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The change in concentration of ochratoxin A and antioxidant capacity during the production of grape juice

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ABSTRACT

Ochratoxin A (OTA), a dangerous fungal toxin, has widely contaminated grapes and its by-products. To estimate dietary intake, information about OTA behavior during processing is demanded. There is currently much data to predict the fate of the OTA in different types of wine, but more is needed for grape juice This study evaluated the effect of grape juice-making steps on OTA contents antioxidant compounds, and activity capacity. OTA extraction and analysis were performed by immunoaffinity column and high-performance liquid chromatography. The juice was made from grapes artificially contaminated with OTA at a level of 5.0 μg/kg. Statistically significant changes in responses were found after the washing, preheating, juicing, clarification, and pasteurization processes. The processing of the grapefruit into grape juice led to a total decline of 62.0 % in OTA level. The remaining OTA content in the final grape juice in the present study was lower than the maximum residue limit for this product established by European Union (EU) legislation (2 μg/kg). Moreover, the overall reduction of 56.2 %, 53.9 %, and 54.3 % in the total phenolic, total flavonoid, and antioxidant capacity were observed, respectively.

1. Introduction

Grapes (*Vitis* sp.) are one of the fruits most produced and consumed globally, with an annual production of more than 67 million tons of berries (Evangelista et al., 2020; Granato et al., 2016). Approximately 2.3 million tons of this content is manufactured in Iranian territory. According to the Food and Agriculture Organization (FAO) statistics, Iran ranks 10th in global grape production (Chatrabgoun et al., 2020). Due to its high moisture and sugar content, the fresh grape is highly susceptible to microbiological spoilage. Therefore, grapes should be quickly processed into processed products such as raisins, wine, juice, and verjus within 1–2 weeks (Ashtiani et al., 2020; Chatrabgoun et al., 2020; Cosme et al., 2018).

The grape juice market is developing because it is a suitable economic alternative for countries that produce grapes (Cosme et al., 2018; Dutra et al., 2021). So, the total world production of grape juice in 2012 is estimated to be between 11 and 12 million hectoliters, where the main producing and consuming countries are the United States of America, Spain, and Brazil, respectively (Cosme et al., 2018). The chemical composition of grape juice is influenced by the grape type and processing technique (Dutra et al., 2018). However, protein, sugars, acids, vitamins, minerals, and antioxidant compounds are the main components of grape juice (Evangelista et al., 2020; Granato et al., 2016).

In vitro, studies have shown the biological activities of grape juice, such as anticancer, anti-inflammatory, antimicrobial, and cardioprotective (da Silva et al., 2019). These biological activities contribute to consumers' acceptance of grape juice (Cosme et al., 2018).

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In addition, the characteristic taste, aroma, and color of grape juice have been considered fresh and delightful sensory properties (Dutra et al., 2021).

Despite all the mentioned benefits of grape juice, some chemical contaminants such as mycotoxins, pesticides, and heavy metals have been reported in this product (Granato et al., 2016; Welke, 2019). However, mycotoxins are the most toxic food chemical contaminants that threaten human and animal health (Aranega and Oliveira, 2022; Bangar et al., 2022; El-Sayed et al., 2022; Pires et al., 2022).

Ochratoxin A (OTA) is a low molecular weight compound that is, the most known mycotoxin in grapes and its by-products (Hocking et al., 2007; Khaneghah et al., 2019). Sohigh-quality after cereals, wine and grape juice are the most important sources of OTA in the human diet (Gil-Serna et al., 2018). Besides grape and grain, OTA is found as a fungal toxin in a wide range of food crops, including nuts, pulses, spices, coffee, meat, milk, and baby foods (El-Sayed et al., 2022; Er Demirhan and Demirhan, 2022; Khoshnamvand et al., 2019; Safaei et al., 2023). Thus, the main human health harm from exposure to OTA is more due to low-level contamination of a wide range of foods than to high-level ingestion of a single food source (Ratola et al., 2005).

Aspergillus carbonarius is ,responsible for OTA production in grape juice (Welke, 2019). The sources of this mold are soil and rotten bunches in the vineyard (Hocking et al., 2007). Moreover, *P. verrucosum* and *P. nordicum* are OTA-producing species (Gil-Serna et al., 2018). Some conditions in the grape berries and vineyard, such as high sugar content, soft skin, and high relative humidity in the growing season, favor OTA production by molds (Welke, 2019).

OTA molecular chemical structure consists of a dihydro-isocoumarin moiety linked with the amino acid phenylalanine through an amide bond (Khaneghah et al., 2019). The chlorine group and methylated lactone ring are structures associated with OTA toxicity (Shen et al., 2020). OTA induces different toxic effects such as hepatotoxic, nephrotoxic, neurotoxic, and immunotoxin for laboratory and farm animals (Ponsone et al., 2009; Welke, 2019). Whereas, based on how it harms cells is classed as a teratogen, mutagen, and carcinogen (Khaneghah et al., 2019). OTA has been categorized as a group 2B carcinogen for humans by the International Agency for Research on Cancer (IARC) (Organization and Cancer, 1993).

Some studies have documented the occurrence of OTA in grapes and wine. For instance, Gholampour (2012) assessed the OTA of the sold grape juice in Iran markets; the OTA was detected in 32 of 100 samples (32 %), with an average concentration of 8.14 μg/kg, This amount is beyond the EU legislation (2 μg/kg) (European Commission, 2006; Gholampour, 2012). Moreover, the maximum value of 91.3 μg/L of OTA in white grape juice samples supplied in Germany was determined. This mycotoxin was seen in 77.8 % of the samples (21 out of 27) (Majerus et al., 2000). Terra et al. (2013) investigated OTA in wine and grape juice. OTA was found in 13 (38.24 %) wine samples ranging from <0.03 to 0.62 μg/L. However, OTA was not found in grape juice samples (Terra et al., 2013).

For the removal and detoxification of OTA, various studies have been performed. The role of chemical methods (chlorine dioxide and cyprodinil), biological strategies (probiotics and enzymes), and physical techniques (adsorption and radiation) in eliminating OTA has been documented (Wang et al., 2022a). However, Information on OTA persistence and transformation during foodstuff processing would be useful in developing an effective food safety program, including defining critical control points (CCP) and implementing corrective actions (Ponsone et al., 2009). In some studies, the effect of processing to reduce OTA by 90.7 %, 86.5 % and 75.19 % in white wine, red wine, and vinegar, respectively, has been identified (Freire et al., 2020; Heshmati et al., 2021; Ponsone et al., 2009). Some processing, including alcoholic and malolactic fermentation, applied in wine manufacture were not utilized in grape juice production. Therefore, the fate of OTA during grape juice production differed from its fate in vinegar and wine generation. A study has recorded the fate of OTA during grape juice production. In this study, authors showed a 73 % reduction of OTA after different times of grape juice extraction (30, 60, 90, and 120 min) (Dachery et al., 2017). research study investigated the effect of steam extraction on OTA. So far, no comprehensive study has been conducted to investigate the effect of all stages of grape juice production on OTA. Therefore, the present study was conducted for the first time to investigate the fate of OTA and antioxidant compounds and capacity in grapes in the entire unit operation and the steps applied for grape juice processing (washing, preheating, dewatering, clarification, and pasteurization).

2. Materials and methods

2.1. Materials

The OTA standard was supplied by Sigma (St. Louis, MO, USA). Immunoaffinity columns (IAC) were bought from Libios (Pontcharra-Sur-Turdine, France). Phosphate buffer saline (PBS), acetonitrile, methanol, and sodium chloride were obtained from Merck (Darmstadt, Germany). A Milli-Q system (Millipore, Milford, MA, USA) was used for ultra-pure water production. Pectinase made of *Aspergillus niger* was prepared by Behnogen (Tehran, Iran). Bentonite was purchased from Mojallali Inc. (Tehran, Iran).

2.2. Spiking OTA into grape

Fresh grapes (various Fakhri) were purchased from a local store and transferred to the laboratory. At first, the OTA content of fresh grapes was measured. The concentration of OTA in fresh grape samples was lower than HPLC's limit of detection (LOD). Therefore, because no natural OTA contamination was observed, fresh grape samples were spiked with OTA at a 5 μg/kg level. The chosen spiked OTA level (5 μg/kg) was based on 50 % of the maximum acceptable limits (10 μg/kg) for this mycotoxin in grapes (ISIRI, 2012), and the high contamination level of OTA reported in the literature (Mehri et al., 2022; Varga and Kozakiewicz, 2006). First, 1 mL of standard mycotoxin solution (1000 μg/L) was diluted with 9 mL of methanol. Then, 500 μL of stock solution was added to 100 g of grape samples to obtain a sample containing 5 μg/kg OTA. The grapes were agitated by a mechanical mixer (Model 40979, Gastroback GmbH, Hollenstedt, Germany) for 1 h to distribute OTA. Samples were stored overnight in the refrigerator to infiltrate the toxin. OTA concentration at the spiked samples was 5.0 μg/kg.

2.3. Grape juice production

At first, grape samples were immersed in tap water for 5 min, then their stems were separated. Samples were preheated by a laboratory heater (62.8 °C for 10 min). Then to increase the juicing efficiency, pectinase (0.01 %) was added to samples and placed for 30 min at ambient temperature. Afterward, the cloudy grape juice was obtained by a juicer machine (Pars Khazar, Tehran, Iran). For clarification, bentonite (1.5 %) was mixed with grape juice on a magnetic stirrer (MTOPS, HS15–03 P model, Korea) for 10 min and precipitated after 2 h. After this period, saturated bentonite was separated from the juice by Whatman filter paper (No. 2). Finally, the grape clear juice was pasteurized at 85 °C for 5 min (Fuleki and Ricardo-da-Silva, 2003). According to Fig. 1, the OTA content of all collected samples (samples A–F) was measured in three replicates.

The reduction of OTA in each stage (%) was calculated according to the following Equation:

The reduction (%) of OTA in each stage = [(OTA concentration before each stage - OTA concentration after each stage)/ OTA concentration before each stage] × 100 (1)

The reduction of OTA compared to fresh grapes was obtained by the following equation:

The reduction (%) of OTA compared to fresh grapes = [(OTA

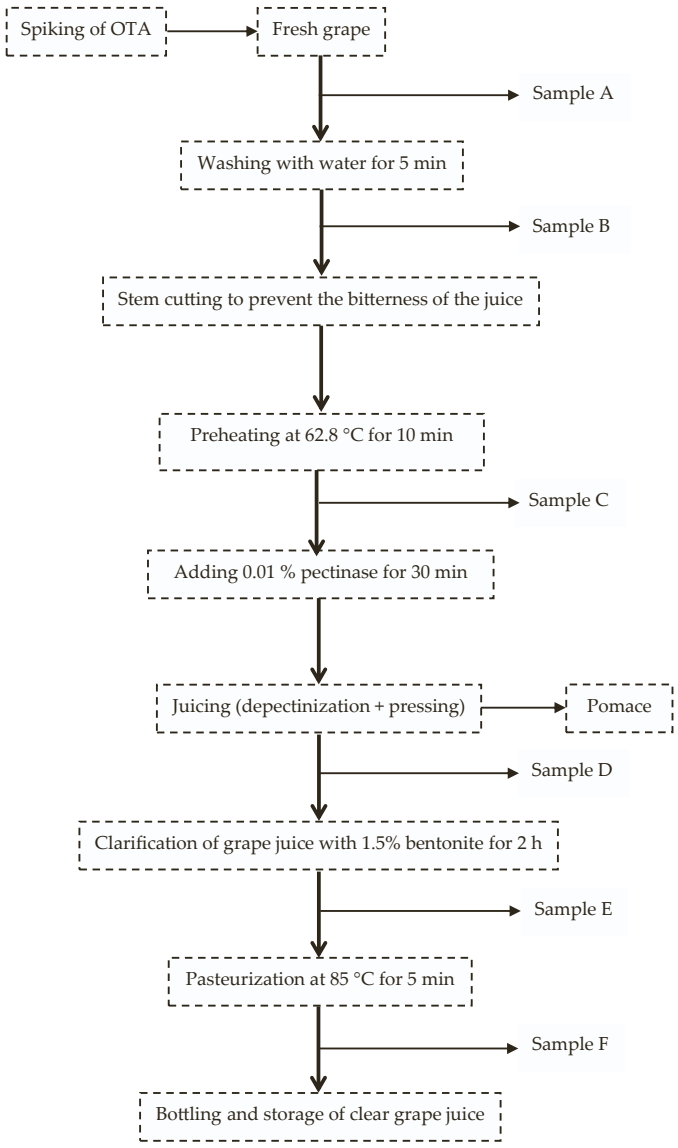

Fig. 1. Different stages of grape juice production.

concentration of fresh grape - OTA concentration after each stage)/ OTA concentration of fresh grape] × 100 (2)

In the above equations, OTA concentration was considered as μg/kg.

2.4. Extraction and clean-up of OTA

OTA measurement was performed according to our previous study with slight modifications (Behfar et al., 2022). First, 40 g of the sample was mixed with methanol (23 mL), deionized water (150 mL), and sodium chloride (2.5 g) by a magnetic stirrer for 10 min. After filtering the solution through filter paper (Whatman No. 1), 23 mL of the filtrated solution was added to 128 mL of PBS. The fluid was centrifuged (4000 RPM for 10 min) (Hettich, Tuttlingen, Germany). Fifty mL of the sample was passed through IAC. The column was washed with methanol until the vial volume reached 2 mL. Finally, the injection volume was set at 40 μL.

2.5. HPLC characteristics

OTA analysis was performed by reverse-phase high-performance liquid chromatography (Waters, 2695, Milford, MA, USA) equipped with a fluorescence detector (model 2475, Milford, MA, USA) and C18 column (250 mm × 4.6 mm, i.d., 5 μm) at 25 °C (Waters, ODS1, Milford, MA, USA). A mixture of water, acetonitrile, and methanol (5:3:2, v/v/v) at a 1 mL/min flow rate was used as the isocratic mobile phase. The excitation and emission wavelengths were determined as 335 nm and 465 nm, respectively.

2.6. HPLC method validation

Method performance parameters for OTA are cited in Table 1. The HPLC method's LOD and limit of quantification (LOQ) were considered threefold and tenfold the signal-to-noise ratio (S/N), respectively. Linearity was established using a calibration curve of the peak area versus the working standard concentration. First, the samples were found to be free of OTA. Then, recovery (accuracy) and precision (repeatability) were performed by spiking and remeasurement of three OTA levels in each foodstuff (2, 4, 6 μg/kg in grape and 0.75, 1.5 and 3 μg/kg in grape juice). Intra- and inter-day precision was determined by measuring a minimum of three replicates on three consecutive days.

2.7. The determination of TPC, TFC, and antioxidant capacity

The TPC, TFC, and Antioxidant capacity were measured according to the previous study (Behfar et al., 2022). Antioxidant capacity was determined by Reducing Antioxidant Power Assay (FRAP) and reported as μmole ferrous sulfate equivalent/kg dry weight (μmole $FeSO_4$ E/kg). The TPC and TFC were reported as μg gallic acid equivalent per g of dry matter (μg GAE/g) and μg catechin equivalent/g of dry matter (μg CE/g), respectively.

2.8. Statistical analysis of data

The statistical analysis was performed by SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). An ANOVA test examined the difference in OTA content and reduction level among various steps of grape juice processing. $P < 0.05$ was considered a significant level.

3. Results and discussion

3.1. Assurance of method quality

The parameters evaluated in method validation and its related results are shown in Table 1. The recovery values of OTA in grape samples contaminated with this mycotoxin at concentrations of 2, 4, and 6 μg/kg were in the range of 94.7–98.5 %, with LOD and LOQ of 0.3 μg/kg and 1 μg/kg, respectively. The highest grape juice OTA recovery was 106 % at the concentration of 1.5 μg/kg, and the lowest was 99.6 % at 0.75 μg/

Table 1
Validation data of ochratoxin A (OTA) in grape and grape juice by HPLC method.

Foodstuff	Spiking level (μg/kg)	Recovery		Linearity (μg/kg)	LOD (μg/k)	LOQ (μg/kg)
		Intra-day ± RSD (%)	Inter-day ± RSD (%)			
Grape	2	95.2 ± 7.9	98.2 ± 5.9	0.35 - 2	0.3	1
	4	96.3 ± 11	97.3 ± 12			
	6	94.7 ± 11	98.5 ± 15			
Grape juice	0.75	101 ± 14	99.6 ± 6.8	0.20 - 5	0.15	0.47
	1.5	106 ± 13	102 ± 11			
	3	103 ± 16	101 ±			

LOD: Limit of detection LOQ: Limit of quantification

kg. The LOD and LOQ amount of OTA in grape juice were 0.15 μg/kg and 0.47 μg/kg, respectively. The maximum relative standard deviations (RSD) of the intra and inter-day precision of OTA were 15 % for grape and 16 % for grape juice, which complies with Commission Regulation (EC) No. 401/2006 (RSD $\leq$ 20). According to EC, the mean recoveries of analytical methods for OTA should be between 70–110 % at levels 1 μg/kg to 10 μg/kg and 50–120 % at levels $<$ 1 μg/kg (Commission, 2006).

3.2. Fate of OTA during grape juice manufacture

Table 2 shows the effect of different stages of grape juice production (Fig. 1) on the OTA amount.

After washing grapes with tap water for 5 min, the average concentration of OTA (sample B) was 4.1 ± 0.2 μg/kg, which showed a significant reduction of 18 % compared with fresh grape samples ($P < 0.05$). Other reports have previously demonstrated that the grape OTA content reduces after the rinsing or washing process. For instance, the OTA content in grapes variety of Asgari contaminated with this mycotoxin at 5 μg/kg, soaked in water for 5 min and rinsed for 20 s, was reduced by 16.53 % (Heshmati et al., 2020). Heshmati et al. (2021) indicated that washing grapes Fakhri cultivar (spiked to 2.49 μg/kg toxin) with tap water for 20 s resulted in lower OTA dissipation (9.63 %) levels. The lower reduction compared to the present results may be due to the shorter washing time. The log P is the partition coefficient between octanol and water used to describe the role of chemical structure hydration; the higher the amount the weaker the hydration (Bergström and Larsson, 2018). OTA with a log P value of 3.66 is a compound relatively soluble in water a few amount of which was reduced during the washing process (Karlovsky et al., 2016). Therefore, the washing step used to decontaminate grape berries from microorganisms, leaves, and soil could reduce this mycotoxin.

In the grape juice industry, grapes are preheated before pressing to maximize the yield of juicing and preserve the color (Featherstone, 2016). By calculating the difference in OTA value between samples B (4.1 ± 0.2 μg/kg) and C (4.0 ± 0.1 μg/kg), the lowest decrement in this study (2.4 %) was related to the preheating stage (Table 2). According to the authors' knowledge, there are no studies on the effect of preheating on grape OTA content. However, the impact of the preheating process on other mycotoxins has been studied in other fruits. Eskandari et al. (2014) showed that the preliminary cooking of apple juice decreased 48.2 % of patulin. They used a higher temperature (90 °C) and longer time (20 min) than our investigation (Eskandari et al., 2014). Another study indicated that preheating tomatoes at 95 °C for 30 min removed 27 % of the patulin (Van de Perre et al., 2014).

The pectinase enzyme was used to break the pectin structure in the cell wall and increase the total yield of juice (Verma et al., 2018). As shown in Table 2, the juicing step had a significant impact on OTA loss (25.0 ± 2.5 %), which was more than the diminution of OTA during the juicing of grape for vinegar (18.86 %) and pekmez (8.32 %) production (Heshmati et al., 2020; Heshmati et al., 2021). The utilization of the pectinase enzyme in the present study has led to OTA increased removal efficiency. The relation between the OTA reduction and enzyme activity could be related to the ability of some enzymes for hydrolytic cleavage of the amide bond and converting OTA into OTα (Wang et al., 2022b). in rats, the removal half-live of OTα is 9.6 h and OTA is 103 h. Therefore, a lower elimination half-live of OTα compared to OTA is considered to be the route for OTA detoxification (Abrunhosa et al., 2010). In addition, hydrogen bonding between the amine group of pomace protein and the hydroxyl group of OTA is responsible for mycotoxin losses during the juicing operation (Giubertoni et al., 2020; Heshmati et al., 2021). According to Aroud et al. (2021), the OTA level decreased from 64 μg/L to 54 μg/L (15.62 % removal) in apple juice with 7 mL/hL pectinolytic enzymatic treatment after 2 h.

Clarification is one of the most effective steps in the grape juice production process due to the removal of suspended solid compounds (Behfar et al., 2022). Bentonite is known as a common clarifier because it is the main agent for reducing turbidity and removing protein and phenol (Dıblan and Özkan, 2021). As observed in Table 2, the OTA concentration in the cloudy grape juice was 3.0 ± 0.1 μg/kg, while its content in the clarified sample was decreased to 2.2 ± 0.1 μg/kg. Among the steps of grape juice processing, the clarification had the highest impact (26.7 ± 3.1 %) on OTA reduction. Var et al. (2008) reported that the clarification of white wine (containing OTA in the concentration of 10 ng/mL) with bentonite (0.4 mg/mL) decreased the content of this mycotoxin by 6.2 %, which was lower than our results (Var et al., 2008). Leong et al. (2006) investigated the impact of a dose of 2.5 g/L of bentonite on the OTA of Semillon wine containing 8 μg/kg OTA. They reported a removal rate of 67 % for OTA, which was higher than that in our study (Leong et al., 2006). As shown above, the data regarding the impact of clarification on OTA grape juice differed. It appeared that the data discrepancies were related to the difference in matrix type, concentration of bentonite, as well as the initial OTA amount. Bentonite separates OTA from grape juice according to two mechanisms: 1- the negatively charged surfaces of OTA are adsorbed electrostatically by the positively charged proteins, and this protein bonded to OTA intercalates between clay layers of bentonite (Behfar et al., 2022; Dıblan and Özkan, 2021). 2- hydrogen bond between the carbonyl group of OTA and hydrogen in the bentonite crystal (Heussner and Bingle, 2015; Kohl, 1960).

The heating was applied as a traditional way to sterilize and preserve food (Wang et al., 2022b). Our results showed that 13.6 ± 1.1 % of OTA was destroyed by the thermal treatment at 85 °C 5 min (Table 2). Some authors indicated that 11.28 % of OTA was decreased during grape vinegar pasteurization at 68 °C in 30 min, which is near the value seen in our study (Heshmati et al., 2021). In contrast, it has been shown that the OTA of apple juice is stable with heating at 72 °C for 30 s; this lack of decline was most likely due to their use of a lower temperature and shorter time (Aroud et al., 2021). Because of OTA's high thermostability it contains many double bonds between carbons in the structure of molecular. However, the main OTA degradation products are created by partial isomerization at the C3 position, including 14-(R)-OTA and 14-decarboxy-OTA (Vidal et al., 2015). Also, temperature leads to making OTα-amide due to the racemization of the phenylalanine moiety (Sueck et al., 2019). Thermal degradation products of OTA, such as 14-(R)-OTA, 14-decarboxy-OTA, and OTα-amide, have less toxicity (Karlovsky et al., 2016). In addition, OTA is bound to polysaccharides through ionic interactions during thermal processing, which is not identifiable (Bryła et al., 2021). In general, OTA levels in juice produced from table grapes fortified with toxin were reduced from 5.0 μg/kg to 1.9 μg/kg. However, the toxin remains during the juice-making process and is present in the final commodity. However, the amount of OTA measured in grape juice was less than 2 μg/kg, which has been

Table 2
Change in OTA concentration during the preparation of grape juice.

Processing stage	OTA content (μg/kg)*	Reduction of OTA during each stage (%)	Reduction of OTA compared to fresh grapes (%)
Fresh grapes	5.0^{a}	-	-
Washing	4.1 ± 0.2^{b}	18.0 ± 1.3^{c}	18.0 ± 1.3^{e}
Preheating	4.0 ± 0.1^{b}	2.4 ± <0.1^{e}	20.0 ± 2.6^{d}
Juicing (depectinization + pressing)	3.0 ± 0.1^{c}	25.0 ± 2.5^{b}	40.0 ± 3.8^{c}
Clarification	2.2 ± 0.1^{d}	26.7 ± 3.1^{a}	56.0 ± 5.1^{b}
Pasteurization	1.9 ± 0.1^{d}	13.6 ± 1.1^{d}	62.0 ± 4.5^{a}

* Values within the same column with different superscript letters indicated significant differences ($P < 0.05$).

determined by EU legislation as the maximum residue limit (European Commission, 2006). The findings regarding OTA reduction during grape juice production were lower than the results of Aroud et al. (2021). They showed that the OTA level in apple juice declined from 64 μg/L to 11 μg/L (Aroud et al., 2021). Considering the health benefits derived from juice consumption, its easy accessibility in all positions, and the extreme reduction in mycotoxin influenced by processing techniques, a scaling-up of the production technology for grape juice is recommended.

In general, OTA concentration (1.9 μg/kg) in the pasteurized grape juice was decreased to 62.0 % in comparison with fresh grape (5.0 μg/kg). OTA reduction value during grape juice processing (62.0 ± 4.5 %) was greater than that of during pekmez processing (37.5 ± 6.5 %) and lower than that during grape vinegar production (75.2 ± 5.7 %) that was reported in our previous studies (Heshmati et al., 2020; Heshmati et al., 2021).

3.3. Fate of TPC during grape juice manufacture

The washing as the first stage in the grape juice process had no significant effect on TPC (Fig. 2. A). In agreement with our data, the soaking of Yuja with tap water for 10 min did not affect the TPC (Sung et al., 2011). The berries' cuticular wax layer mainly contained aliphatic compounds, which renders the berry waterproof (Yang et al., 2021). This hydrophobic layer prevents the loss of polyphenols during washing.

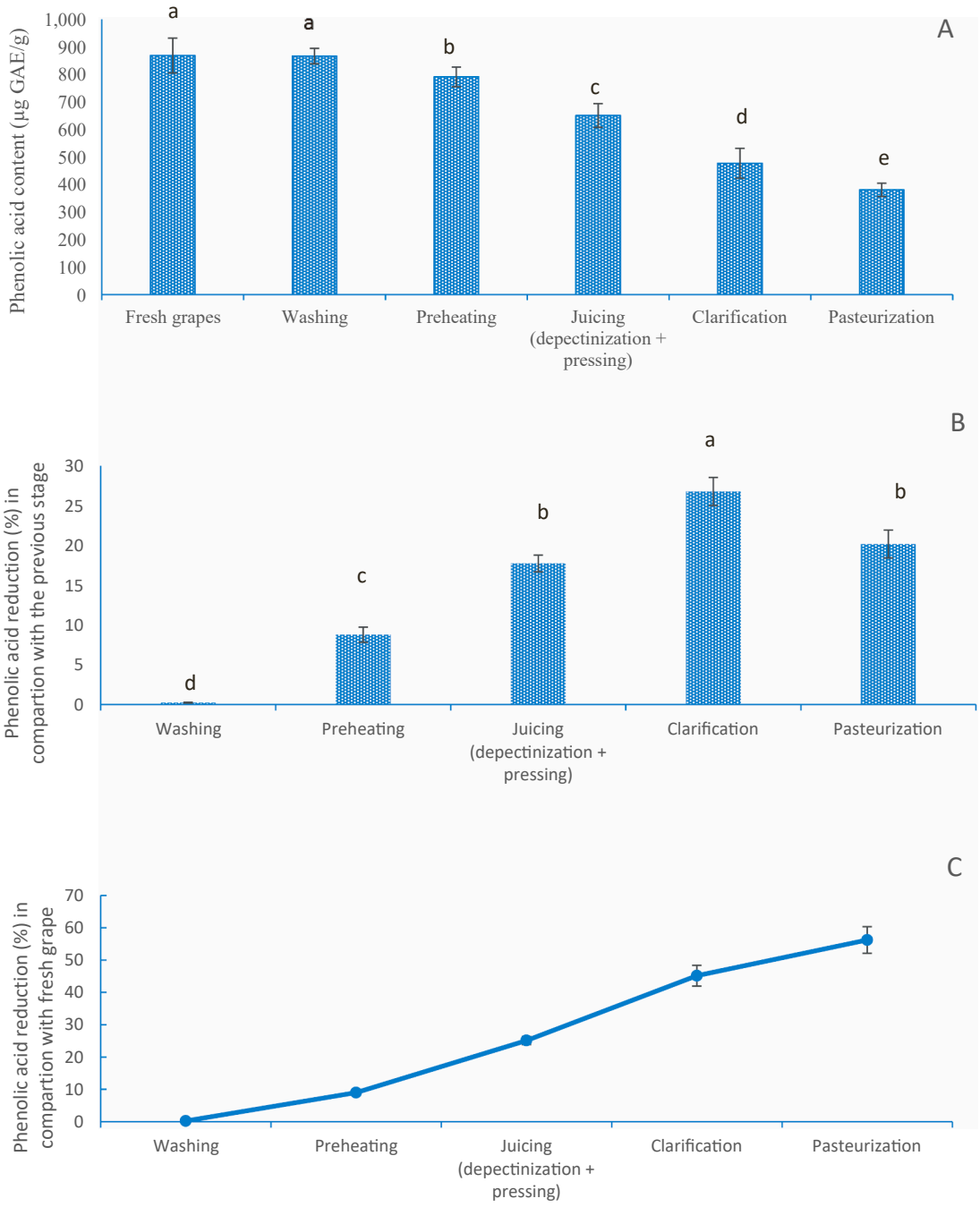

Fig. 2. The change of phenolic acid amount during grape juice production (A), the reduction of phenolic acid level s during each stage in comparison with the previous stage (B), the reduction of phenolic acid levels during each stage in comparison with fresh grape (C). The different letters above columns indicate statistically significant differences ($P < 0.05$).

As presented in Fig. 2. A, the TPC of preheated grapes (790.8 μg GAE/g) is lower than those in raw samples (868 ± 63 μg GAE/g). At higher temperatures, polymerization reactions between phenolic compounds resulted in the reduction of TPC (Suzme et al., 2014). However, Mannozzi et al. (2018) expressed that preheating apple mash at 80 °C led to an increase in TPC, which is in contrast to our results.

During juicing (serpentinization + pressing), 650.6 μg GAE/g of TPC was transferred into fruit juice and the remains (17.7 %) were discarded by pomace. Pectinase can release the cinnamic acid derivatives such as coumaric and ferulic acids from their ester linkages and metabolize into volatile phenols, which explains the decline of cinnamic acid derivatives from the impact of pectinase (Shah et al., 2015). In a study done by Balík et al. (2009), the TPC of grapes was reduced by 7.5 % during juicing.

Fig. 2. B shows the clarification step led to the most reduction in the TPC of juice (26.8 %). In the previous studies, the reduction of TPC during clarification of black carrot juice and red wine was 10 % and 4.5 %, respectively (Dereli et al., 2015; González-Neves et al., 2014). Bentonite cations such as interact electrostatically directly with negatively charged phenolic acids (Dıblan and Özkan, 2021).

As Fig. 2 shows, 20.2 % of TPC was lost during pasteurization. This reduction might be related to the Maillard reaction and the decarboxylation of phenolic acids in the presence of high moisture (Zhao et al., 2019). There are some different findings about the influence of pasteurization on juice TPC. Ma et al. (2013) heated carrot juice at

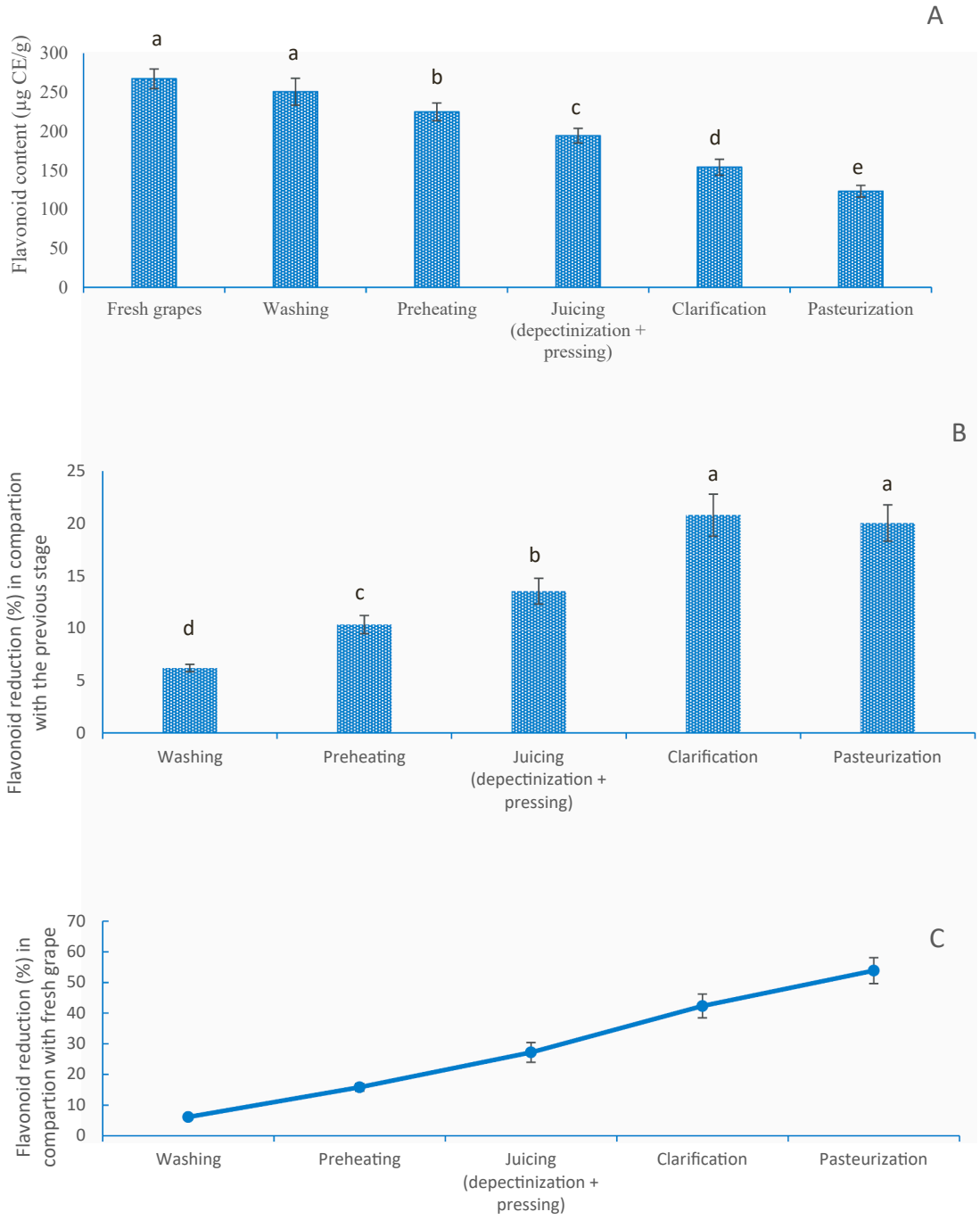

Fig. 3. The change of flavonoid amount during grape juice production (A), the reduction of flavonoid levels during each stage in comparison with the previous stage (B), the reduction of flavonoid levels during each stage in comparison with fresh grape (C). The different letters above columns indicate statistically significant differences ($P < 0.05$).

100 °C for 30 s and observed 59.8 % removal of TPC. In contrast, the pasteurization of black carrot juices caused a 1.1- 2.3-fold increase in the TPC (Dereli et al., 2015).

Processing the grape juice led to an overall reduction of TPC by 56.2 ± 4.1 (Fig. 2 C), which is lower than that in carrot juice concentrate (70 %) and pomegranate juice (68 %) (Farahmand et al., 2017; Suzme et al., 2014).

3.4. Fate of TFC during grape juice manufacture

During washing, TFC decreased by about 6.2 % (Fig. 3). Anthocyanins as one of the most important classes of flavonoids are water-soluble pigments and the washing step leads to the remove of these compounds (Zhao et al., 2019).

From Fig. 3. B could conclude that the preheating and juicing (depectinization + pressing) cause TFC significant dissipation by 10.3 % and 13.5 %, respectively. Suzme et al. (2014) reported an increase in TFC (7.3 % after 1 min of preheating in black carrots at 85–90 °C) that is contrary to our results. The cooking will destroy the microstructure of grapes and induce a better extraction of anthocyanins (Zhao et al., 2019). However, activation of the peroxidase enzymes during preheating leads to anthocyanins enzymatically degradation (Suzme et al., 2014).

Fig. 3. B reveals the loss of 20.8 % TFC after clarification by bentonite at a 1.5 % level. In literature, it has been mentioned that bentonite could not lead to a marked loss of monomeric flavanols in red wine (Gonçalves and Jordao, 2009). However, Cosme et al. (2020) observed similar results in the fining of red wine using bentonite. Dordoni et al. (2015)

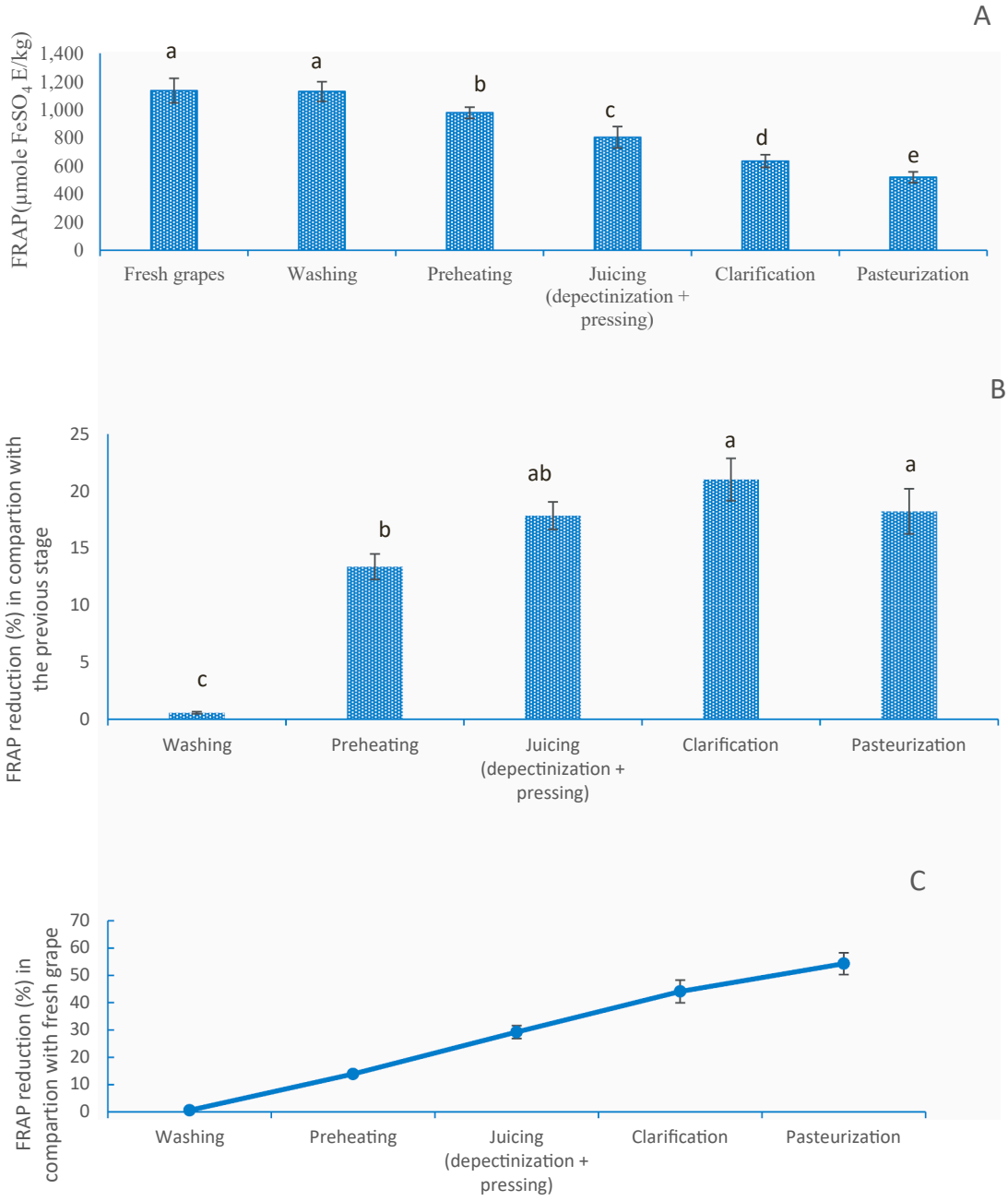

Fig. 4. The change of FRAP levels during grape juice production (A), the reduction of FRAP levels during each stage in comparison with the previous stage (B), the reduction of FRAP levels during each stage in comparison with fresh grape (C). The different letters above columns indicate statistically significant differences ($P < 0.05$).

suggested that the hydrogen bond between the hydroxyl groups of the benzene ring of anthocyanin and bentonite is linked to the elimination of flavonoids, including anthocyanins.

Due to an increase in the temperature at pasteurization, the central pyran ring starts to oxidation, and thus flavonoids in juice are reduced (Zhao et al., 2019). In a study, pasteurization of black carrot juice at 90–95 °C for 90 s led to 33 % dissipation in the monomeric anthocyanin content (Suzme et al., 2014). Igual et al. (2011) illustrated the pasteurization treatment operating at 95 °C for 91 s caused a decrease of 6 % in the TFC of grapefruit juice. These differences in TFC removal levels were related to the differences in fruit variety, environmental factors, and juice processing techniques (Capanoglu et al., 2013).

3.5. Fate of antioxidant capacity during grape juice manufacture

Our result showed washing didn't have a significant impact on antioxidant capacity. The stability of antioxidant capacity during the washing stage may be related to the protective effect of the berries' cuticular wax of polyphenols. In a previous study similar to our findings, no significant effects of washing treatments with chlorine and water on the antioxidant activity of Spanish lettuce were reported (Kenny and O'Beirne, 2009). However, a 40 % reduction in antioxidant capacity during the chickpea soaking for 2–22 h was reported (Aharon et al., 2011).

The preheating process for 10 min at 62.8 °C showed a 13.4 % reduction in antioxidant capacity (Fig. 4. B). In a recent study, nuts preheated at 120 °C for 30 min showed a significant decrease in antioxidant potency (Amoussa et al., 2021). Mojica et al. (2015) presented an opposite finding. They stated that the antioxidant capacity of pinto beans in precooked is higher than that of raw specimens.

The antioxidant capacity after grape juicing reached 976.9 to 802.6 μmole/kg (Fig. 4. A). This decline (17.8 %) may be due to insoluble phenolic compounds, which are bonded to cell wall structures and discarded by pomace (Nickel et al., 2016). Our findings were similar to the study done by Balík et al. (2009) which reported a decrease of approximately 18 % in the antioxidant potential after the grape pressing system.

The reduction of antioxidant capacity during clarification in this study (21 %) was similar to the previous study (Bakardzhiyski, 2022; Gonçalves and Jordao, 2009). Dissipation of antioxidant capacity during pasteurization (18.2 %) can be attributed to the reductone formation during the Maillard reaction (Sharma et al., 2022). In this regard, Escudero-López et al. (2016) showed pasteurization process produced a significant decrease in the values of orange juice antioxidant capacity by 38 %. However, Dereli et al. (2015) found a lower loss (1.0 – 2.7 %) of antioxidant capacity during pasteurization of carrot juice at 90 °C for 10 min

Fig. 4. shows antioxidant capacity in final grape juice (518.4 μmole/kg) decreased to 54.32 % in comparison with raw grape (1134.2 μmole/kg). The higher decline level was observed by Capanoglu et al. (2013). They mentioned a nearly 85.33 % diminish in antioxidant capacity during the washing, pressing, pasteurization, clarification, filtration, and evaporation processes of grape juice (Capanoglu et al., 2013). Grapes are a good natural source of antioxidants with strong activity for scavenging free radicals leading to decreased mortality from age-related diseases (Capanoglu et al., 2013; Kim et al., 2017). Hence, the behavior of antioxidant capacity during the production process of grape juice is an important issue.

4. Conclusions

OTA was significantly reduced during grape juice manufacture, leading to safer fruit juice production than table grapes. The OTA, TPC, TFC, and antioxidant capacity reduction during the various steps of grape juice manufacture ranged from 2.4 to 26.7 %, 0.2 to 26.8 %, 6.2 to 20.8 %, and 0.6 to 21 %, respectively. The total dissipation of OTA, TPC, TFC, and antioxidant capacity was 62.0 %, 56.2 %, 53.9 %, and 54.3 %, respectively. The OTA decrease can be related to its removal during grape washing, juicing, and clarification or degradation during pasteurization, depectinization, and preheating. This study provides valuable information about the fate of OTA and antioxidant compounds and capacity during grape juice production, which would allow the establishment of best management practices. However, the formation of unknown products due to OTA degradation during food processing is a newfangled concern. The authors encourage more research to identify, quantify, and assess the toxicological properties of degradation products of OTA. This study did not find natural OTA contamination in grape samples. The fate of OTA in naturally contaminated grapes utilized for grape juice could present more exact results about the changes of this mycotoxin.

CRediT authorship contribution statement

Majid Behfar: Data curation; Investigation; Methodology; Software; Formal analysis; validation; Writing – original draft. **Ali Heshmati**: Supervision, funding acquisition, Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; Writing – original draft; Writing – review & editing. **Amir Mohammad Mortazavian**: Supervision, Project administration; Validation; Writing – review & editing. **Zahra Hadian**: Investigation; Methodology; Supervision. **Nabi Shariatifar**: Supervision, Data curation; Formal analysis; Project administration; Writing – original draft. **Amin Mousavi Khaneghah**: Conceptualization, Supervision, Visualization, Writing – original draft. Writing – review & editing.

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

No data was used for the research described in the article.

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