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Key Words

Bioactive compounds; Fruit smoothie; High pressure; Antioxidant activity; Anti-inflammatory activity

Abbreviation

HHP: High Hydrostatic Pressure; BC: Bioactive Compounds; DF: Dietary Fiber; AOX: Antioxidant Capacity; PC: Phenolic Compounds; TSP: Total Soluble Polyphenols; TC: Total Carotenoids; TDF: Total Dietary Fiber; FRAP: Ferric Reducing Antioxidative Power; FSB: Fetal Bovine Serum; PBS: Phosphate-Buffered Saline; GADPH: Glyceraldehyde-3-Phosphate Dehydrogenase

Research Article

Bioactive Compounds and in Vitro Anti-Inflammatory Properties in a Fruit-Smoothie Processed by High Hydrostatic Pressure

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Abstract

A fruit-smoothie was analyzed in order to evaluate the effect of High Hydrostatic Pressure (HHP) on the release of Bioactive Compounds (BC) after different pressure and time treatments (500 and 600 MPa for 45 and 90 s), conditions commonly used in the food industry. Vitamin C, bioactive compounds (BC; phenolic compounds, carotenoids) and Dietary Fiber (DF) content, as well as, Antioxidant Capacity (AOX) and anti-inflammatory properties were analyzed in the samples. The vitamin C, BC content, and AOX was minor in the Non-HHP fruit-smoothie; while 500 MPa treatments showed greater increase of the content of BC and AOX. Regard to anti-inflammatory effect, it was observed that HHP fruit-smoothies caused a modulating effect in various inflammation markers; at 500 MPa for 45 s generated the modulation in the expression of NF-kB, TNF-α, IL-8, COX-1 and COX-2 markers. Therefore, it was possible to obtain valuable information about the effect of HHP on fruit-smoothie BC and its potential anti-inflammatory effect.

Introduction

The consumption of beverages from fruits and vegetable milks has become popular among people looking for a healthy diet. Fruit smoothies are a popular and convenient way of consuming fruit, they contain a large amount of fruit rich in nutrients and Bioactive Compounds (BC) responsible for health effects [1,2]. The term "smoothie" is given to a blended fruit drink characterized by a pulpy consistency, containing one or more fruits, yogurt, cow's milk or, vegetable milk [3-5]. Fruits such as Mango (Mangifera indica) and Jackfruit (Artocarpus heterophyllus) are tropical fruits, which have proven to be a good source of vitamin C and BC as Phenolic Compounds (PC), and carotenoids (CC) mainly [6-8]. Other variants such as the addition of vegetable milk facilitate the consumption of the lactose intolerant population [9], and the contribution of flavonoids such as γ-tocotrienol, and α-tocopherol (homologue of vitamin E) that come from the grain of rice. The BC act as antioxidants and offer some protection against oxidative stress-related diseases and in the state of inflammation [10,11]. Concerning human health, chronic inflammation is considered to be a critical factor in many chronic degenerative human diseases [12]. Several authors have reported the ability to modulate the expression of the inflammatory markers and reducing the expression of interleukins by different BC of mango [10,13,14], and jackfruit [15-17, 11]. High Hydrostatic Pressure (HHP) is a non-thermal technology that has shown positive effects on nutritional compounds and sensory characteristics, in different fruit-products, specifically in beverages [18]. HHP allows the inactivation of microorganisms and enzymes [19-22], a range of 580 MPa is recommended by the FDA for commercially products (FDA, 2012). Furthermore, HHP opens a possibility for functional foods by improving the extraction and maintaining of BC [9, 21, 22]. In this sense, the aim of this study was to evaluate the effect of HHP on BC in a fruit-based drink (mango, jackfruit, and rice drink), its Antioxidant Capacity (AOX) and anti-inflammatory properties using an HT-29 cell model.

Materials and Methods

Chemicals

All solvents, standards, salts, and acids were purchased from Sigma Chemical Co. (St. Louis, MO), J. T. Baker (Mexico City) and Santa Cruz Biotechnology (Santa Cruz, CA, USA). The human colon cancer cell line HT-29 was obtained from the American Collection of Type Cultures (ATCC HTB-38TM) and was provided by the Laboratory of Functional Foods and Nutraceuticals (Instituto Tecnológico de Durango, Mexico).

Fruit-smoothie preparation

Jackfruit and Mango ('Ataulfo') were acquired from a local market located in Tepic Nayarit, Mexico, in consumption maturity and the commercial rice beverage (Nature's heart terrafertil®, USA) was obtained from a local supermarket. The smoothie was prepared mixing mango (24 %), jackfruit (6 %) and the rice beverage (70 %). Fruits were blended in a homogenizer (Model 38BLC10, Waring Commercial®) for 5 min. The samples were packed in polyethylene bags of 8 in. x 11 in. (Food Saver®) and vacuum sealed (MULTIVAC®, C100, Sepp Haggenmüller GmbH & Co. KG, Germany). Packed samples then were cooled at 6 °C until HHP treatments were applied.

High hydrostatic pressure processing

All the treatments were performed in an industrial HHP (Verfruco of Mexico S de RL de CV Company), located in Uruapan, Michoacán, Mexico. The fruit smoothies were subjected to treatments using an HHP equipment (Hiperbaric Wave Model 6000/135 NC), with a capacity to 135 L and 600 MPa. A 2x2 factorial design was used, where the factors were pressure (500 and 600 MPa) and time (45 and 90 s). A non-pressurized sample (Non-HHP) was included as fruit smoothie control. After obtaining the smoothies with HHP treatment and control samples were freeze-dried (LABCONCO, Freezone, USA), ground

(Nutribullet, NBR0804B, USA), sieved (0.5 µm), and then stored in sealed bags at −20 °C until analysis.

Vitamin C analysis

The vitamin C content was determined according to the method of previously an extract was elaborated starting from 1 g of dried sample and 9 mL of extraction solution (5 % metaphosphoric acid and 10 % acetic acid glacial). After coupling with 2, 4-dinitrophenyl hydrazine at 37 °C for 3 h, the extract was treated with H2SO4 solution (85 %) to produce a red color complex and the absorbance was spectrophotometrically measured at 521 nm. Vitamin C content was calculated using a calibration curve of standard ascorbic acid. Results of vitamin C were expressed as grams of ascorbic acid per 100 g of sample (mg AA /100 g sample).

Bioactive compounds analysis

Preparation of organic extracts for evaluation of total soluble polyphenols content and antioxidant capacity: The organic extract was prepared starting from dried samples (0.5 g) with methanol-water (50:50 v/v, 50 mL/g of sample, 60 min) followed by an extraction with acetone-water (70:30 v/v, 50 mL/g of sample, 60 min). After each extraction step, samples were centrifuged (3000 g for 15 min at 25 °C), and extracts were collected.

Total soluble polyphenols content: Total Soluble Polyphenols (TSP) content was determined according to [23, 24]. Briefly, 250 µL of extract was mixed with 1000 µL of sodium carbonate solution (75 g/L), and 1250 µL of Folin-Ciocalteu reagent (100 mL/L) were combined in glass tubes and then mixed using a vortex. The solution was incubated at 50 °C in the dark for 15 min. Then, 270 µL of sample extracts or gallic acid were placed in the wells and the absorbance was measured at 750 nm using a multidetector microplate reader (Biotek, Synergy HT, Winooski VT, USA) with Gen5 software and the results were expressed as grams of gallic acid equivalents (g GAE /100 g of sample) using a gallic acid (0-0.2 mg/mL) standard curve.

Total carotenoids content analysis: Total Carotenoids (TC) were determined by spectrophotometric method according to the procedure of [25]. It was estimated in acetone-petroleum ether extracts. 0.5 g of lyophilized sample was used to make the extraction. The absorbance at 448 nm of the resultant sample was measured in spectrophotometer single cell holder (Jenway® model 6705, UK). β-carotene was used as standard and results were expressed as mg of β-carotene equivalents per 100 g of sample (mg Eβ-carotene /100 g of sample).

Total dietary fiber analysis: The beverages were analyzed in the Total Dietary Fiber (TDF) using the AOAC enzymatic-gravimetric method (method 991.42) modified [26], all data were expressed as g TDF /100 g of sample.

Antioxidant capacity (AOX)

The AOX was evaluated in the organic extract by three methods: A) 2, 2-Azinobis Radical 3-Ethylbenzthiazoline-6-Sulfonic (ABTS) [27], B) 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) [28], and C) Ferric Reducing Antioxidative Power Assay (FRAP). All data were expressed as Trolox Equivalents (mmol TE /100 g of sample). The absorbance was measured in a microplate reader Biotek (Synergy HT®, Winooski VT, and EE.UU.) with the Gen 5 software (Biotek®, Winooski, Vermont, USA).

Anti-inflammatory assay

The anti-inflammatory effect of the HHP fruit-smoothies was evaluated, using the techniques implemented by [29].

Cell culture: Human colon cancer HT-29 (colorectal adenocarcinoma) cell suspension of 1.2 x 106 was grown at preconfluence into 100 mm plates with the medium RPMI (Roswell Park Memorial Institute) medium supplemented with 10 % Fetal Bovine Serum (FSB), penicillin (50 U/mL) and streptomycin (50 µg/mL) for 72 h (5 % CO2 at 37 °C) in a humidified atmosphere. Cells at 70 % of confluence were used for assays, which were carried out between passages number 10-15.

Cell viability: Cell viability was assessed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method. The cells were seeded into 96 well plate at a density of 9.6 x 103 cells per well. After the experimental treatments, cells were thoroughly washed with PBS (Phosphate-Buffered Saline) three times to avoid any interference of the oak with the MTT. Then, 0.1 mL of MTT reagent was added to the plate and was incubated for 4 h (5% CO2 atmosphere). The MTT reduced by the viable cells to formazan product was dissolved in 0.2 mL of DMSO (Dimethyl sulfoxide solution) and absorbance was measured at a test (570 nm) and reference wavelengths (690 nm) using a microplate ELISA reader (ELx800™, BioTek® Instruments, Inc. Winooski, Vermont, USA). The percentage of mitochondrial enzyme activity was determined by the ratio of the mean absorbance for each treatment and control obtained.

Experimental treatments: Several concentrations (0.125, 0.5, 0.75 and 1.5 mg/mL) from the lyophilized samples of HHP and Non-HHP fruits smoothies were prepared in FSB-free DMEM (Dulbecco's Modified Eagle Medium). Subsequently, the treatments were applied and allowed to incubate for 24 h in a 5 % CO2 atmosphere. After the incubation period, the effect of pH and precipitation of the treatments on the possible cytotoxic effects generated by the changes in the physicochemical factors in the cellular microenvironment was evaluated. Only those concentrations that did not change the culture media conditions were selected for further experimentation. The final dose established for the experiments was that which did not induce changes in the physicochemical parameters of the culture and which did not show cytotoxicity (0.125 mg/mL).

Western analysis: For the immunoblot analysis, pre-confluent cultures were washed with PBS 1X and treated with medium alone (RPMI) and HHP and Non-HHP (0.125 mg/mL) fruits smoothies in DMSO as vehicle for 3 h at 37 ºC. Cells were removed by adding a mixture of trypsin and verseno dissolved in phosphate buffer (pH 7.8), and then centrifuged at 3000 rpm for 5 min. The pellet was recovered and suspended with phosphate buffer (pH 7.8) added with protease inhibitor (Bio-Rad), and then the cells were lysed on ice for a maximum speed using an Ultra-turrax cell (IKA T 10 basic). The suspension was centrifuged at 12,000 rpm; the supernatant was recovered to determine protein concentration and immunoblot analysis.

Protein concentration was determined using the Bio-Rad Bradford dye binding protein assay kit according to the manufacturer's instructions. Fractions of cellular proteins were subjected to SDS-PAGE and electrotransferred into nitrocellulose membrane for 1 h at 100 V. Membranes were immersed for three hours at 4 °C in 10 mL blocking solution which contained 5% (v/v) non-fat milk in TBS-T (20mM Tris/HCl-buffered saline/100 mM NaCl, 0.2%, 0.1% Tween 20) at pH 7.6. Membranes were washed three times with 10 mL TBS-T (20 mM Tris/HCl, 100 mM NaCl, 0.2% (v/v) Tween-20, pH 7.6), followed by incubation with the first antibody with an appropriate dilution prepared in 10 mL of TBS-T: for COX-1 and COX-2 (1:1000), for total NF-KB p65 (C-20) and IL-8 (1:200) and for TNF-α (1:800) for 2 h. After washing for three times with 10 mL TBS-T, membranes were incubated for 3 h with a secondary antibody, mouse anti-rabbit IgG or mouse anti-goat IgG, conjugated with alkaline phosphatase diluted 1:2000 in 10 mL of TBS-T. Subsequently, the membrane was washed two times with TBS-T, stained and revealed. Subsequently, it was quantified from a densitometer analysis using the TOTALLAB software (TotalLab Ltd, Newcastle, Inglaterra). GADPH (Glyceraldehyde-3-Phosphate Dehydrogenase) was used as charge control and DMSO as a control reference 100% depending on the intensity of the band.

Statistical analysis

Experimental values are given as mean ± SD. Statistical significance was determined by one-way ANOVA ($P < 0.05$) and an analysis of Fisher's LSD means to determine significant differences between all the HHP treatments and Non-pressurized sample. An analysis of correlation analysis ($p<0.05$) and the principal components analysis (PCA) was carried out ($P <0.05$) to correlate all the response variables analyzed for the different treatments applied. Statistical analyses were performed using Statistica 8.0 (StatSoft, Inc., Tulsa, OK).

Results and Discussion

Effect of HHP on vitamin C and BC content of fruit-smoothies

(Table 1) shows vitamin C and BC (TSP, TC and TDF) content in Non-HHP fruit-smoothie and HHP fruit-smoothie treatments. The content of TSP was ranged from 0.164 to 0.182 g GAE/100 g FW sample. It can be observed that TSP content increased in fruit-smoothie samples after HHP processing. Maximum TSP content (0.182 g GAE/100 g sample) was obtained in samples treated at 500 MPa for 45 s, showing an increase of 10.97% in the TSP content. HHP favors the extraction of TPS and its increase in the smoothie matrix. This increment could be explained by the rupture generated in the cell membranes by the pressure applied [30]. Consequently, these BC could be more bioavailable when smoothie is consumed and then generate a beneficial effect on health. TC content was ranged from 1.006-1.049 mg Eβ-carotene/100 g of sample (Table 1), with non-significant differences ($p>0.05$) between HHP and Non-HHP smoothies. However, the highest content was obtained at 500 MPa for 45 s (1.049±0.028 mg Eβ-carotene/100

g). HHP treatments at different pressure levels and holding times did not cause an increase in the carotenoid extraction, but maintained same concentration as the Non-HHP smoothie. The vitamin C content was ranged from 14.35 to 17.73 mg AA/100 g of sample, being the highest content at 500 MPa for 45 s with significant difference ($p<0.05$) between samples.

Table 1: Effect of High Hydrostatic Pressure (HHP) on Total Soluble Polyphenols (TSP), vitamin C, Total Carotenoids (TC) and Total Dietary Fiber (TDF) content of a fruit-smoothie.

Treatments	TSP (g GAE/100 g FW)	TC (mg E β-carotene/ 100 g FW)	Vitamin C (mg EAA/100 g FW)	TDF (g/100 g FW)
Non-HHP	0.164±0.009 c	1.006±0.014 a	14.35±0.289 d	2.049±0.040 a
500 MPa-45 s	0.182±0.005 a	1.049±0.028 a	17.73±0.206 a	2.057±0.024 a
500 MPa-90 s	0.178±0.005 b	1.091±0.033 a	16.97±0.179 b	2.065±0.021 a
600 MPa-45 s	0.175±0.007 b	1.029±0.025 a	15.42±0.132 c	2.050±0.081 a
600 MPa-90 s	0.169±0.010 b	1.022±0.017 a	15.10±0.252 c	2.058±0.036 a

Values are means ± standard deviations of triplicate measurement. For different treatments, means in each column with different letters were significantly different ($P < 0.05$). FW-fresh weight.

Similar behavior was observed in soy-smoothies with increase in total polyphenol and total carotenoid contents after HHP (650 MPa/3 min) [9]. In strawberry juices it was reported an increment of phenolic compounds and vitamin C content during HHP processing, at different treatment conditions (400 MPa/3 min, 500 MPa/3 min, 600 MPa/3 min, and 85 °C/2 min), demonstrating that different pressures and time used did not have effect on the contents of these compounds [31]. In wheatgrass juice it was observed that there were no increases regarding the total phenolic content and vitamin C content with HHP treatment (0.3161 mg GAE/100 mL and 9.03 mg/100 mL, respectively) compared to the control group (0.3418 mg GAE / 100 ml and 9.21 mg / 100 ml, respectively). These results indicate that HHP promotes the extraction of antioxidant substances, enables the retention of functional components, and reduces the destruction of natural nutrients in fruits and vegetables [32,8].

TDF content was not affect by HHP treatment ($p>0.05$). Dietary fiber is a complex covalent structure of carbohydrates with high molecular weight and negligible compressibility of covalent bonds that are not affected by HHP [8]. TDF in the studied smoothies were from 2.049 to 2.065 g/100 g of sample. The daily recommended value for fiber is 25 g per day on a 2,000-calorie diet (FDA, 2016). The formulation used for fruit smoothie gives us 8 % of daily recommendation. A high DF intake has been related to several physiological and metabolic effects, attenuating blood glucose responses, assisting with cholesterol lowering and impacting on the prevention and treatment of obesity, atherosclerosis, coronary heart diseases, colorectal cancer and diabetes [33].

Effect of HHP on AOX of fruit smoothies

AOX of Non-HHP and HHP fruit smoothies are shown in (Figure 1).

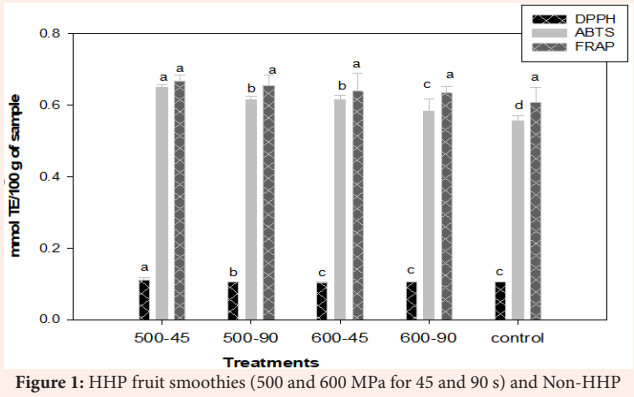

Figure 1: HHP fruit smoothies (500 and 600 MPa for 45 and 90 s) and Non-HHP (control) fruit-smoothie antioxidant capacity ($P<0.05$).

Different assays show different results and particular mechanisms of action. To elucidate the AOX of smoothie samples, DPPH, ABTS, and FRAP assays were used. Non-significant differences were observed between Non-HHP and 600 MPa samples, to 45 s and 90 s, on the AOX by DPPH. However, HHP samples at 500 MPa showed a high AOX, being the highest AOX at 500 for 45 s (0.11 ± 0.005 mmol TE/100 g of sample). The AOX measured by ABTS detected significant differences between Non-HHP and HHP samples, where the lowest AOX was observed in the Non-HHP sample (0.51 ± 0.01 mmol TE/100 g of sample) and the highest was observed in 500 MPa for 45 s sample (0.65 ± 0.007 mmol TE/100 g of sample). Contrary to DPPH and ABTS, AOX by FRAP showed non-significant differences ($P<0.05$) between Non-HHP and HHP fruits smoothies samples. This can be explained, because antioxidants may respond differently to different radical or oxidant source. Furthermore, an individual antioxidant may, in some cases, act by multiple mechanisms in a single system or by a different single mechanism depending on the reaction system [34,28].

Since fruits are rich in BC, they have high antioxidant activity and are a good source of nutrients, therefore, the use of HHP in fruits and beverages reduces the destruction of antioxidant components and favors their AOX [8]. In soy smoothies a slight increase of AOX by FRAP was observed of 2.9% at 550 MPa and 6.6% at 650 MPa and a slight increase by DPPH of 3.8% at 550 Mpa, and 5.4% at 650 MPa [9, 4]. Reported higher antioxidant activities at lower operating pressures compared to higher pressures in fruit smoothies (450 vs. 600 MPa). HHP at 500 MPa for 10 min led to better retention of antioxidant activity with the ORAC value of 481.68 mmol TE/mL in mulberry juice [20]. The variability of the effects of HHP depends not only to the treatment conditions (pressure, temperature, time) but also the type of food (whole fruit, juice or puree) and food matrix (hydrophilic compounds, lipophilic, hydrophobic, etc.) [3,35,20]. On the other hand, AOX by ABTS and DPPH methods was strongly correlated ($P<0.05$) with the content of vitamin C of HHP fruits smoothies. Vitamin C and TSP are correlated with major bioactive compounds that contribute to radical scavenging activity [9]. The fruit-smoothie that showed a higher AOX also contained a higher content of vitamin C and TSP, so that its AOX could be related by a possible synergistic effect of vitamin C with phytochemicals as polyphenols and carotenoids, these last one also contained in the fruit-smoothies.

Accumulation level of pro-inflammatory and anti-inflammatory proteins in vitro

HHP fruits-smoothies modulated the levels of pro-inflammatory and anti-inflammatory proteins in HT-29 cells (Figure 2).

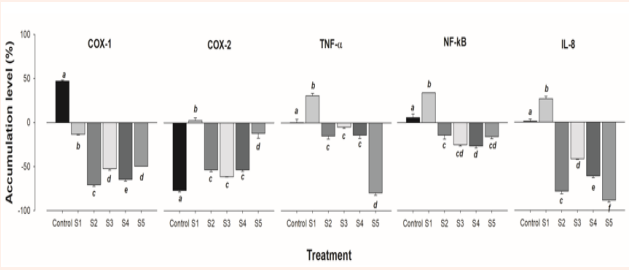

Figure 2: Differential levels of pro-inflammatory and anti-inflammatory proteins in HT-29 cells treated with HHP fruits-smoothies (S1: non-HHP, S2: 500 MPa - 45 s, S3: 500 MPa – 90 s, S4: 600 MPa – 45 s and S5: 600 MPa – 90 s). The density of the bands of COX-1, COX-2, TNF-α, NF-kB and IL-8, were semi-quantified by ImageJ and expressed as arbitrary units relative to control group. Values with different letter(s) are significantly different ($P < 0.05$). GADPH was included as loading control.

HHP fruits smoothies differentially modulated the levels of pro-inflammatory proteins in HT-29 cells. All HHP fruits-smoothies decreased COX-1, COX-2, NF-κB, TNF-α, and IL-8 expression compared with Non-HPP. Unexpectedly, COX-1 expression decreased with the HHP fruit-smoothies, which would be expected that COX-1 will not be altered as the control (GADPH). However, most of the treatments were effective in reducing oxidative stress, if we measure it indirectly through levels of accumulation of TNF-α, which results in an anti-inflammatory effect by decreasing IL-8 levels. There are a few related studies on high pressures, bioactive compounds, and anti-inflammatory activity [2]. Investigated the effects of HHP extract of ginseng (PEG) on obesity and inflammation in rats fed a high-fat diet, where the levels of pro-inflammatory genes such as TNF-α, IL-6, and MCP-1 were down-regulated.

Applying a principal component analysis (Figure 4A) were observed the factors that represent 89.06 % of the variability obtained in the response variables.

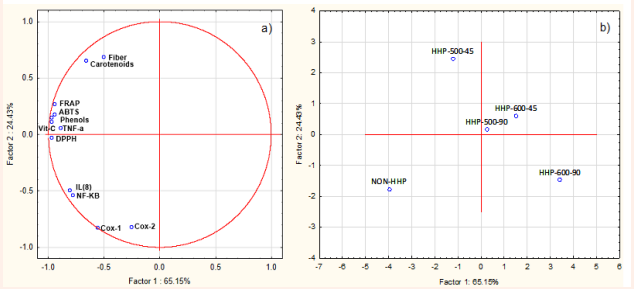

Figure 4(A): Analysis of the main components of the vitamin C and BC (carotenoids, dietary fiber, soluble polyphenols), AOX (FRAP, ABTS, DPPH), and the inflammation markers (TNF-a, IL-8, COX-2, COX-1, NF-Kß) of the HHP fruit-smoothies. b) Principal components analysis of the Non-HHP and HHP fruits smoothies (500 MPa - 45 s, 500 MPa-90 s, 600 MPa – 45 s and 600 Mpa-90 s).
Figure 4(B): Shows the behavior of the treatments according to the two factors that are previously described.

It is observed that there are two behavior patterns, the first (Factor 1) that explains the 65.15 % of the variability describes that as the AOX (ABTS, FRAP, and DPPH), the content of TSP and TNF-α marker decrease, likewise, a decrease is observed in the biological markers IL-8, COX-2, COX-1, and NF-kβ. Factor 2 with 24.43 % of the variance describes an increase in the content of carotenoids and fiber, also reveals low variation in AOX, TSP, vitamin C, and in the TNF-α marker. This behavior indicates that IL-8, NF-KB, COX-1, and COX-2 markers decrease could be associated with carotenoid and fiber content increment. HHP treatment at 600 MPa for 90 s presents a greater correlation with the decrease of the NF-KB, COX-1 and COX-2 markers, while at 600 MPa for 45 s treatment follows the trend according to (Factor 2), where appreciates a low variation concerning the AOX and content of TSP. The HHP treatment at 500 MPa for 90 s does not follow any of the trends indicated in the response variables, while treatment at 500 MPa for 45 s maintains a positive correlation with the (Factor 2), thus presenting higher concentrations of carotenoids and fiber. These values demonstrate that 500 MPa for 45 s, 600 MPa for 45 s, and 600 MPa for 90 s treatments are the most suitable for decreasing the concentrations of inflammation markers. About the results obtained, HHP extract of ginseng reduced mRNA levels of pro-inflammatory genes such as TNF-α and IL-6 in rats fed a high-fat diet, and it was attributed to the increase of total phenolic content polyphenols by HHP [2, 1]. Determined that the consumption of HHP extract of red ginseng in rats induced obesity generates a regulation of pro-inflammatory genes (TNF-α and IL-6).

The anti-inflammatory effect obtained is attributed to the BC (carotenoids, vitamin C, and phenolic compounds) present in the HHP fruit-smoothies since BC can act directly in the inflammatory signaling cascade, so that prevent the activation of different transcription factors as the factor nuclear kB (NF-kB), which triggers signaling cascades that releases cytokines and inflammation [36]. Also, several authors have reported that these compounds are capable to inhibit the expression of these markers [37,13] and numerous studies in different cell lines or animal models suggest a protective role of the synergistic effect of the bioactive compounds resulting beneficial biological functions, such as the inhibition of proliferation of human cancer cells is related to the anti-inflammatory effect [38, 39]. In this connection a correlation analysis ($p < 0.05$) between the concentration of BC, AOX with the anti-inflammatory effect reflected that carotenoids and vitamin C influence repressed the expression of NF-kB and COX-2, as well as, FRAP and DPPH antioxidant activity influenced significantly ($p < 0.05$) in suppressing the expression of IL-8, COX-2, and NK-Kb. Regarding the TSP content in fruit smoothies and its relationship to the anti-inflammatory activity, HHP smoothies can have a significant impact on the inflammatory state and modulation of the expression gene of inflammatory markers TNF-α, TLR4, IL-6 [40].

The anti-inflammatory properties of vitamin C can be attributed to its ability to modulate the expression of the marker NF-kb and reducing the expression of interleukins [41]. The biological activity of mango can be attributed mainly to gallic acid, which is the major polyphenolic compound and which has been shown to reduce the expression of the biological markers responsible for inflammation [37]. On other hand, some authors report that jackfruit contained carotenoids can be important for the prevention of several chronic degenerative diseases, such as cancer and inflammation [42]. Studies have also explored the anti-inflammatory role of jackfruit, phytochemical investigations of ethyl acetate extracts of jackfruit led to the isolation of the phenolic compounds artocarpesin [5,7,2′,4′-tetrahydroxy-6-(3-methylbut-3-enyl) flavone] [43-46], which was further shown to possess potent anti-inflammatory effects in RAW264 [6]. Also, three phenolic compounds were characterized of jackfruit as artocarpesin [5,7,2′,4′-tetrahydroxy-6-(3-methylbut-3-enyl) flavone], norartocarpetin (5,7,2′,4′- tetrahydroxyflavone), and oxyresveratrol [trans-2,4,3′,5′-tetrahydroxystilbene] exhibited potent anti-inflammatory activity [7]. On the other hand, the rice drink could also be contributing to the polyphenol content and flavonoids such as ferulic acid, γ-tocotrienol and α-tocopherol (homolog of vitamin E) [47-49].

Conclusions

HHP processing is an efficient treatment for the preservation of the BC present in a fruit-smoothie. Only a small number of studies have assessed the effect of HHP on mixtures of fruits, such as fruit smoothies, its biological effect on health, and the anti-inflammatory activity. This study demonstrated that high pressure conserves and/or increases BC, enhancing as consequence the AOX in fruit-smoothie beverages. The HHP fruits smoothie treatments were very effective in reducing oxidative stress with decreased COX-2, NF-κB, TNF-α, and IL-8 expression. Therefore, the high pressures are a promising technology for the conservation of nutritional properties in different food-matrix and open possibilities to functional foods, which when consumed may be generating beneficial effects for health.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

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