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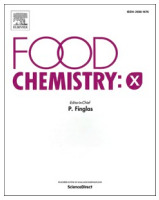

CO_2-moderate-pressure enhances phytonutrients and prolongs shelf-life of flowable smoothie formulated from quadrable functional vegetables

Ibrahim Khalifa [a,b,1], Remah Sobhy [a,c,1], Xiaobo Zou [a,*], Asad Nawaz [d], Noman Walayat [e], Putri Widyanti Harlina [f], Tarek Kh. Abdelkader [g], Mukhtar Ahmed [h], Sajid Maqsood [b,i,**]

[a] Agricultural Product Processing and Storage Lab, School of Food and Biological Engineering, Jiangsu University Zhenjiang, Jiangsu 212013, China
[b] Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al-Ain, 15551, United Arab Emirates
[c] Department of Biochemistry, Faculty of Agriculture, Benha University, 13736 Moshtohor, Egypt
[d] College of Chemistry and Bioengineering, Hunan University of Science and Engineering, 425199 Yongzhou, Hunan, China
[e] College of Tea Science and Tea Culture, Zhejiang Agriculture and Forestry University, Hangzhou 311300, China
[f] Department of Food Industrial Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Bandung 45363, Indonesia
[g] Agricultural Engineering Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt
[h] Department of Zoology, College of Science, King Saud University, 2455, Riyadh 11451, Saudi Arabia
[i] International Research Center for Food Nutrition and Safety, Jiangsu University, Zhenjiang 212013, China

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ABSTRACT

The effect of non-thermal (HPP and semi-HPP-CO_2) and thermal (flash pasteurization, FP) treatments on phytonutrients of flowable smoothie prepared from quadrable vegetable blends (FQVS) was investigated using multidimensional methods. First, FQVS gained an acceptability sensorial index (85.7%) compared with other formulas. FQVS/semi-HPP-CO_2 showed a greater microbial stability during storage (0–30 d) compared to HPP and FP. Fructose and glucose highly declined than sucrose in all smoothies, where semi-HPP-CO_2 steadily declined this reduction during storage. LC/MS-MS analysis showed that semi-HPP-CO_2 preserved most of FQVS's phytonutrients and their antioxidant effects measured by ORAC and oxidative enzymes inhibition assays. Semi-HPP-CO_2 acquired the lowest apparent viscosity among different FQVS smoothies, showing its post-processing flowability behavior. Most importantly, semi-HPP-CO_2 predicted a reduced power consumption for HPP and reduced the gas emission. In conclusion, blending different vegetables assisted with semi-HPP-CO_2 could be a novel approach to produce storage-stable smoothies with adequate amounts of phytonutrients and sensorial scores.

1. Introduction

The consumption of vegetables has been widely reported to reduce the risk of cardiovascular diseases mostly due to their high concentrations of antioxidative phytochemicals (Aravind, Wichienchot, Tsao, Ramakrishnan, & Chakkaravarthi, 2021), which had several positive health functions (Vivarelli et al., 2023). Eating such foods is crucial for maintaining good health because humans are unable to produce these phytonutrients (Khalifa, Zhu, Li, & Li, 2018). Meanwhile, consuming whole vegetables is much better than single dietary phytonutrient from their natural sources, mainly due to the interactive impacts of co-existing phytonutrients in whole vegetables (Mehany et al., 2021). Although research relevant to interactive impacts among phytonutrients mounted up, research on vegetable matrix models is of value and need due consideration.

Sweet potatoes (*Ipomoea batatas*), carrots (*Daucus carota* subsp. *Sativus*), pumpkins (*Cucurbita maxima*), and sugar beets (*Beta vulgaris*) are one of the outstanding examples of functional vegetables. These vegetables have high energy, dietary fiber, biologically functional phytochemicals, minerals, and vitamins, which make them a very nutritious vegetable that is ideal for usage as a functional food component (Steed & Truong, 2008). For examples, carrots, as a commercially significant

* Corresponding author.
** Corresponding authors at: Department of Food Science, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al-Ain, 15551, United Arab Emirates.
E-mail addresses: zou_xiaobo@ujs.edu.cn (X. Zou), sajid.m@uaeu.ac.ae (S. Maqsood).
[1] Equally contributed.

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crop, are widely consumed either as raw products or after additional processing (López-Gámez, Elez-Martínez, Martín-Belloso, & Soliva-Fortuny, 2021). Pumpkins are cultivated often in many nations and eaten as a vegetable, but they may also be used to a variety of baked goods, rice cakes, and sweets to increase its protein, peptide, dietary fiber, vitamin, and mineral content (Janowicz et al., 2023). Sugar beet's pulp is low in sugar and contains a lot of fiber, carbohydrates, proteins, minerals, and red pigments (Mohdaly, Hassanien, Mahmoud, Sarhan, & Smetanska, 2013). Formulation of several vegetables that had plenty of phytochemicals with multiple health-promising effects is a novel way to amend the market prospects of vegetable-based products such as smoothy and puree.

Herein, we targeted to use four vegetables rich in different phytochemicals (Fernandes, Mateus, & de Freitas, 2023). The structure of the cell wall and chromoplasts affects how phytochemicals are absorbed, where they are typically kept in vacuoles or attached to dietary fiber (Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2018). Thus, increasing their bio-accessibility typically involves tissue rupture and particle size reduction. One of the examples of such techniques is high-pressure processing (HPP) that could enhance the phytochemicals releasing and stability (Ravichandran, Jayachandran, Kothakota, Pandiselvam, & Balasubramaniam, 2022). HPP employs the high pressure to render harmful or spoilage germs inactive (Nawawi et al., 2023). HPP preserves the physicochemical characteristics, phytochemicals, and tastes of products than the traditional thermal methods (Zou et al., 2023). HPP-CO_2 is an advanced and developed method of HPP that enhances its sustainability, wide-applicability, thermal-death efficiency against microorganism, and preserving effects on heat-sensitive phytochemicals (Bu et al., 2022). Most importantly, shelf life of such vegetable formulas is often short, impeding their applications that could be increased *via* the thermal processing, but such treatment significantly deteriorates the phytonutrients, aside its impact on the products' freshness and quality. To date, no studies on formulating quadrable matrixes of vegetables as a flowable smoothie treated with semi-HPP-CO_2 were reported. We hypothesized that CO_2 could reduce the pressure needed for treating smoothie by HPP, and a synergetic effect among phytochemicals could be achieved after blending among four functional vegetables.

Thus, we aimed at formulating a flowable smoothie from sweet potatoes, carrots, pumpkin, and sugar beet blends and treating this mixture using semi-HPP-CO_2. Total soluble solids (TSS), pH-value, sugar fractions, ascorbic acid (AA), total phenolic content (TPC), total flavonoids (TF), total carotenoids (TC), antioxidant activities (AOA), polyphenols fractions, instrumental color parameters, rheological, enzymatic activities, microbiological, sensorial evaluation, and carbon finger assessment were thereafter measured throughout storage compared with HPP and FP. We do believe that our results may help to develop a super vegetable smoothie with high shelf life that could be further industrialized with low-gas emissions.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteau reagent, gallic acid (GA), Trolox (TE), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), and other solvents with analytical grades were acquired from Aladdin Co., (Shanghai, China). MillQ-H_2O was used in this study.

2.2. Flowable quadrable vegetables smoothie's preparation treated with semi-HPP-CO_2

Sweet potatoes, pumpkin, carrots, and sugar beets were all acquired from a local market (Zhenjiang, China) that were immediately delivered to the lab at School of Food and Biological engineering, Jiangsu University and then directly handled manually. Formulas of flowable quadrable vegetables' smoothie (FQVS) were selected based on previous pre-experiments, where the sensory palatability test was carried out with characteristics of appearance, color, texture, flavor, and inclusive satisfactoriness were considered. The hedonic test was employed for the sensory analysis of different formulas. Four randomly chosen samples of each formula from the middle, which were kept at RT for 24 h were analysed by 25 untrained panelists comprising of research fellows and graduate students from Jiangsu University using a 9-point hedonic scale (1 = extremely dislike and 9 = awfully like) (Selvakumaran, Shukri, Ramli, Pak Dek, & Wan Ibadullah, 2019). The acceptance index (AI) was expressed as:

$$\text{AIScore}100/9 \quad (1)$$

Fig. 1 portrayed vegetables formulas and their sensory analysis. These vegetables were hand washed, where non-edible parts were removed. Before processing, vegetables were cleaned by soaking for 5 min in Chlorinated-H_2O, drying, and then manual peeling. All samples were homogenized and placed at −80 °C for 5 min to prevent the deterioration of phytochemicals. They were then steam-cooked in a 5-L pot with a steam holder (Home Essentials, Kmart, MI, US) at 90 °C for 3 min under the atmospheric pressure. To minimize moisture loss after steaming, the samples were kept in containers with covers until they reached room temperature. To create a uniform smoothie, all components were combined for 60 s in a homogenizer (JTC OmniBlend, Guangdong, China) in the presence of MillQ-H_2O with a ratio of 1:1.4, *w*/w. The flowable smoothie was packaged into polyethylene terephthalate pouches. The flowable smoothie were then flash pasteurized using a UHT sterilization unit (ST-20, Shanghai Sunyi Tech. Co., Ltd., Shanghai, China) at 110 °C for 8.6 s, and then directly cooled which later consisted as a control smoothie. We selected formula number 6, where the quadrable blending of vegetables (FQVS) showed an AI-value of 85.7% compared to other formulas with AI-values of 59.9, 63.8, 61.9, 74.86, and 77.65% for formulas of #1, #2, #3, #4, and #5, respectively. FQVS formulas were then treated by FP, HPP, and semi-HPP-CO_2. The smoothie was stored in an ice bath, and the pH was then maintained by adding CO_2 (99.9% purity, Benlai Biological Technology Co., Ltd., Guangzhou, China) at a rate of 2.2 L min^{-1} from the bottom of the sample and then sealed. Smoothie were cooled at 4 °C then processed *via* SHPP-57DZM-600 HPP treating machinery (Sanshuihe Tech., Co., China) by imperiled to high-pressure (raising of 200 Mpa min^{-1}, discharge duration of 5 s) for 10 min at RT (Scheme 1). The HPP treatment was same without using CO_2. The pressure used for HPP, and semi-HPP-CO_2 was 600 and 300 Mpa, respectively. FQVS were kept under dark at 4 °C after each treatment for 30 days, and the parameters were regularly analyzed every 10 d.

2.3. Microbiological analysis

In a sterile saline solution, 1 mL of smoothie was diluted (1:10 *w*/w). After 48 ± 2 h of incubation at 37 ± 1 °C, the total aerobic bacteria (TAB) were counted using plate count agar (Himedia, India). After being cultured on rose Bengal agar (Himedia, India) for 5 d at a temperature of 28 ± 1 °C, the number of yeast and mold (Y&M) was then counted. The smoothie' microbial counts were then calculated as a log of CFU mL^{-1} (Khalifa, Barakat, El-Mansy, & Soliman, 2015).

2.4. TSS, pH, and sugar fractions analysis

With a Brix Refractometer (TD-45, Beijing Jinkelida Electronic Technology Co., Ltd., China), TSS were calculated at a temperature of 25 ± 1 °C, and the outcomes were given in °Brix. Each FQVS underwent two dilutions to determine its pH-value. First, MillQ-H_2O was added to the entire smoothie to generate the same dilution utilized in smoothie manufacturing, bringing the dry matter level down to 18%. Then, MillQ-H_2O was used to dilute each sample one to one. A Tissumizer (Tekmar,

Formulas	Ingredients (%)						Visual appearance
	Sweet potato	Carrots	Pumpkin	Sugar beets	Ascorbic acid	Lemon juice conc.	
#1	100	0	0	0	0	0	
#2	99.45	0	0	0	0.05	0.50	
#3	69.45	30	0	0	0.05	0.50	
#4	69.45	0	30	0	0.05	0.50	
#5	89.45	0	0	10	0.05	0.50	
#6	59.45	15	15	10	0.05	0.50	

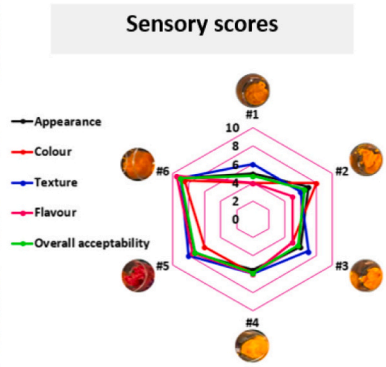
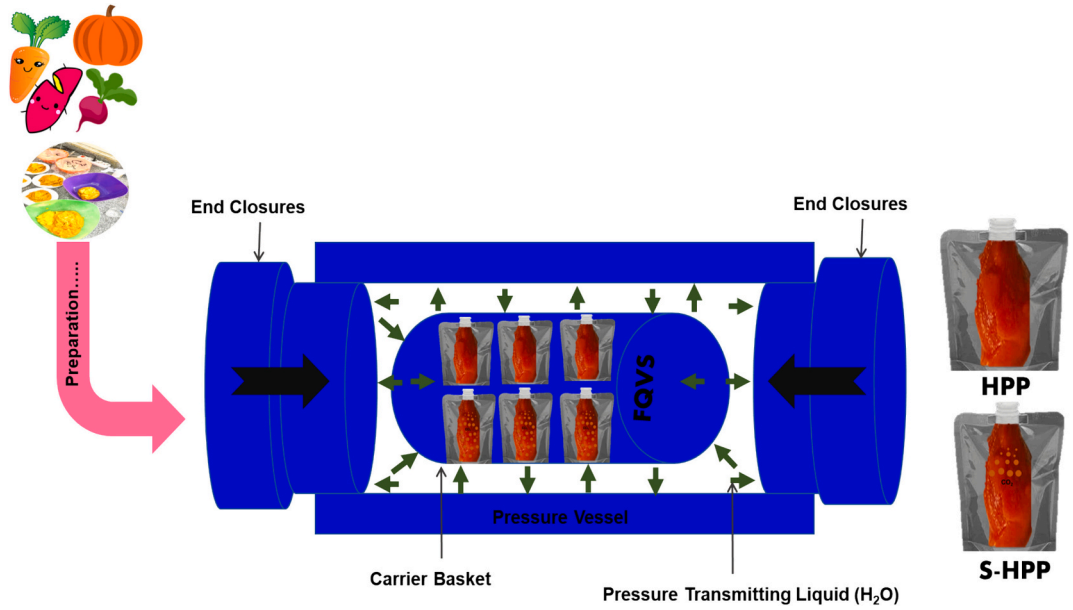


Fig. 1. The different vegetables formulation and their visual appearance and sensory scores.



Scheme 1. The Schematic representation of producing FQVP with HPP-vessel in the presence of CO_2.

CT, U·S) was used to homogenize the samples, and a Fisher Scientific Accumet AR50 pH meter was used to test the pH-value. LC-20AT-HPLC with an evaporative light-scattering detector was utilized to measure the levels of fructose, glucose, and sucrose. Using a Shodex Asahipak NH2P-50 4E (4.6 mm × 250 mm, 5 μm) column at 40 °C, the analytes were separated. 70% acetonitrile was utilized as the mobile phase, and the elution flow ratio was 1 mL min^{-1}. The injection volume was 10 μL with the aid of a refractive index detector (Bu et al., 2022; Sarantakou, Andreou, Paraskevopoulou, Dermesonlouoglou, & Taoukis, 2023).

2.5. Chromatic characteristics

Dimensions of color Hunter Associate Laboratories Inc., Reston, VA, US, D25/DP9000 Tristimulus Colorimeter was utilized to assess Hunter L^*, a^*, and b^* values (Khalifa, Sobhy, Morsy, & Xiaobo, 2023). A 35-mm Petri dish was filled with the prepared smoothie, roofed, and pushed *versus* the top to eliminate air bubbles. Smoothie's measurements were made at 3 distinct sites, with a duplicate performing for each smoothie, after the colorimeter was regulated *versus* a reference white tile ($L^* = 92.78$, $a^* = -0.73$, $b^* = -0.13$) at room temperature (25 ± 3 °C). Arc tan (b*/a*) and $[a^{*2} + b^{*2}]^{1/2}$ were used to determine the hue angle (h°) and the chroma (C*), where averages of all readings were presented. The color changes (ΔE*) were expressed based on the alterations before and after storage (Khalifa, Sobhy, et al., 2023).

2.6. Antioxidative phytonutrients

LC-20AT-HPLC with a PDA-detector was used to determine the ascorbic acid (AA) concentration of each FQVS formula (Niu et al., 2022). With an insertion volume of 10 μL and an elution flow rate of 1 mL min^{-1}, ascorbic acid was split on an Agilent Zorbax SB-C18 (4.6 mm × 250 mm, 5 μm). The $(NH_4)2HPO_4$ solution had a mobile phase with a pH of 2.7 and 0.1 M L^{-1}. The recognition wavelength was set at 254 nm, and the column's temperature at 30 °C. AA was then calculated as mg 100 g^{-1} FW.

A modified Folin-Ciocalteu technique was used to quantify TPC of

each FQVS formula (Khalifa, Barakat, El-Mansy, & Soliman, 2016). After being diluted in 4 mL of MillQ-H_2O and being added 0.5 mL of the Folin reagent, the smoothie, and standards (0.25 mL) were permitted to react for 3 min. The reaction was then run for 1 h after inserting 0.5 mL of 1 N Na_2CO_3. Using a Varian spectrophotometer (Cary WinUV Model 300, PA, US), samples were scanned for absorbance at 725 nm. A blank containing 0.25 mL MillQ-H_2O was used to calibrate the spectrophotometer. The blank was also mixed with the same volume of MillQ-H_2O for dilution, Folin-Ciocalteu reagent, and Na_2CO_3 solution. TPC was then expressed as mg GA equivalents (100–500 mg 100 g^{-1}) per 100 g fresh weight (mg GAE 100 g^{-1} FW).

TF of each FQVS formula was then measured (Khalifa, Li, Mamet, & Li, 2019). $NaNO_2$ solution (1 mL) and 6 mL of TPC extracts' solution were combined. 5 min later, 1 mL of 10% Al $(NO_3)_3$ was inserted into the blend. After 6 min, the blend received 4 mL of a 1 M L^{-1} NaOH solution, and the mixture underwent a 10-min reaction at 45 °C. The blend was then centrifuged at 1000 $_{\times g}$ for 10 min, and the supernatant's absorbance was determined at 505 nm. TF was then conveyed as mg rutin equivalents (10–100 mg 100 g^{-1}) per 100 g (mg RE 100 g^{-1} FW).

TC of each formula was examined using a spectrophotometric technique. 25 mL of acetone was added to 15 g of each formula. The blend was centrifuged at 3000 $_{\times g}$ for 15 min after extraction in the dark for 24 h. After that, 40 mL of petroleum ether was added to a 500 mL separation funnel along with the supernatant. Acetone was removed using MillQ-H_2O, and this process was repeated 2–3 times until all traces of acetone were removed. Lastly, anhydrous Na_2SO_4 was used to remove MillQ-H_2O from the extract solution. At 450 nm, the extract solution's absorbance was measured, and TC was calculated as μg 100 g^{-1} FW using the equation of Niu et al. (2022). The fractionation of polyphenols of each formula was also done using the UPLC-ESI-MS by applying our recent protocols (Khalifa et al., 2020).

The technique reported by Khalifa et al. (2019) for measuring oxygen radical absorbance capacity ($ORAC_{FL}$) was also used. A Safire monochromator-based microplate reader from Tecan US was used to assess the intensity of the fluorescence. In 96-well clear Costar polystyrene flat bottom plates (Corning, Acton, MA, US), samples were loaded after 100-times dilution. Fluorescein solution, standard, and sample were each added to 60-μL wells along with 70-μL of PBS. PBS was used as the sample in the blank wells. 15 min at 37 °C were spent incubating the plate before rapidly adding 60 μL of AAPH to each well. Between each 1-min reading, the plates were rocked orbitally for 5 s. Excitation and emission filter wavelengths for the measurements were 485 and 520 nm, respectively. ORAC-values were then obtained using a linear regression over the range of 6.25 to 100 μmoL TE using the regression eq. $Y = mx + b$. X is the net area under the fluorescence decay curve, and Y is the concentration. Following is how the area under the curve was determined:

$$AUC = \left(0.5 + f_5/f_4 + f_6/f_4 + f_7/f_4 + \ldots + f_i/f_4\right) \times CT \quad (2)$$

where CT is the cycle time in min, f_4 is the first fluorescence measurement at cycle 4, fi is the fluorescence reading at cycle 1, and so on. The area under the curve for the blank values was subtracted from the sample and standard curves to get the net area under the curve. Units of ORAC readings were mmol TE 100 g^{-1} FW.

2.7. Viscosity testing

A stress monitored ATS Stresstech Rheometer (Rheosystems, Bordentown, NJ, US) fitted with toothed cup and bob geometry (40 mm) was used to assess the created smoothie' rheological characteristics (Bu et al., 2022). Smoothie were pre-trimmed at 20 s^{-1} for 30 s before assessing to minimize moisture loss and were then topped with a thin coating of mineral oil. At temperatures ranging from 1 to 100 s^{-1}, shear ratio sweeps were done at 25 °C. The samples were given 60 s to adjust to each temperature change. Apparent viscosity readings were compared to a commercial smoothie as a reference. The findings were utilized to model the behavior of smoothie *via* Herschel–Bulkley model:

$$\sigma = \sigma_0 + K\gamma^n \quad (3)$$

where σ is the shear ratio, σ0 is the yield stress, γ is shear ratio, K is the uniformity coefficient, and n is the flow behavior index. $n < 1$, $= 1$, and > 1 where smoothie be pseudoplastic or shear-thinning, Newtonian, and a dilatant or shear-thickened fluids, respectively.

2.8. Enzymes activities analysis

The polyphenol oxidase (PPO) and peroxidase (POD) enzymes activity were measured using the modified assays of Fernandez, Denoya, Agüero, Jagus and Vaudagna (2018). The enzyme's activity (1 unit) was measured as a change in absorbance of 0.001. The reaction mixture for the PPO test contained 0.05 mL of enzyme extract and 2 mL of 0.07 M catechol in a SPB solution (0.05 M, pH 6.5). The test mixture was mixed, and the absorbance at 420 nm using UV–visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu). The reaction mixture for the POD test included 2.7 mL of SPB (0.05 M, pH 6.5), 0.04 mL of enzyme extract, 1.0% (*w/v*) *p*-phenylenediamine, 1.5% (w/v) H_2O_2, and 0.1 mL of SPB. The test blend was homogenized, and UV–visible spectrophotometer was utilized to quantify the absorbance at 485 nm, their activity was then calculated:

$$\text{Residual activity} = \frac{\textit{Activity in treated purees}}{\textit{Activity in untreated purees}} \times 100\% \quad (4)$$

To elucidate the anti-enzyme effects of S-HPP-CO_2, CO_2 (PubChem CID: 280) was docked with the crystal structure of PPO (PDB: 2P3X) or POD (PDB: 1U2J) resolution of 7 Å gained from https://pubchem.ncbi.nlm.nih.gov/ and https://www.rcsb.org/, respectively. They were then optimized by combining fractional charges, and energy was minimized using Protonate-3D and MMFF94X force fields. PPO or POD was used as a receptor after removing H_2O, structure refining, energy minimization, and 3D protonation *via* MOE software version 2015. After that, enzyme-CO_2 conjugates were then docked *via* MOE docking tool by identifying the potent binding spots *via* MOE site discoverer tool and afterward engaged by the docking process. The process was repeated for the composites, and thereafter 4–5 appropriate docked postures were created, employing a scoring function London dG, and refining by Force field algorithm. The MOE-LigX tool was utilized to evaluate the most suitable ligand-receptor binding based on Root-Mean-Square Deviation (Khalifa, Nilsuwan, Prodpran, & Benjakul, 2023).

2.9. Calculating the energy consumption and CO_2-emssion during processing of the smoothies

The consumed energy varies depending on the production cycle of manufacturing. Thus, in this experiment, all FQVS were homogenized by a homogenizer with a power of 1200 W and steam-cooked in a 5-L pot with a steam holder with a power of 12,000 W. Then, the proposed treatments differ in their electricity consumption according to the used power and exposed time, since power which was applied to FP, HPP, and S-HPP-CO_2 were 2500, 5000, and 3330 W, respectively. Thus, energy consumption (kW. h) was calculated by the below equation (5) for 500 g as experimental weight for FQVS and the result were scaled up to the production of one ton of FQVS as follows:

$$\text{Energy consumption (kW.h)} = \sum_1^n \frac{power(W) \times time(h)}{1000} \quad (5)$$

Since, n is the manufacturing process. Estimating impact of different manufacturing processes on environment is a function of consumed energy, energy type, and generation source of it, thus, emissions factor to estimate CO_2 in Kg from electrical energy consumption is estimated based on International Energy Agency, as follow:

$$CO_2 \text{ emission (kg)} = \text{Consumed energy(kW.h)} \times \text{emission factor (kg/kW.h)} \quad (6)$$

While emission factor is 0.95,0.75 and 0.55 kg/kW.h for generated electricity from coal, petroleum oil and natural gas, respectively (*Energy, G. J. I. P., France*, 2019).

2.10. Statistical analysis

All investigations, unless otherwise noted, were done at least 3 times. Data were supplied as a mean ± SD, and a *p*-value of <0.05 was utilized to evaluate statistical significance. Each factor was related *via* an ANOVA, monitored by a Tukey's multiple difference post-test *via* SPSS ver., 26 (IBM, US). The principal component analysis (PCA), bubbles Pearson correlation, and heat map analysis with 0.05, 0.01, and 0.001 significance levels were achieved *via* OriginPro 2023 (Origin Lab, Co., US).

3. Results and discussion

3.1. CO_2-semi-high-pressure efficiently declined the microbial counts of FQVS

The microbial counts, namely Y&M and TAB, in FQVS treated with FP, HPP, and semi-HPP-CO_2 were counted in term of evaluating the shelf life of the produced smoothie. Initial counts of Y&M and TAB were about 3.15×10^3 and 1.42×10^5 CFU g^{-1}, respectively in FQVS-FP smoothie. The inhibitory impact of temperature, *i.e.,* FP, on aerobic bacteria steadily improved with adjusting the temperature used *versus* time. HPP has a limited ability to inhibit bacterial growth in low-acid foods despite being commonly used in plant-based foods (Georget et al., 2015), especially in our case of study where we used blends of vegetables to formulate FQVS-smoothie. The inactivation of microorganisms may not be as effective after typical HPP treatment as FQVS is a low-acidic food. Thus, FQVS was processed using semi-CO_2-HPP treatment, where its microbial counts were portrayed in **Figs. S1A—B**. TAB decreased by 8.45% when HPP pressure reached 0.1 Mpa (**Fig. S1A**), while 200 Mpa completely inhibited Y&M (**Fig. S1B**), well matched with a previous study (Bu et al., 2022), where Y&M showed a pressure-sensitive than bacteria. Additionally, FQVS/semi-HPP-CO_2 at 200 Mpa showed lower TAB values than FQVS/HPP at the same pressure by 15.55%. TAB in FQVS/ semi-HPP-CO_2 at 300 Mpa were entirely inactivated, whereas HPP required a pressure of 600 Mpa to have the same effect, lower than the pressure needed to gain the same effects in Durian smoothie (Bu et al., 2022). The buildup of CO_2 on the cell's surface and high-pressure entry into the cell that results in membrane damage and cell death might be the cause of the more effective deactivation of microorganisms in semi-CO_2-HPP (Wang, Pan, Xie, Yang, & Lin, 2010). TAB of FQVS/FP, FQVS/HPP, and FQVS/ semi-HPP-CO_2 throughout 30 d of cold-storage were also noted, where an increasing tendency was shown in all formulas (**Fig. S1C**). Y&M was not found in pineapple juice treated with traditional pasteurization and/or HPP (Wu et al., 2021), agreeing with our findings during storage. The recovery of the microorganisms harmed by thermal processing may be the cause of the rise of TAB in FQVS. The FQVS/FP had the greatest TAB on 30 d of storage (3.11×10^5 CFU g^{-1}), followed by HPP (1.15×10^3 CFU g^{-1}). Accordingly, FQVS/S-HPP-CO_2 had a greater ability than others to regulate its microorganism throughout storage, showing the successful of binding CO_2 and S-HPP on the shelf life of small- or middle-acid food like FQVS.

3.2. The chemical constitutes of FQVS

The TSS, pH, and sugar fractions of FQVS/FP, FQVS/HPP, and FQVS/semi-HPP-CO_2 formulas after one month of cold storage were examined to further estimate the application potential of semi-HPP-CO_2 on FQVS manufacturing. First, it was determined how differently TSS and pH-values of each FQVS's formula changed during the storage. The TSS and pH-values of all FQVS's formulas decreased steadily during the storage (**Fig. S2**). The TSS declined by 9.9, 12.94, and 12.62% in FQVS/FP, FQVS/HPP, and FQVS/semi-HPP-CO_2, respectively. Meanwhile, pH-values decreased by 16.88, 10.57, and 4.91% in FQVS/FP, FQVS/HPP, and FQVS/ semi-HPP-CO_2, separately. Previously, TSS and pH showed a declining tendency during storage, mostly due to the microbial activity toward carbohydrates and generating organic acids (Mukhopadhyay, Sokorai, Ukuku, Fan, & Juneja, 2017), agreeing with our microbial results and confirmed that semi-HPP-CO_2 could maintain TSS and pH *via* inhibiting the microorganism. Likewise, sugar fractions declined throughout the storage, where FP significantly decreased compared to HP-based treatments. The reduction in fructose and glucose showed to be higher than that in sucrose in all formulas. Meanwhile, HP especially with CO_2 declined this steadily reduction during the cold storage (**Fig. S3**). This shows that semi-HPP-CO_2 could de-facilitate the Maillard reaction that is responsible for the decline in fructose and glucose. FQVS's cell integrity was compromised following semi-HPP-CO_2, leading to the liberation of molecules from compartmentalized structures and noncovalent binding (Elizondo-Montemayor et al., 2015). As a result, pressure-based treatments had higher sucrose concentrations. The levels of fructose, glucose, and sucrose in treated FQVS decreased to variable degrees after storage. Additionally, the period where microbes first developed was mostly responsible for the initial declines of the FQVS's sugar levels.

3.3. Instrumental color features of FQVS

Color is a crucial aspect that consumers assess when determining if smoothie is acceptable. The values of L*, a*, and b* of FQVS/FP, FQVS/HPP, and FQVS/semi-HPP-CO_2 formulas were examined to thoroughly analyze the impact of the selected treatments on FQVS's quality. The utilization of quadrable vegetables, namely carrots, pumpkins, sweet potatoes, and sugar beets, increases the color attributes of FQVS smoothie (**Fig. S4**). Semi-HPP-CO_2 increased each of L*, a*, b* values compared with both HPP and FP formulas, where the thermal-based formula had bottomed out. The chroma (C) of FQVS/ semi-HPP-CO_2 formula was also peaked, where its hue angle (H°) value was bottomed, signifying more of a blueish purple. These results highlighted the deleterious impact of FP on the color properties of processed vegetables' products (Xu et al., 2018). Meanwhile, HP-based treatments especially semi-HPP-CO_2 enhanced redness (a*), yellowness (b*), and lightness (L*) of FQVS alongside with its effect on the C-values, confirming that semi-HPP-CO_2 could release the pigments responsible for the color and their homogeneity. The smoothie of blueberry treated with traditional thermal processing had lower L* and b* values than the one treated with HPP (Zhang et al., 2021). Polyphenol oxidase and peroxidase may be rendered inactive by CO_2 as well, explaining why FQVS/semi-HPP-CO_2 had the greatest L* value. The color changes (ΔE*) were calculated based on the fluctuations in each of L*, a*, b* values throughout the storage. The redness, yellowness, and lightness values of all FQVS exposed a shrinking trend during 30-d of storage (**Fig. S4**), mostly caused by microorganisms' production of chemicals adducts that had a detrimental impact on the lightness and yellowness of FQVS (Bu et al., 2022). Meanwhile, ΔE* of FQVS/ semi-HPP-CO_2 was lower than other samples, agreeing with our results on the microbial counts of FQVS.

3.4. The antioxidative phytonutrients of FQVS

Fig. 2 shows the change in TPC of various treated FQVS during storage. Different treatments reduced TPC of FQVS compared with the non-treated one, where FQVS/ semi-HPP-CO_2 formula had the lowest declining rate compared to the thermal-based treatment ($p < 0.05$). This implies that TPC of semi-HPP-CO_2 had the best retention than others. TPC then steadily increased during storage with an increasing ratio of 8.12, 3.89, and 1.86% in FQVS/FP, FQVS/HPP, and FQVS/ semi-HPP-

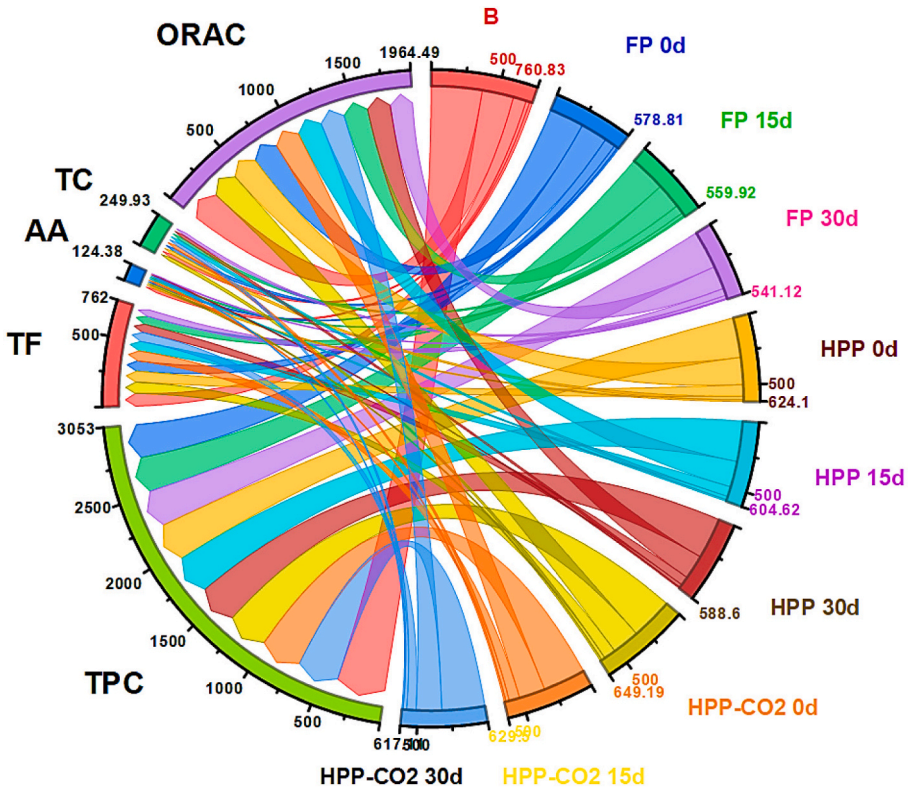


Fig. 2. The chord diagram analysis for phytonutrients included TPC, TF, TC, and AA as well as their antioxidant capacity using the $ORAC_{FL}$ method of FQVP/FP, FQVP/HPP, and FQVP/semi-HPP-CO_2 formulas throughout 30-d of cold storage. B: is the FQVP before treatment.

CO_2 formulas, respectively. This increase is mostly due to the microbial activity that converted the bound polyphenols to be soluble and simple polyphenols like phenolic acids that finally increase TPC based on the Folin-Ciocalteu assay where gallic acid was utilized as a standard. These results also showed the inhibitory impact of semi-HPP-CO_2 *versus* the microbial activity and agreeing with our results.

The most prevalent polyphenols in the human diet are flavonoids that are broadly present in fruits and vegetables (Khalifa et al., 2018). Similarly, TPC, TF of the treated FQVS was lower than that of the untreated one (Fig. 2). FQVS/ semi-HPP-CO_2 formula showed the highest TF retention rate (90.81%), followed by HPP (87.75%), and the FP-based formula showed the lowest the lowest TF content (81.63%), representing the preserving impact of semi-HPP-CO_2 on TF. A similar pattern of TPC during storage was noted, where TF drastically declined during 30-d of storage. The degradation ratios of FQVS/FP, FQVS/HPP, and FQVS/ semi-HPP-CO_2 formulas were 33.75, 22.09, and 20.22%, separately, showing again the storage-preserving impact of semi-HPP-CO_2 on TF than others ($p < 0.05$). A similar finding was also noted on mulberry juice and multi-vegetable smoothies (Hurtado et al., 2019), where the microorganisms are mainly responsible for TF-degradation during storage.

In our study, sweet potatoes, carrots, pumpkins, and sugar beet were used to prepare the FQVS formulas, where all are rich in carotenoids. Thus, we measured the TC of each formula. Carotenoids, like their biologically active counterparts, had been shown to have the potential to lower the prevalence of several malignancies and cardiovascular diseases (Khalifa et al., 2018). The TC content of FQVS did not alter ($p > 0.05$) after each treatment compared with the non-treated one (Fig. 2). Additionally, FQVS/semi-HPP-CO_2 formula has the utmost TC (25.24 g 100 g^{-1}). Protein-carotenoid complexes may undergo permanent protein denaturation because of HPP, releasing carotenoids especially in the presence of CO_2. As a result, FQVS treated with semi-HPP-CO_2 had a higher content of TC during storage ($p < 0.05$). The TC of FQVS, however, showed a negative connection with FP process. The overall TC of all treated FQVS remained constant throughout storage that is consistent with other studies (Bu et al., 2022).

Ascorbic acid (AA) of fruits and vegetables is frequently recognized as a key indicator for evaluating oxidative degradation. AA was gradually degraded during storage with degradation ratios of 37.56, 34.01, and 24.29% in FQVS/FP, FQVS/HPP, and FQVS/ semi-HPP-CO_2 formulas, respectively (Fig. 2). This demonstrates the negative effects of storage on AA, where semi-HPP-CO_2 significantly ($p < 0.05$) declined this effect and increased the storage retention of AA than the normal HPP or the thermal-based treatment. Additional anti- or pro-oxidants, pressure-tempted enzyme beginning, aerobic and non-enzymatic anaerobic processes, as well as the potential reality of other anti- or pro-oxidants, might all help explain the AA degradation of all FQVS formulas (Sakhale, Pawar, & Ranveer, 2012). Meanwhile, our retention values of AA were lower than that of a related study on the CO_2-assisted HPP on in Durian fruit smoothie models with a retention rate around 39–53% (Bu et al., 2022). AA also showed higher retention rates in HPP (92–95%) treated smoothie drinks compared to thermally (88%) processed ones (Sarantakou et al., 2023). Meanwhile, higher the pressure better the AA retention, explaining that CO_2 might accelerate the pressure processing, thereby increasing the AA retention.

The AOA of vegetables could also be used as a key indicator to evaluate the smoothie quality and confirm AA-degradation. Fig. 2 illustrates how various processing techniques affect the AOA of FQVS's measured using $ORAC_{FL}$ assay. The AOA of FQVS/FP, FQVS/HPP, and FQVS/semi-HPP-CO_2 formulas were all lower than those of untreated FQVS, according to the findings of the $ORAC_{FL}$ assays. The FQVS that had been exposed to FP exhibited the lowest AOA ($p < 0.05$). It was discovered that HPP treatment preserved more AOA than FP treatment but both FP and HPP treatments might reduce the AOA of vegetable products (Sulaiman, Farid, & Silva, 2017). The high AOA in FQVS/ semi-HPP-CO_2 formula is due to its extra content of phytochemicals including polyphenols and carotenoids where these components still existed in this formula. All the treated formulas displayed declining trends in AOA

during storage, where FQVS/ semi-HPP-CO_2 formula had peaked, and FP had bottomed out ($p < 0.05$).

The polyphenols components were identified and quantified through UPLC-ESI-LC/MS to further understand the individual polyphenol alterations in FQVS following FP, HPP, and semi-HPP-CO_2 technologies. Before treatment, 29 different polyphenols were found in FQVS (Figs. 3A-B). This vast number of occurred polyphenols in FQVS mostly due to the blending among 4 different vegetables we used where carrots, pumpkins, sweet potatoes, and sugar beets differed in their polyphenol's fractions. Peonidin-3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside (3.08 mg 100 g^{-1}), sesaminol 2-O-β-d-glucoside (3.04 mg 100 g^{-1}), rosmarinic acid (3.01 mg 100 g^{-1}), and vanillic acid (3.07 mg 100 g^{-1}) were the key polyphenols found in the control FQVS (Fig. 3A). Meanwhile, peonidin-3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside was noted as the highest anthocyanin fractions. Likewise, rosmarinic and vanillic acids had peaked, due to their plentiful existence in FQVS formula. Sesaminol 2-O-β-d-glucoside was also identified (Yang et al., 2022). The phenolic profiles of FQVS/FP, FQVS/HPP, and FQVS/semi-HPP-CO_2 formulations were closer to those of FQVS before treatment (Fig. 3A), whereas FP significantly decreased most phenolics in FQVS. Vanillic acid had the most reduction ratio following FP, HPP, and semi-HPP-CO_2 with a reduction value of 64.49, 62.86, and 59.28%, respectively. Meanwhile, gallic acid and peonidin-3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside were the most stabilized polyphenols after treatments. Semi-HPP-CO_2 significantly enhanced the stability and retention ratios of polyphenol fractions than both of HPP and/or FP in FQVS models. The varied structural characteristics of these phenolics were the reason for their varying stability toward thermal and nonthermal treatments, as supported by their correlation analysis (Fig. 3B). Additionally, certain phenolics may form strong covalent connections with dietary substrates, and FP would have a limited capacity to break these glycoside bonds (Zou et al., 2023). Pelargonidin derivative and cirsilineol were the two most polyphenols degraded during storage, where semi-HPP-CO_2 significantly declined this degradation by 88.87% compared to other treatments. Meanwhile, FP reduced the polyphenol fractions in FQVS during storage. The disintegration of a cell wall structure and the hydrolysis of polysaccharides brought on by an extra temperature may have contributed to the rise in glycoside content (Rodríguez-Roque et al., 2015). Similar results were shown when quercetin glycosides in red raspberry juice were treated with HTST (Wentao Zhang et al., 2021).

3.5. The apparent viscosity of FQVS

The rheological characteristics have a significant role in determining the quality, hence it was investigated how various treatments affected the apparent viscosity of FQVS. All FQVS smoothies showed a reduction in apparent viscosity as shear rate increased during storage (Fig. 4) that was consistent with a pseudo-plastic flow characteristic. The n-values of all FQVS smoothies were also tabulated (Fig. 4, upper right corner), when the viscosity was fitted, showing that FQVS was a pseudoplastic fluid. Another smoothie yielded comparable rheological properties results (Bu et al., 2022). Significant changes were noted between FP, HPP, semi-CO_2-HPP, and untreated smoothie at low shear ratios (0.01 to 1 s^{-1}) but not at high shear rates. Semi-CO_2-HPP acquired the lowest apparent viscosity among FQVS ($p < 0.05$), where HPP showed a minimal ostensible viscosity than the control one. The smoothie's apparent viscosity is definitely allied with its polysaccharide content (Ollé, Baron, Lozano, & Brillouet, 2000). The polysaccharide subject of the samples after semi-CO_2-HPP was lower (14.26 g kg^{-1}) than that of others (before: 21.71; FP: 18.67; HPP: 15.98 g kg^{-1}), implying the lowest apparent viscosity being obtained in FQVS-based semi-CO_2-HPP. Semi-CO_2-HPP smoothie's apparent viscosity was consistently lower during storage than others. Additionally, appearance of microorganisms significantly accentuated difference in apparent viscosity among these formulas. This phenomenon may be explained by a rise in the number of bacteria in FQVS that can produce polysaccharides which chiefly responsible for TSS, thereby increasing polysaccharides quantity of FQVS.

3.6. The enzymes activity and sensory scores

With relative activities of 34.57 ± 1.72 and 19.89 ± 0.99% for PPO in the FQVS/HPP and FQVS/ semi-HPP-CO_2 formulas, respectively, FP completely inactivated PPO (Fig. 5A). In contrast, FP induced a progressive decrease in POD by 43.96 ± 0.43%, whereas FQVS/HPP formula showed a greater relative activity of 81.98 ± 4.09%. Meanwhile, POD-activity in FQVS/semi-HPP-CO_2 formula was 56.98 ± 2.84%, showing the stability of POD than PPO, where HPP-CO_2 showed much better than the common HPP treatment. High pressure altered the enzyme shape by compaction and an alteration in molar volume, and this, along with an increase in temperature, led to the loss of enzyme functioning. Meanwhile, to discover the role of CO_2 on the enzyme's activities, a lib-dock analysis was performed to evaluate their interaction simultaneously. As 2D- and 3D-portrayed in Fig. 6, CO_2 successfully interacted with the structure of both PPO and POD enzymes, verifying the forming of CO_2 + enzyme conjugates. This interaction was aided by H-bonds, hydrophobicity alteration, and electrostatic forces. For example, 5 and 7 amino acids of PPO and POD enzymes are involved in this interaction. Particularly, Lys283, Asp322, Val321, Asn280, Trp279, SerA-B6626, Lys627, AsnA-B628, and ThrA-B676 interacted with CO_2 by van der Waals, π-π forces, and H-bonding. The binding score of PPO-CO_2 and POD-CO_2 are −2.3 and − 2.1 with binding energies of −3.4 and − 2.9 kcal/mol, respectively showing their binding affinity simultaneously (Khalifa, Nilsuwan, et al., 2023). This interaction might be responsible for the inhibition effects on PPO and POD enzymes showed in FQVS/ semi-HPP-CO_2 formula. PPO and POD were reported to have different active site residues namely Arg, His, Glu, Ala, Trp, Gly, and Asn (Patil, Akki, Raghu, Kulkarni, & Akamanchi, 2023), showing that CO_2 could deactivate PPO or POD *via* binding with some of their catalytic residues (Asn). FQVS/ semi-HPP-CO_2 and/or FQVS/HPP formula also showed no noteworthy difference ($p > 0.05$) with the FQVS/FP formula, where most of them get higher than 9 scores of all parameters tested, confirming that HPP and/or semi-HPP-CO_2 did not alter the sensory evaluation of FQVS smoothie (Fig. 5B).

3.7. Energy consumption and CO_2 emissions

As shown in Fig. 7, FP shows the least electricity energy consumption while 1000 kg of FQVS's production consumed around 1359.4 kW.h. Meanwhile, manufacturing of FQVS using HPP was the highest with an energy consumption of 1740 kW.h, mostly because the time needed to produce it where a high energy needs to maintain the mechanical press for a certain time. Most importantly, semi-HPP-CO_2 is remarkably decreasing the consumed energy by about 9.6% of which consumed in HPP. This is mostly due to the lower pressure used in the case of semi-HPP-CO_2, where adding CO_2 minimized the pressure needed and thus the energy needed. In addition, using semi-HPP-CO_2 also preserved phytonutrients and shelf-life better than both HPP and FP, showing its advantage compared with the negligible high energy needed than FP (Fig. 7). Furthermore, CO_2-emissions to the environment are a function of electric energy consumption. Thus, as the same trend of FP, HPP, and semi-HPP-CO_2 energy consumption, CO_2 emissions are resealed as illustrated in Fig. 7. And the amount of released CO_2 emissions was the highest for coal generation. Consequently, S-HPP-CO_2 is highly recommended for acceptable energy consumption and CO_2-emissions.

3.8. The correlation analysis

Based on PCA, the first and second principal components (PC_1 and PC_2) had individual values of 63.5 and 29.96% and a cumulative variant contribution proportion of 93.46% (>75–85%), respectively. PC_1 positively associated with most of the FQVS features, namely L*, b*, a*, C,

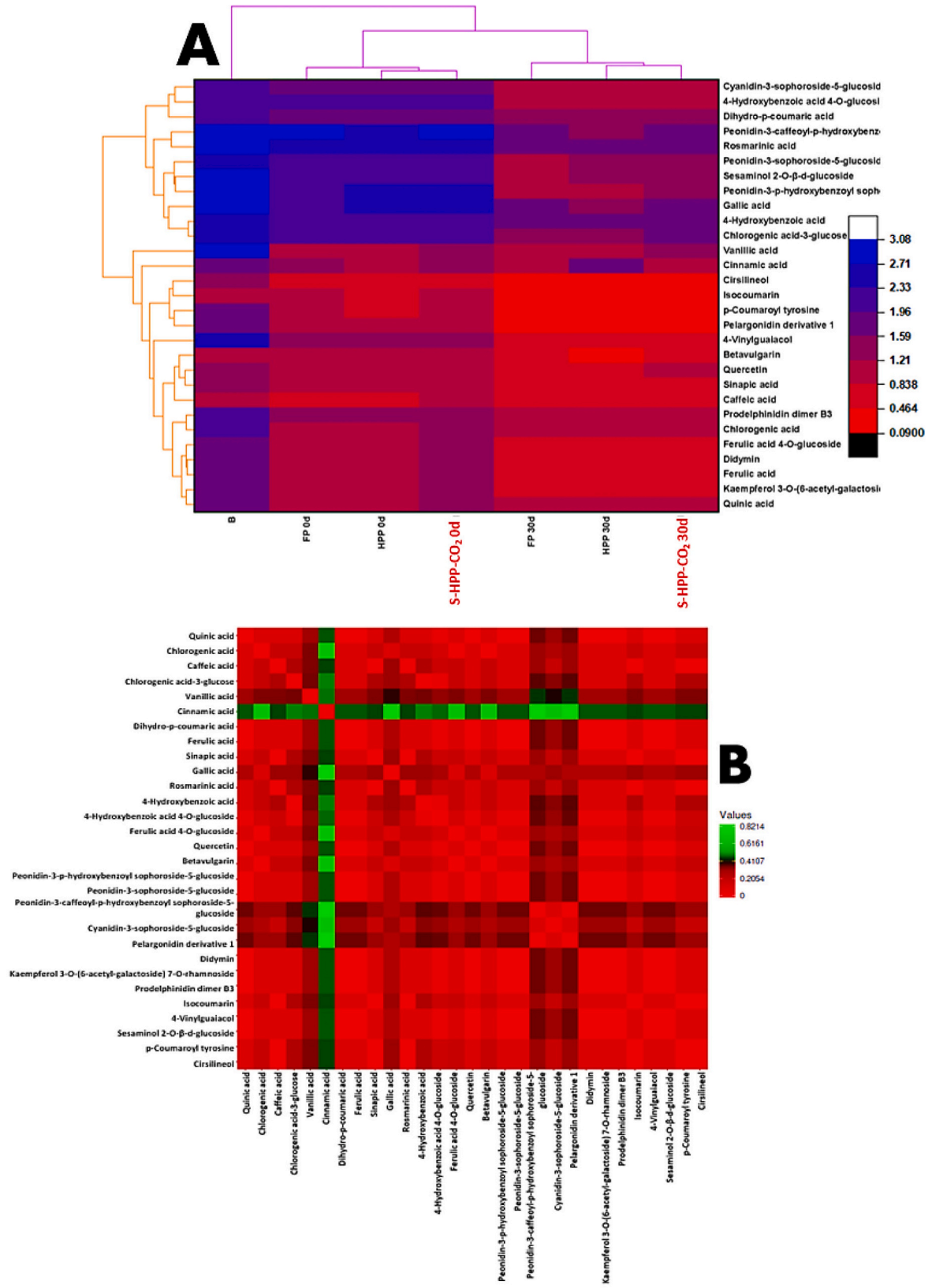


Fig. 3. The heat map analysis for polyphenols' fractions **(A)** and their Spearman rank correlation **(B)** of FQVP/FP, FQVP/HPP, and FQVP/semi-HPP-CO_2 formulas before and after 30-d of cold storage. B: is the FQVP before treatment.

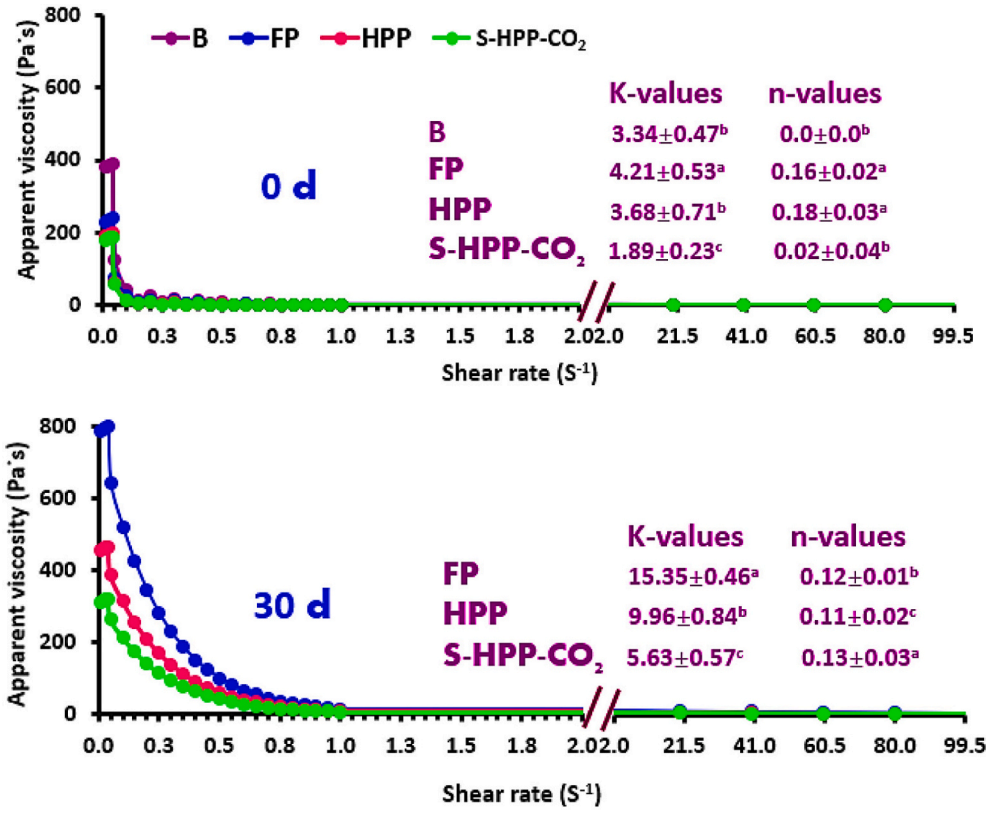


Fig. 4. Alterations in apparent viscosity of FQVP/FP, FQVP/HPP, and FQVP/semi-HPP-CO_2 formulas before (0 d) and after storage (30 d) and their K & n values. B: is the FQVP before treatment.

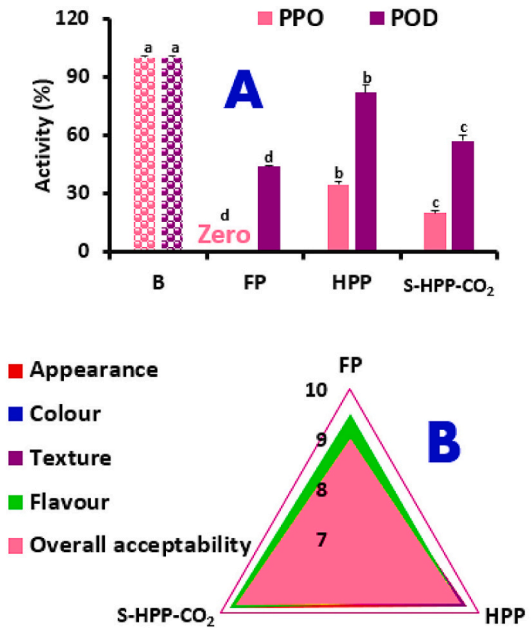


Fig. 5. Activities (%) of PPO and POD enzymes **(A)** and sensory scores **(B)** of FQVP/FP, FQVP/HPP, and FQVP/semi-HPP-CO2 formulas. B: is the FQVP before treatment.

pH, POD, PPO, texture, color, appearance, AI (%), TF, TPC, sucrose, AA, glucose, ORAC, TC, TSS, fructose, and flavor (Fig. 8A). Meanwhile, PC_1 negatively correlated with H°, ΔE, and TAB of FQVS. Most notably, PC_1 positively allied with both HPP, and semi-HPP-CO_2 treated FQVS, displaying their favored smoothie parameters and agreeing with our investigational results. Meanwhile, semi-HPP-CO_2 was more applicable than those of other FQVS-treated samples, where this smoothie located near to most of the quality, phytonutrients, and sensorial parameters. PC_2 positively correlated with some sensorial, phytonutrients, and color parameters, showing their influence on each other, where the polyphenols showed an inhibition against some enzymes and the sensory evaluation is related mianly with the color changes. Moreover, the location of semi-HPP-CO_2 treated FQVS was in the lower right-hand quadrant that was linked with the most of the biological and sensorial quality, and composition of the processed smoothie, signifying once more their quality. Instead, FP was in the lower left-hand quadrant, pointing out that their quality would have faded. Bubbles Pearson correlation pictured by blue and red bubbles, where more shade color and less size mean the superior connection among variables and contrariwise. As statistically analyzed in Fig. 8B, the bubbles Pearson correlation noticed that an extremely high significance ($p < 0.001$) between L* and TC, and PPO with L* had occurred, implying their impact on each other. The following parameters were also semi-highly significantly correlated ($p < 0.01$): (ORAC with pH-values) and (H° and ΔE with sucrose). Most importantly, many other parameters are positively correlated ($p < 0.05$), proving the valuable association among FQVS features we chosen to proof the quality of the manufactured smoothie. These parameters include (pH or ORAC with glucose), (sucrose with L*, a*, b*, TC, and PPO, and negatively with C), (L* with a*, H°, ΔE, and PPO), (a* with b*, H*, ΔE, and TC), (b* with H°, ΔE, and PPO, and negatively with C), (H° with ΔE, TC, and PPO), (C negatively with ΔE, where ΔE with TC and PPO), (TPC negatively with TC), (TC with PPO), and (color with texture sensorially). Previously we reported the positive correlation between the bioactive components including polyphenols and other flavonoids either anthocyanidins or non-anthocyanidins ones and their activities such as antioxidant and anti-enzymes effects in other different models (Khalifa et al., 2016, 2019; Khalifa, Sobhy, et al., 2023).

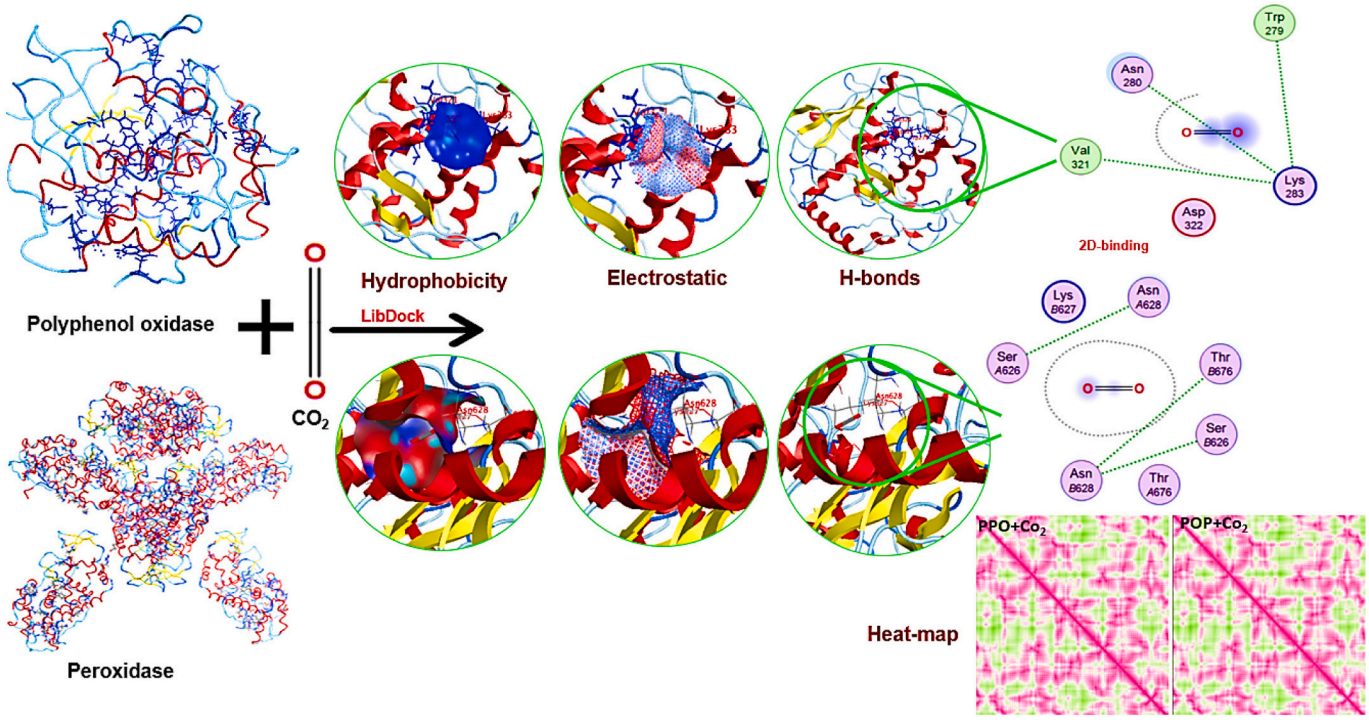


Fig. 6. The Lib-docking interaction between CO_2 with PPO and POD enzyme conjugates with a focus on the noncovalent interaction possibility including hydrophobicity changes, electrostatic forces, and H-bonding.

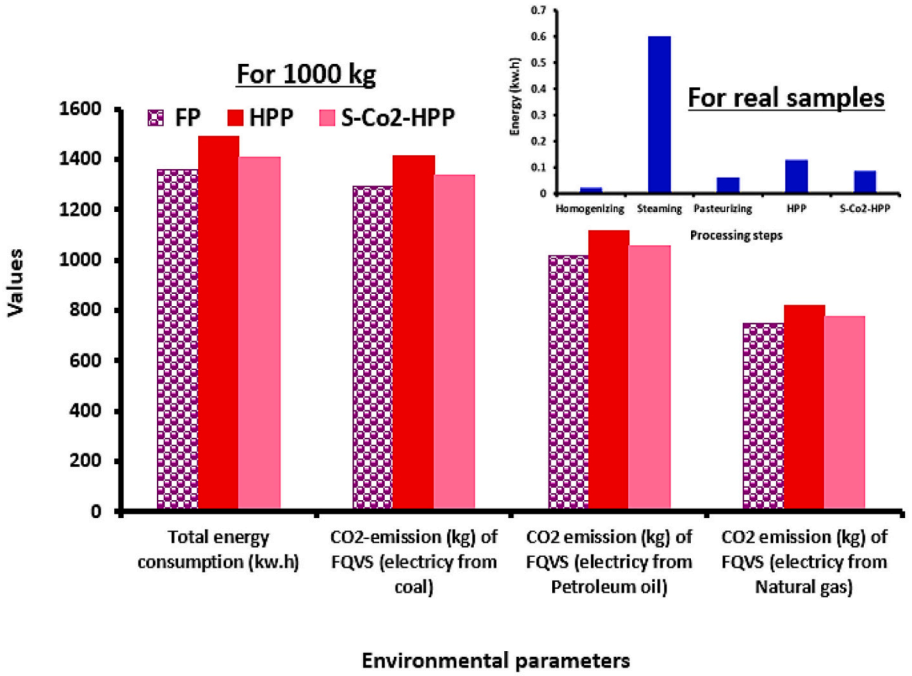


Fig. 7. Energy consumption and CO_2-emssions of producing 1000 kg of FQVS calculated based on the real sample based on different electricity sources.

4. Conclusions

The polyphenol content of sweet potatoes, carrots, pumpkins, and sugar beets is higher than that of other foods that are known to be reliable sources of physiologically active compounds. The blending of quadrable vegetables was employed with the aid of non-thermal processing and compared with thermal treatments. The results demonstrated that the flowable FQVS smoothie exhibited a good viscosity behavior, with high antioxidative polyphenols, carotenoids, and ascorbic acid. With its flowability, acceptable sensory scores, instrumental color parameters, and microbiological load, FQVS smoothie especially the one treated with semi-HPP-CO_2 has potential as a functional food product. Most importantly, semi-HPP-CO_2 showed an environmentally friendly trend compared with HPP. The findings of this study provide novel insights into the best use of non-thermal processing compared to conventional thermal processing, which may result in the loss of

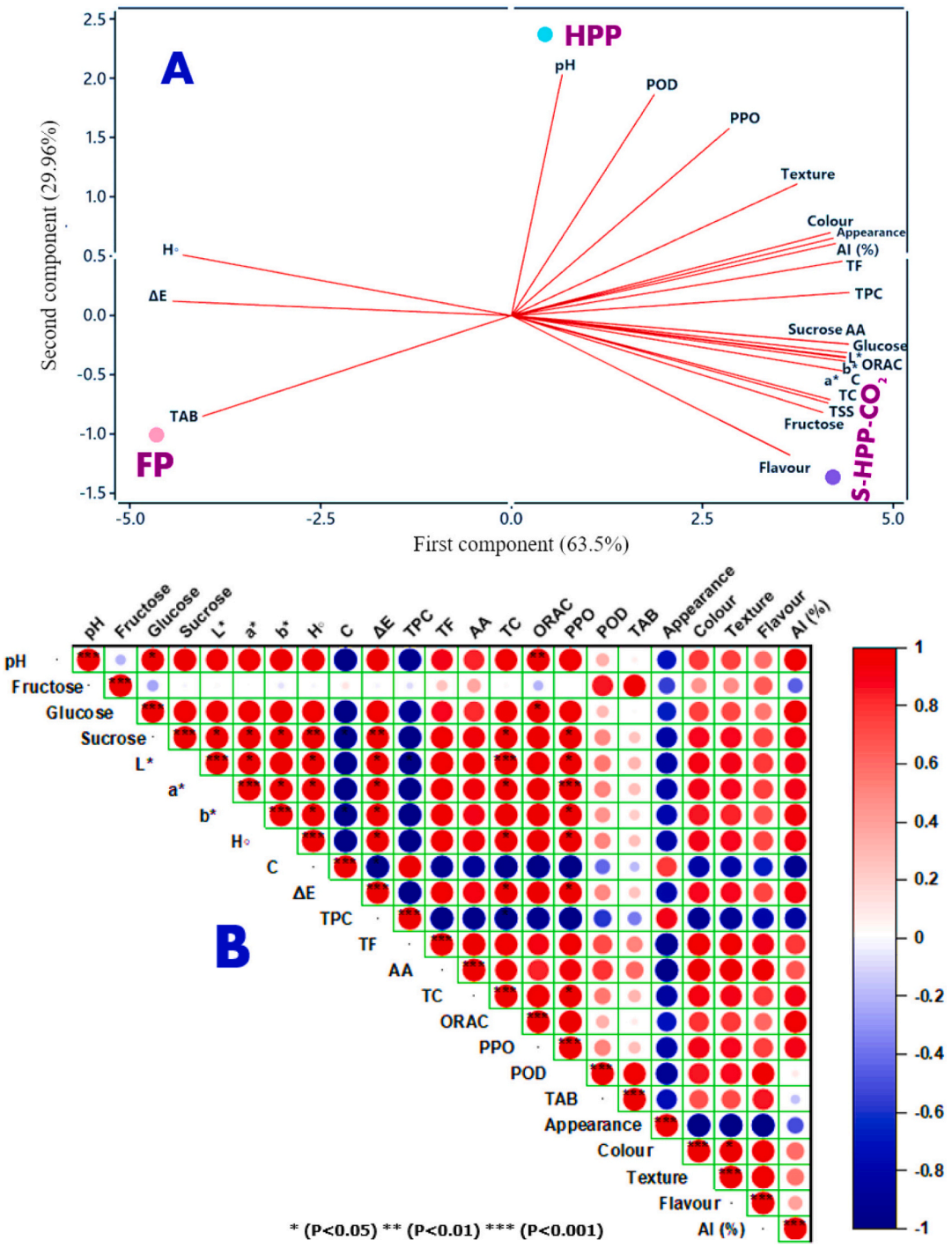


Fig. 8. Principal component (A) and bubbles Pearson correlation (B) analysis of FQVS parameters. *, **, and *** are the significant levels of 0.05, 0.01, and 0.001, respectively.

essential nutrients and affect the quality attributes of the final product.

CRediT authorship contribution statement

Ibrahim Khalifa: Writing – original draft, Methodology, Investigation, Conceptualization. **Remah Sobhy:** Writing – original draft, Methodology, Investigation, Conceptualization. **Xiaobo Zou:** Writing – review & editing, Supervision. **Asad Nawaz:** Writing – review & editing, Data curation. **Noman Walayat:** Writing – review & editing, Formal analysis. **Putri Widyanti Harlina:** Writing – review & editing, Software. **Tarek Kh. Abdelkader:** Writing – review & editing, Visualization. **Mukhtar Ahmed:** Writing – review & editing, Validation, Project administration. **Sajid Maqsood:** Writing – review & editing, Data curation, Resources, Visualization, Funding acquisition.

Declaration of competing interest

No conflict of interest among authors.

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.fochx.2024.101663.

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