)	TPC	YM
Raw	0	*$5.30^{d} \pm 0.07$	$6.00^{f} \pm 0.43$
	1	$5.54^{c} \pm 0.05$	$6.15^{e} \pm 0.00$
	2	$5.77^{b} \pm 0.01$	$6.25^{d} \pm 0.07$
	4	$5.95^{b} \pm 0.00$	$6.30^{c} \pm 0.00$
	6	$6.18^{a} \pm 0.01$	$6.40^{b} \pm 0.03$
	8	$6.23^{a} \pm 0.05$	$6.48^{a} \pm 0.04$
Pasteurization	0	2	ND
	1	2	ND
	2	2	ND
	4	2	ND
	6	2	ND
	8	2	ND
Microwave	0	2	ND
	1	2	ND
	2	2	ND
	4	2	ND
	6	2	ND
	8	2	ND

* All values were expressed as the mean ± SD of three determinations. Different letters between rows indicate statistically significant differences between the samples (p-value <0.05). TPC = Total plate count, YM = Yeast and mold count.

Table 5
The identified compounds in kiwi juice (*Actinidia deliciosa* Hayward) perceived by GC–MS.

No.	Rt. (min)	Identified compound		Area (%)	Mw
		Scientific name	Common name		
1	4.99	Butanoic acid, 4-(2-oxocyclopentyl)-	4-(2-oxocyclopentyl) butanoic acid	3.21	170
2	5.29	Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexyloxy)-3-(1-piperidyl)-	–	**8.26**	297
3	6.92	*O*-Cymene	–	2.76	134
4	7.41	Dodecanoic acid, 3-hydroxy-	Lauric acid	1.36	216
5	8.00	Trans-2-undecen-1-ol	–	1.00	170
6	8.28	Tetradecanoic acid, 2-hydroxy-	Myristic acid	1.33	244
7	8.87	Melezitose	–	**6.65**	504
8	8.97	2-Hydroxypentadecyl propanoate	–	3.61	300
9	10.26	2-Hydroxyhexadecyl butanoate	2-Hydroxybutanoic acid	3.13	328
10	10.37	2-Propyl-tetrahydropyran-3-ol	–	5.26	144
11	11.16	Terpinen-4-ol	–	2.78	154
12	14.79	Phenol, 2-methyl-5-(1-methylethyl)-	Carvacrol	3.90	150
13	17.06	Decanedioic acid, 3,8-dioxo-, dimethyl ester	Sebacic acid, dimethyl ester	3.95	258
14	23.03	d-Melibiose	–	4.55	342
15	23.56	Desulphosinigrin	–	3.70	279
16	29.08	Hexadecanoic acid, methyl ester	–	3.23	270
17	29.94	n-Hexadecanoic acid	Palmitic acid	**9.52**	256
18	30.43	Hexadecanoic acid, ethyl ester	–	2.47	284
19	33.68	9-Octadecenoic acid (z)-	Oleic acid	**14.66**	282
20	34.15	Octadecanoic acid, ethyl ester	Stearic acid, ethyl ester	3.96	312
21	35.95	Heptacosane	–	**9.73**	380

3.4. Microbial counts

Sugarcane juice, as nutritious as it is, is susceptible to spoilage by many microorganisms, particularly fungus and lactic acid bacteria, as well as *Escherichia coli*, enterococci, and coliform. This contamination results from interactions with fecal matter and offers a serious risk of infection if the raw juice is consumed (Dhansu et al., 2023). Total plate counts (TPC) as well as yeast and mold counts were evaluated in this study to assess the efficacy of the tested processing methods: pasteurization and microwave in the presence of kiwi. The results (Table 4) reveal that raw samples had a high microbial load as a total count, as well as yeast and fungi, which rose significantly as kiwi concentration increased. Juice contamination can occur at numerous phases of processing, including poor staff handling, infected sugarcane or collecting containers, roller drum crushers, the filter, and the addition of ice to the juice (Arif et al., 2019). The results also showed that the treated samples met the acceptable microbiological limit (no >2 log CFU/ml) in the packed juice samples, which is supported by other research studies on different juices (Lima Gomes et al., 2020; Nayak et al., 2020). That means pasteurization (85 °C) for 5 min or microwave treatment (for 3 min) preserves the required juice qualities, making it biologically safe for consumption.

3.5. GC–MS analysis

In recent years, GC–MS investigations of medicinal plants have become more common, as this approach has proven to be a beneficial way of analyzing nonpolar components, including volatile essential oils, fatty acids, lipids, and alkaloids (Ozen et al., 2019). GC–MS analysis was undertaken to identify the major components of kiwi juice that play an important role in the inactivation of POD and PPO. A total of 21 distinct chemicals from various functional groups were discovered in this study (Table 5), primarily among alcohols, fatty acids, acids, esters, and sugars. In descending order, the most prevalent chemicals were oleic acid (14.66%), heptacosane (9.73%), palmitic acid (9.52%), propan-2-ol, 1-(2-isopropyl-5-methylcyclohexyloxy)-3-(1-piperidyl) (8.26%), and melezitose (6.65%). Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexyloxy)-3-(1-piperidyl), 2-propyl-tetrahydropyran-3-ol, terpinen-4-ol, and trans-2-undecen-1-ol are among the alcohols found. The latter is a white liquid with a fatty, green, slightly citrus-like odor that is thought to be responsible for the scent of kiwi fruit (Surburg & Panten, 2006). Esters of sebacic acid, palmitic acid, and stearic acid have also been identified as significant contributors to the kiwifruit aroma. Heptacosane is a straight-chain alkane with 27 carbon atoms that has beneficial health effects, such as overcoming multidrug resistance in myeloid leukemia (Labbozzetta et al., 2022).

Lauric acid, myristic acid, palmitic acid, and oleic acid are among the fatty acids found in kiwi juice. Lauric acid (C12), myristic acid (C14), and palmitic acid (C16) are saturated fatty acids. Oleic acid, on the other hand, is a monounsaturated fatty acid (18:1 cis-9) that causes moderate PPO inhibition even at very high doses (80 mM) (Sellés-Marchart et al., 2007). Palmitic acid was recently discovered to be an inhibitor of PPO in fresh Chinese cabbage (*Brassica pekinensis*) at concentrations of 0.03–0.05 g/l (Gao et al., 2023). Butanoic acid, 4-(2-oxocyclopentyl), a straight-chain alkyl carboxylic acid often called butyric acid, was also detected. Carvacrol is found in many plants, such as oregano and wild bergamot, and it is classified as a phenolic monoterpenoid that possesses an antioxidant effect (Sharifi-Rad et al., 2018). Desulphosinigrin, on the other hand, is a glucosinolate molecule with anticancer and antibacterial activity (Youssef et al., 2023). Sugars like melezitose and melibiose were also discovered. Melezitose, commonly known as melicitose, is a type of non-reducing trisaccharide sugar made up of two glucose molecules and one fructose molecule (Seeburger et al., 2020). It showed a potential effect on extending the shelf life of fresh pineapple when coated with a melezitose-chitosan coat (5 mg/l) (Malikul Ikram et al., 2023). Based on the GC–MS analysis, it could be concluded that kiwi juice may inhibit PPO and POD in cane juice not only because of its ascorbic acid and citric acid content but also because of the presence of additional constituents such as oleic acid, palmitic acid, and melezitose.

Table 6
The free binding energies (ΔG) calculated in kcal/mol of the identified phytochemicals within peroxidase, and polyphenol oxidase binding sites computed by molecular docking study.

No.	Compounds	Target enzyme	
		Peroxidase (1BEM)	Polyphenol oxidase (3WKY)
		Binding energy ΔG (kcal/mol)	
	Co-crystallized ligand (MES)	−42.1891	–
	Co-crystallized ligand (NAG)	–	−17.4470
1	Butanoic acid, 4-(2-oxocyclopentyl)-	−39.0381	−15.4668
2	Propan-2-ol, 1-(2-isopropyl-5-methylcyclohexyloxy)-3-(1-piperidyl)-	−54.9224	−31.4578
3	*O*-Cymene	−26.3744	−9.08243
4	3-Hydroxydodecanoic acid	−48.0445	−21.2251
5	*2*-Hydroxytetradecanoic *acid*	−52.6203	−23.7995
6	Melezitose	63.2361	−30.9556
7	2-Hydroxypentadecyl propanoate	−57.9690	−25.8458
8	2-Hydroxyhexadecyl butanoate	−60.9430	−26.8132
9	2-Propyl-tetrahydropyran-3-ol	−30.1516	−12.2717
10	Terpinen-4-ol	−30.5877	−12.7816
11	Carvacrol	−31.7107	−12.5558
12	Dimethyl 3,8-dioxodecanedioate	−48.0871	−24.8721
13	Melibiose	−57.0282	−28.9100
14	Desulphosinigrin	−46.3573	−20.6172
15	Methyl palmitate	−52.7925	−25.0814
16	Palmitic acid	−56.0220	−23.1077
17	Ethyl palmitate	−54.8196	−27.3566
18	Oleic acid	−61.1395	−23.9819
19	Stearic acid ethyl ester	−57.7158	−30.1610
20	Heptacosane	−66.9916	−31.4595
21	Ascorbic acid	−33.2669	−15.6941
22	Citric acid	−34.9296	−11.6051

Ozen et al. (2019) discovered 20 distinct chemicals in green light genotype kiwi fruit, the most abundant of which were oleic acid methyl ester, palmitic acid methyl ester, and stearic acid methyl ester, in descending order. Heptacosane and carvacrol were also detected in the same genotype at percentages of 0.34 and 6.47%, respectively. Yang et al. (2016) reported that the major fatty acids in kiwi fruit were oleic acid, linoleic acid, stearic acid, and palmitic acid. Additionally, Zhao et al. (2021) found esters, the volatile chemicals that mostly display the fruity or floral smell in kiwifruit, in three kiwi cultivars. Most of the esters found were straight-chained, although butanoate, hexanoate, and acetate esters are also being identified. The varied scent profiles of kiwifruit varieties may be due to variances in content and different sorts of chemicals.

GC-mass spectrometry was also applied to assess the impact of treatments such as pasteurization and microwave heating on the chemical composition of sugarcane juice in the presence of kiwi juice. A comparative statistical analysis was conducted between the treated juice and the untreated (raw), as presented in (**Supplementary 1**). The findings indicate that heat treatment induces changes in the concentration of specific essential kiwi constituents within the juice. While some compounds decreased in concentration, others remained unaffected by the applied heat treatments.

The analysis reveals that oleic acid, a major component of kiwi (14.66%), and palmitic acid remain unaffected by both treatments, suggesting that they are thermostable in the treated juice and maybe the fatty acids responsible for enzyme inhibition. Heptacosane, one of the components of kiwi juice (9.73%), is highly sensitive to heat treatment, and while its concentration increases in untreated (raw) juice with kiwi addition, both microwave and pasteurization methods used to preserve cane juice significantly reduce its levels even in the presence of kiwi. This indicates that heptacosane is thermolabile. However, the impact of

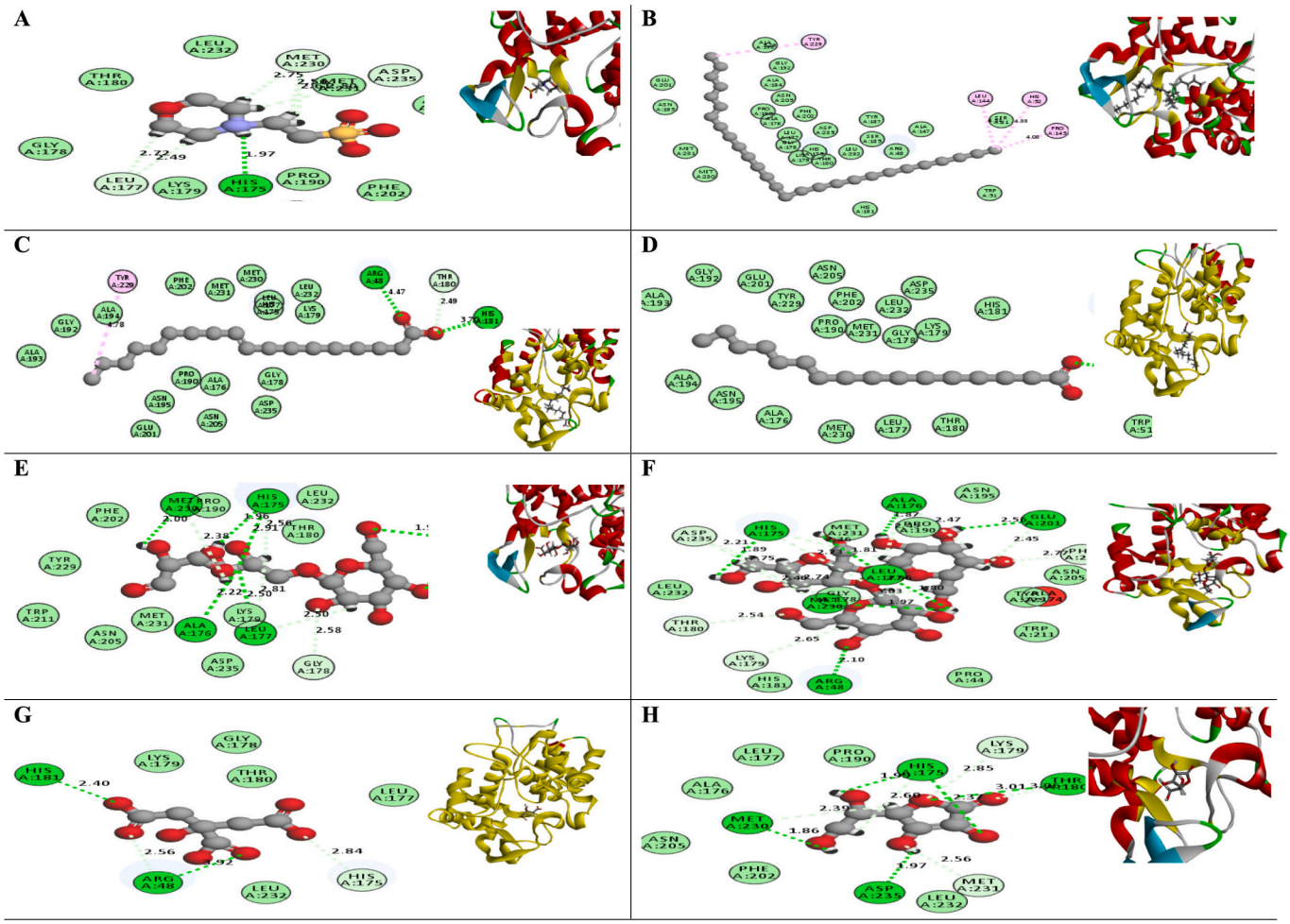

Fig. 1. Two-dimensional (2D) and three-dimensional (3D) ligand–enzyme interactions of the selected compounds with peroxidase (1BEM): (A) Co-crystallized ligand (MES) (B) Heptacosane (C) Oleic acid, (D) Palmitic acid, (E) Melibiose, (F) Melezitose (G) Citric acid, (H) Ascorbic acid.

heat on stearic acid is moderate.

3.6. Molecular docking

To confirm the effect of each constituent of kiwi juice on POD and PPO inactivation in sugarcane juice, in silico molecular docking was performed as a fast screening approach. The results of the docking studies (Table 6) demonstrate that many of the identified molecules in kiwi juice have a strong affinity toward peroxidase (1BEM) and polyphenol oxidase (3WKY) compared to the co-crystallized ligands. The interactions of heptacosane, the main identified fatty acids (oleic and palmitic), and sugars (melezitose and melibiose) beside citric and ascorbic acids were drawn in 2D and 3D, as shown in Figs. 1 and 2. The co-crystallized ligand (MES) of POD recorded a binding energy score of −42.1891 kcal/mol, whereas the co-crystallized ligand (NAG) of PPO recorded −17.4470 kcal/mol. Heptacosane showed the greatest inhibition activity against POD and PPO, −66.9916 and − 31.4595 kcal/mol, respectively. The π-π interaction was observed with the following amino acids in peroxidase (1BEM): TYR A:229, LEU A:144, HIS A:52, and PRO A:145 (Fig. 1 **B**). However, the binding behavior with polyphenol oxidase (3WKY) was recognized through hydrogen bonding with only MET A:656 (Fig. 2 **B**). The two main fatty acids, oleic and palmitic acids, both have significant inhibition activity, but oleic acid has a higher inhibitory effect against POD and PPO (−61.1395 and − 23.9819 kcal/mol, respectively) through hydrogen bonding with TYR A:229, ARG A:48, THR A:180, and HIS A:181 in POD (Fig. 1 **C**) and only ASN A:427 in PPO (Fig. 2 **C**). Additionally, melezitose and melibiose also showed a great affinity for the binding site of PPO (−30.9556 and − 28.9100 kcal/mol, respectively); however, melezitose showed the strongest affinity with POD (−63.2361 kcal/mol) through hydrogen bonding with HIS A:175, LEU A:122, GLU A:201, ARG A:48, ALA A:176, and MET A:231 amino acids (Fig. 1 **F**). On the other hand, the organic acids such as ascorbic and citric showed lower inhibition activity compared to the co-crystallized ligand of both enzymes, as demonstrated by their recorded interaction energy. Citric acid binds with POD in HIS A:181 & A:175, and ARG A:48 (Fig. 1 **G**), and with PPO in THR A:488 and A490 (Fig. 2 **G**). This could demonstrate the role of the synergistic activity of the identified constituents in kiwi juice.

4. Conclusion

The current work aimed to investigate how adding kiwi juice (*A. deliciosa*, Hayward) followed by pasteurization or microwave treatment affected the various qualitative attributes of sugarcane juice (*Saccharum officinarum*). Based on the obtained information, kiwi juice addition significantly increased vitamin C content, acidity, reducing sugars, phenolic compounds, and antioxidant power in raw cane juice. It also significantly reduced PPO and POD activities, the most important causes of cane juice discoloration, which resulted in an increase in b* color attribute (yellowness). It is feasible to infer that excellent quality and safe sugarcane juice may be produced using pasteurization rather than microwave treatment after the addition of 2 ml of kiwi juice per 100 ml. This additive maintains the color of cane juice and inhibits natural browning enzymes. In addition, pasteurized samples had more

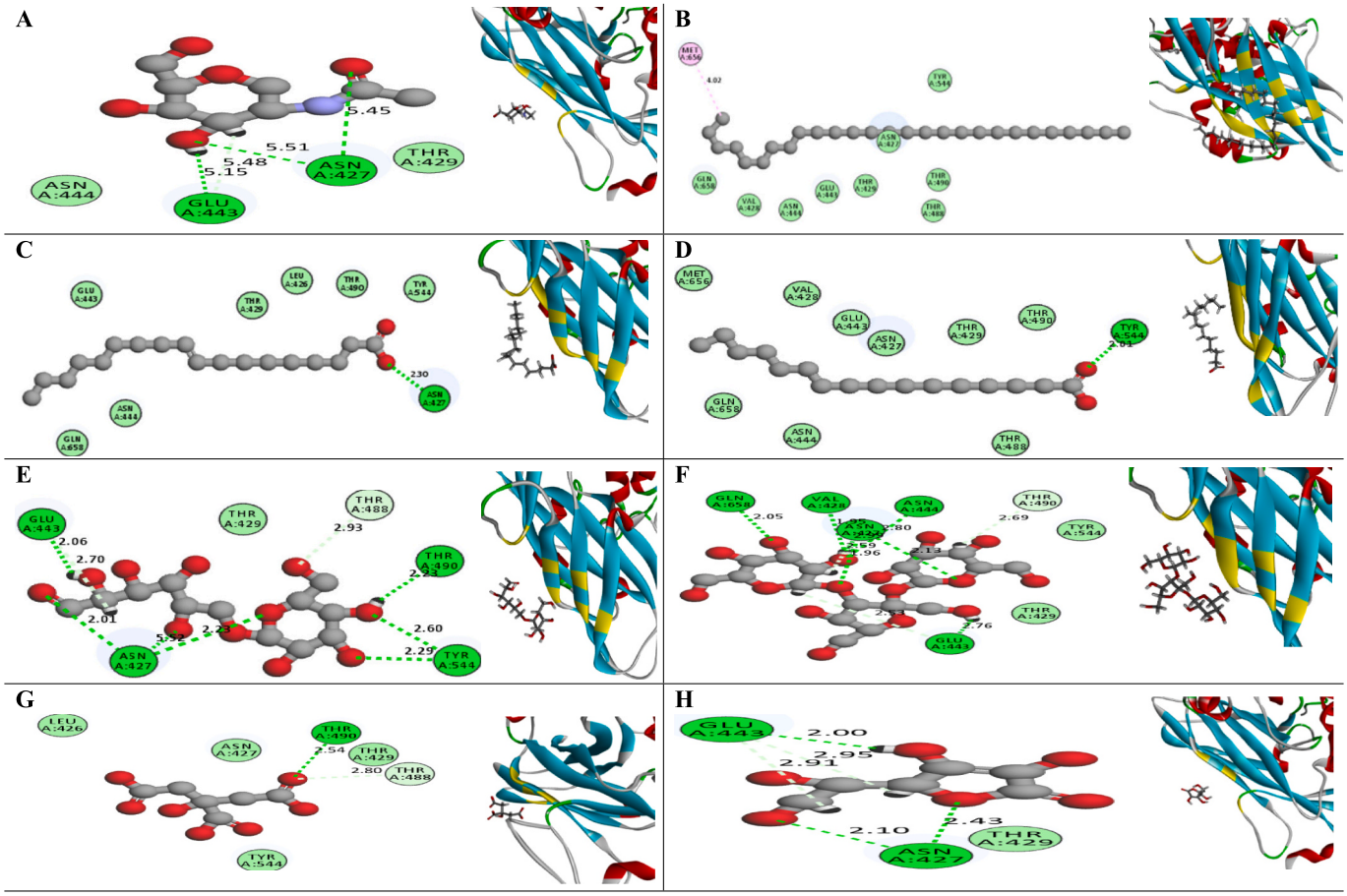

Fig. 2. Two-dimensional (2D) and three-dimensional (3D) ligand–enzyme interactions of the selected compounds with polyphenol oxidase (3WKY): (A) Co-crystallized ligand (NAG) (B) Heptacosane, (C) Oleic acid, (D) Palmitic acid, (E) Melibiose, (F) Melezitose, (G) Citric acid, (H) Ascorbic acid.

phenolic compounds; hence, sugarcane juice would be a less expensive and more effective option to enhance our intake of polyphenol compounds and vitamin C in our diet. Molecular docking recognized the most effective compounds in kiwi juice against browning enzymes, namely heptacosane, melezitose, and oleic acid, rather than ascorbic or citric acids. Oleic acid remains unaffected by treatments, while heptacosane is significantly reduced even in the presence of kiwi. The mechanism by which each major compound of kiwi juice acts as an inhibitor of these enzymes during storage, as well as the kinetics of both enzymes, remains to be explored.

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Heba Sayed Mostafa: Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Conceptualization. **Fatma Fakher Ramadan:** Methodology, Investigation, Data curation. **Hagar Ahmad Emam:** Methodology, Investigation, Data curation. **Engy Raafat Shaker:** Methodology, Investigation, Data curation. **Wafaa Mostafa El Kady:** Writing – original draft, Visualization, Validation, Software. **Aya Khaled Sayed:** Methodology, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.140133.

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