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ORIGINAL PAPER

Development of an Orange-Tangerine Juice Treated by Assisted Pilot-Scale UV-C Light and Loaded with Yerba Mate: Microbiological, Physicochemical, and Dynamic Sensory Studies

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Abstract
An orange-tangerine juice processed by pilot-scale UV-C light (390 mJ/cm^2) assisted by mild heat (UV-C/H,50 °C) and enriched with yerba mate (YM) extract (UV-C/H + E) or encapsulated YM extract (UV-C/H + EE) is proposed. The inactivation of *Escherichia coli*, *Lactiplantibacillus plantarum*, and *Saccharamyces cerevisiae* was assessed. Juice samples were analysed for polyphenol content (TPC), total antioxidant activity (TAA), colour, turbidity, and °Brix along refrigerated storage (4 °C; 20 days). For the UV-C/H + E and UV-C/H + EE juices, dynamic consumer profiling studies were performed. According to the challenge study, UV-C/H induced high microbial inactivation (4.9–5.8 log-reductions). TPC (0.059 ± 0.002 mg GAE/mL) and TAA (6.3 ± 0.2 mg TroloxEq/mL) in the UV-C/H + EE juice were 2.1 and 1.6 times greater than the untreated ones, respectively. The UV-C/H + E juice displayed a slightly lower increase in TPC (0.045 ± 0.020 mgGAE/mL), but similar TAA (7.1 ± 0.3 mgTroloxEq/mL), compared to the UV-C/H + EE juice. Therefore, the encapsulation procedure prevented YM extract from TPC degradation. UV-C/H did not alter juice colour while YM addition increased its turbidity. Frequent consumers of YM tea showed a high willingness to try the juice. A cluster sensory analysis revealed that one group of consumers was interested in the *sour* and *herbal aroma* of the UV-C/H + E and UV-C/H + EE juices. The T-CATA question showed that the *sweetness* and *herbal aroma* of the UV-C/H + E juice were better perceived with sips; whereas, UV-C/H + EE juice perception shifted from *fruity*, *herbal aroma*, and *sweet* to *intense taste*, *bitterness*, and *pleasant aroma*. Thus, YM extract encapsulation also contributed to mask juice bitterness in the first sip while highlighting its sweetness. However, as juice intake proceeded, the panelists perceived juice bitterness more intensely.

Keywords UV-C light · Mild heat · Yerba mate · Juice blend · Dynamic sensory profile

Introduction

Among fruit derivates, juices represent an interesting alternative to increase fruit consumption as it can be an "on the go" option. As to the demand for novel products, juice blends are an interesting way of offering new flavours in the beverage industry. Blending allows to balance some characteristics of single-flavour juices, such as colour, acidity and taste (FAO, 2018), to obtain attractive products for the consumers.

The use of herbal extracts in fruit-based beverages widens product design possibilities as, on the one hand, new flavors can be offered to "adventurous" consumers, who are currently trending the future of the food and beverage industry (Innova Market Insight, 2018). This kind of consumers are generally moving out from their comfort zones to explore bolder flavours and to have multisensory food and beverage experiences from all around the world (Green, 2018). Moreover, as a growing number of people is paying attention to health and sustainability, the development of functional beverages with health claims and/or processed by milder preservation technologies has flourished in the last few years (Aghajanpour et al., 2017). Even though this option has not

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been extensively explored yet, the use of some interesting herbal extracts has been studied (Rodino & Butu, 2019). In particular, several products loaded with yerba mate (YM, *Ilex paraguariensis A., St Hill)* extracts and/or preserved by traditional or emerging technologies have been developed, including carrot-orange juice (Ferrario et al., 2018), soy-based beverage (Frizon et al., 2018), nutraceutical fermented beverage (Ferrari Pereira Lima et al., 2012), mayonnaise (Fenoglio et al., 2020), gelatin (Berté et al., 2011), cheese (Saraiva et al., 2019), and blackcurrant juice (Orjuela-Palacio et al., 2014). Industrialized yerba mate leaves are usually consumed in some countries of South America as *maté* tea bags or the traditional popular hot-water infusion of loose leaves in a typical calabash gourd, a container made from the shell of calabash provided with a strainer or infuser. For coffee and tea consumers, yerba mate represents a proved healthier alternative. It has been demonstrated that yerba mate is a natural source of minerals, caffeine, and other polyphenols with antioxidant capacity (Burris et al., 2012). Its consumption has additional anti-inflammatory and anti-obesity proved effects (Burris et al., 2015; Fayad et al., 2020).

The development of different yerba mate extracts employing diverse extraction procedures has gained interest during the past years. However, some of them, such as stirring at room temperature, are time-consuming (Ballesteros et al., 2017), or either can have a detrimental effect on the extract properties as they involve the use of high temperature (boiling procedure). Therefore, environmentally friendly and fast extraction methods such as the assistance during extraction of high-intensity ultrasound, are being currently studied (Schenk et al., 2021; Tsaltaki et al., 2019). Extracts with high content of bioactive compounds can be obtained through these technologies; however, many of those compounds are sensitive to several environmental factors, such as light and oxygen, and need to be protected from degradation. Encapsulation is a suitable method to overcome this issue. This technique involves the entrapment of the substance that needs to be sheltered within another immiscible substance, which is the wall material that forms the protective coat (Vinceković et al., 2017). Among the different types of wall material, maltodextrin is an interesting option, as it is a low-cost, easily purchased hydrolyzed starch with high water solubility (Fenoglio et al., 2021; Pourashouri et al., 2014). On the other hand, this procedure may be a feasible strategy as it can mask, at least partially, the typical bitter taste of the yerba mate derivates, which makes its application suitable for food products intended to reach a wider range of consumers.

Some alternative technologies for food processing are being studied to totally or partially replace the traditional procedures such as thermal pasteurization which, despite its high microbial inactivation effectiveness, has been proved to significantly alter food quality. UV-C light is an interesting low-cost and safe preservation technology for the decontamination of food and beverages (McLeod et al., 2018). In the UV-C treated products, the microbial cell replication is impeded due to a photochemical reaction that promotes the occurrence of pyrimidine dimers in the DNA (Biancaniello et al., 2018). In turbid beverages, the low penetration depth is the main obstacle during the UV-C treatment as the material interference impedes the UV-C light from effectively reaching the microbial cells, and consequently hinders the disinfection process (Gök, 2021). Even though UV-C has already been approved by the US FDA (US FDA, 2019) for the decontamination of water and clear beverages, its use for the treatment of more complex systems such as turbid juices with suspended particles is currently being studied to expand its usefulness. UV-C effectiveness may be enhanced by the assistance of additional microbial stress factors to achieve the desired product safety while maintaining or even improving its nutritional, sensory and physicochemical quality. In the last decade, different combinations of UV-C light and emerging or conventional stress factors have been successfully applied, with the purpose of better interfering the homeostatic mechanisms of the microbial cells, compared to its single application. The assistance of UV-C light by heat has been widely studied as thermal treatments are commonly applied in the food industry (Guerrero et al., 2022). In general, mild temperature conditions ($\leq$ 50 °C) have been assessed to preserve the food quality detriment caused by severe thermal treatments (Riganakos et al., 2017). However, studies assessing the combination of UV-C and mild heat at pilot scale are scarce (Guerrero et al., 2022).

Although the demand for products enriched with plant-based bioactive compounds with potential health benefits has significantly increased in recent years, sensory studies related to the dynamic perception of herbal flavours have received little attention. This is a relevant aspect that should not be overlooked due to the presence of bitter and astringent notes, generally associated to this kind of product. Until now, no dynamic sensory profiling studies on food and beverages processed by UV-C and enriched with herbal extracts have been reported. It is important to highlight the high relevance of assessing different sensory aspects of those products loaded with herbal extracts along intake as their perception can vary with repeated tasting or sips (Orjuela-Palacio et al., 2014). In addition, there has been no research on the physicochemical properties nor on the sensory aspects of fruit juices treated by UV-C assisted by mild heat on a pilot scale.

The main objective of this work was to develop a functional orange-tangerine juice blend (1:1) processed by pilot-scale UV-C light assisted by mild heat (50 °C) and loaded with encapsulated or not, YM extract. A challenge study was performed to evaluate the inactivation of *Escherichia coli* ATCC 25922, *Lactiplantibacillus plantarum* ATCC 8014 and *Saccharomyces cerevisiae* KE 162 in the UV-C treated

juice blend. Polyphenol content, total antioxidant activity, colour, turbidity, and °Brix were measured along 20-day refrigerated storage (4 °C). Consumer willingness to try was studied. A dynamic sensory profile was also conducted to assess the influence of the YM extract encapsulation on juice perception.

Materials and Methods

Orange-Tangerine Juice Elaboration

For the pilot scale experiments, an orange-tangerine (OT) juice blend (12.4 ± 0.2 °Brix) was prepared by mixing and diluting equal parts (1:1) of frozen concentrated industrial orange and tangerine juices without any additive (ECA Agroindustrias S.A., Concordia, Argentina, and Tradecos S.A., Buenos Aires, Argentina) with tap sterile water (water/juice ratio = 5/1, 65.0 ± 0.1 °Brix).

For the triangle test, fresh oranges (*Citrus sinsensis*, var. Valencia) and tangerines (*Citrus reticulate*, var. Okitsu) were purchased from a local market and sanitized by immersion in 0.02% hypochlorite for 2 min. Afterwards, the fruits were rinsed with sterile water and patted dry with a sterile cloth. The fruit juice was aseptically extracted using a gastronomic squeezer (ANION, Buenos Aires, Argentina) and centrifuged at 1200 rpm for 10 min at 20 °C to reduce pulp content. Finally, the orange and tangerine juices were mixed in 1:1 proportion.

Yerba Mate Extracts

Aged canchada yerba mate leaves (*Ilex paraguariensis, St Hill*) were gently provided by the industrial establishment *La Cachuera* (Apóstoles, Misiones, Argentina). The leaves (20 g) were coarsely ground, mixed with 150 mL of ethanol in a double-wall cylindrical vessel (600 mL) connected to a thermostatically controlled water bath (HAAKE, Model Rotovisco RV12, Germany) and were twice-sonicated (750 W, 20 kHz, 95.2 µm, 25 °C, 10 min) using a high-intensity ultrasonic processor (Vibracell, Sonic Materials Inc., Newtown, CT, USA), as described in Schenk et al. (2021). The extracts were vacuum filtered (pore: 0.45 µm), and the solvent was eliminated at low-pressure conditions (50 °C, 40 min) in a rotary evaporator (Senco, Shanghai, China). Finally, the extract was re-suspended in 50 mL of distilled water and lyophilized (Stokes model 21, Philadelphia, USA) during 24 h to obtain the non-encapsulated YM extract (E).

For the encapsulation, the dried extract E was mixed with maltodextrin (MD) under constant agitation (1 h; 35 °C; dark) in the 2:1 proportion (MD:E) until it was fully homogenous, as described in detail in Fenoglio et al. (2021). Then, the suspension was lyophilized to obtain the dry encapsulated YM extract (EE). The extracts, which were fully analysed in Fenoglio et al. (2021), were characterized in this study by the measurement of some physicochemical, optical structural and stability properties such as moisture content (MC, % db- dry basis percentage, that is g water/100 g solids), water activity (a_w), colour parameters (L*,a*,b*) and total polyphenol content (TPC, mg GAE/mL = milligrams of gallic acid equivalents per mL of extract). E displayed MC = 9.2 ± 1.5% db; a_w = 0.05; L* = 39.1 ± 1.8, a* = -8.8 ± 0.1, b* = 28.2 ± 0.9; TPC = 57.0 ± 1.2 mg GAE/g pure extract; whereas EE showed MC = 4.0 ± 0. %db; a_w = 0.07; L* = 46.1 ± 1.1, a* = -7.9 ± 0.9, b* = 27.3 ± 1.3; TPC = 87.6 ± 2.1 mg GAE/g pure extract (Fenoglio et al., 2021).

Strains and Preparation of Inocula

The challenge studies were performed using *Escherichia coli* ATCC 25922, which has been determined as an adequate surrogate for the pathogen strain *Escherichia coli* O157:H7 (Kim & Harrison, 2009; Sauer & Moraru, 2009), *Saccharomyces cerevisiae* KE 162 and *Lactiplantibacillus plantarum* ATCC 8014. The yeast was chosen as it represents one of the main spoilage agents in fruit juices, due to its tolerance to ethanol, which is the main product of the fermentation initiated by yeasts contamination (Paniagua-Martínez et al., 2018). Additionally, *L. plantarum* is an acid-tolerant bacteria that produces an undesired off-flavour and the swelling of packages (Emamifar et al., 2011).

E. coli, L. plantarum, and *S. cerevisiae* were stored as fresh stock cultures, at 4 °C on Trypticase Soy Agar plus 0.6% (w/w) Yeast Extract (TSAYE), de Man Rogosa and Sharpe Agar (MRSA) and Sabouraud agar (SAB), respectively. Each medium was purchased by Biokar Diagnostics, Beauvais, France. Before each assay, *E. coli, L. plantarum*, and *S. cerevisiae* were grown up to the stationary phase in 20 mL of TSBYE, MRS and SAB broths, respectively and incubated at 37 °C for 24 h *(E. coli* and *L. plantarum)* and 27 °C *(S. cerevisiae).* As a large quantity of inoculum was required for each pilot scale assay, the grown suspension was poured into a larger Erlenmeyer flask containing 980 mL of TSA, SAB or MRS broth, and incubated for additional 24 h. All inocula were harvested by centrifugation (157 g, 10 min) (Labnet, USA), washed twice with peptone water and re-suspended in 100 mL of peptone water to obtain a cell density of 10^6–10^7 CFU mL^{-1}.

UV-C Treatments

The self-developed UV-C unit used for these experiments was described in detail in Fenoglio et al. (2020). Briefly, the pilot-scale UV-C reactor consisted of a stainless-steel chamber inside of which, twelve UV-C lamps (PhillipsT8/36 W; UV-C lamp-efficiency: 41.7%) were disposed surrounding

a fluorinated ethylene propylene (FEP) coiled tube (UV-C transparency: 0.98–0.99; length: 13.9 m; diameter: 19 mm). The UV-C unit (Total input power = 432 W; Total UV-C output = 176.4 W; UV-C energy = 1.67 kJ/ L/pass; Incident UV-C fluence = 390 mJ/ cm^2) also consisted of a power and control board, a centrifugal pump, two stainless steel 50-L tanks, one of which worked as the feed tank and was provided with a mixer, a heating jacket and a temperature sensor. The entire unit was assembled on a skim. The juice was recirculated through the reactor at 380 L/h. The Reynolds number was 3824; thus, the US FDA recommended turbulent flow regime (Re > 2000) was achieved. The Dean number was 833 and the ratio between the diameter tube (D) and the coil (D_c) was 0.05, which guaranteed a proper mixing by the development of secondary vortices as stated in Koutchma (2009). A radiometer probe was placed on the UV-C lamps to determine the emitted UV-C dose as described in Fenoglio et al. (2020). For the estimation of the delivered UV-C dose the method proposed by Rahn Rhan (1997), which involved the use of iodide/iodate chemical actinometer, was adapted to continuous flow and recirculation mode (Antonio-Gutiérrez et al., 2019; Char et al., 2010). The actinometrical solution (20 L) was treated in the UV-C device following the same procedure and conditions applied for the juice treatment. Samples were taken at pre-set intervals between 0 and 19 min, and the absorbance of those aliquots was measured at 353 nm to determine the concentration of the moles of the triiodide ion formed (RahnRhan, 1997).

Before starting the UV-C or UV-C/H treatments, the unit was adequately sanitized using a safe disinfecting solution of Benzalkonium chloride (1%v/$v_{\text{tap water}}$) suitable for food processing devices (Bio Bac, SEIG, Buenos Aires, Argentina), and properly rinsed with sterile water. The UV-C lamps were stabilized by keeping them on during 15 min before processing. Then, the juice blend was poured into the feed tank under continuous gentle agitation. In the case of the UV-C/H treatment, the desired temperature (50 ± 1 °C) was set in the control board and the heating jacket was turned on, the sensor inside the tank monitored the temperature to keep it constant throughout the whole experiment. Once the desired temperature was reached, the inoculum (10^6–10^7) was poured into the juice, under gentle agitation and was recirculated through the reactor for 2 min with the lamps turned off to ensure full homogenization. Immediately after, the lamps were turned on and the juice was treated by consecutive passes. Juice samples were collected at preset intervals between 0 and 19 min (0–390 mJ/cm^2) for the enumeration of survivors. Time setting conditions were selected based on previous studies in which the process was optimized to achieve a high microbial inactivation while compromising, as little as possible, the overall quality of the juice (Fenoglio et al., 2020).

Addition of the Yerba Mate Extracts

The untreated (control, C) and processed by UV-C/H juice samples were immediately loaded with 0.4 g of E or 1.2 g of EE per 100 mL of juice. The YM extracts were added after the UV-C/H treatment to avoid an increase in juice turbidity, which would impede light transmission during the disinfection process. In the case of EE, three times the weight of E were added to load the samples with the same amount of extract as two-thirds (2/3) of the total EE weight consisted of MD, to make proper comparisons among the juice samples. Therefore, six different samples were obtained: control juice without extract load (C), control juice loaded with non-encapsulated extract (C + E) or with encapsulated extract (C + EE), UV-C/H treated juice without extract load (UV-C/H), UV-C/H treated juice with non-encapsulated (UV-C/H + E) or with encapsulated (UV-C/H + EE) extract load.

Microbial Enumeration

Each sample was serially diluted in 0.1% (w/v) peptone water and surface plated by duplicate onto TSAYE for *E. coli,* MRS for *L. plantarum* and Sabouraud Agar for the yeasts using a spiral plater (Autoplate 4000, Spiral Biotech, Norwood, Massachusetts, USA). When treatment resulted in low counts (longer treatment times), 1 mL of sample was directly pour plated into each Petri dish. Plates were incubated for 48 h at 37 °C for *E. coli* and *L. plantarum* and 72 hs at 27 °C for *S. cerevisiae*. Colonies were counted using a grid in the case of spiral plating. Experimental data was displayed as Log N/N_0 (where *N* is the number of CFU/mL at a given time and N_0 is the initial number of CFU/mL) versus treatment time.

Physicochemical Determinations

The physicochemical determinations described in this section were performed by triplicate on the above-mentioned systems: C, C + E, C + EE, UV-C/H, UV-C/H + E, and UV-C/H + EE immediately after the UV-C treatment and/or the extract loading, and, along 20 days of storage under refrigeration (4 ± 1 °C). In addition, the total polyphenol content (TPC) and total antioxidant activity (TAA) of the YM extracts (E and EE) were determined.

A turbidimeter (LaMotte 2020we, Chestertown, Maryland, USA) was used to measure turbidity in the juice samples using AMCO Primary Turbidity (100 NTU) and Formazin standards (4000 NTU).

°Brix were measured with a hand-held refractometer (PR-101 Palette, ATAGO Co. LTD, Japan).

The colour of the juice samples was measured with a handheld tristimulus reflectance Spectrocolorimeter

(Minolta Co. Model CM-508-d, Osaka, Japan) by using a 1.4-measuring aperture with white and black backgrounds. Three millilitres of each sample were poured into a glass-bottom cylindrical vessel (2.5 cm internal diameter × 1 cm height) provided with an opaque wall and black or white background were measured setting the following conditions: illuminant C and 2° standard observer angle. The standards provided by the manufacturer were used to calibrate the instrument before each measurement. The CIE colour coordinates (X, Y, Z) and the components of the CIELAB space, L* (lightness), a* (green–red), and b* (blue-yellow) were recorded.

Different dilutions of the juice blend were prepared and the absorbance at 254 nm ($A_{254\ nm}$) was determined using 1-cm light-path quartz cuvettes in a UV–VIS spectrophotometer (V-630, Jasco, Tokio, Japan). The slope of the regression line obtained by plotting absorbance vs. sample concentration (%v/v) was considered as the absorption coefficient. This coefficient does not correspond to the molar absorptivity, since sample concentration is not expressed as mol/L (Oteiza et al., 2010).

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Singleton et al., 1999). A calibration curve of Gallic acid (ranging from 0.0025 to 0.125 mg/mL, Biopack, Buenos Aires, Argentina) was prepared. Sample concentrations were determined from the calibration curve ($y = 13.2465x + 0.0518$, $R^2 = 0.99$) and expressed as mg of Gallic acid equivalents per millilitre of the sample, GAE/mL, or per gram of pure extract, GAE/g. Briefly, 0.5 mL of juices samples or 0.5 mg of YM extract was 100 times diluted in distilled water and mixed with 2.5 mL of Folin-Ciocalteu phenol reagent (10%v/v, Biopack, Buenos Aires, Argentina), followed by 2 mL of Na_2CO_3 (7.5% w/v, Biopack, Buenos Aires, Argentina) addition. The reactive mixture was allowed to stand for 3 h in darkness, and the formation of blue colour, as indicator of TPC, was quantified at 740 nm using a UV–VIS spectrophotometer (V-630, Jasco, Tokyo, Japan).

The TAA of the juice samples was evaluated using a colorimetric method based on the free radical scavenging sample capacity, which was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical (Merck, Billerica, MA USA) according to the methodology proposed by Gutiérrez et al. (2015). Eighty microliters of sample diluted 1500 times with methanol was mixed with 2200 µL of DPPH (0.02 g/L in methanol). The reaction mixture was led to stand for 30 min at 25 °C in the dark, and absorbance was measured at 515 nm using a UV–VIS spectrophotometer (V-630, Jasco, Tokyo, Japan). A calibration curve was obtained using Trolox (Merck, Billerica, MA USA) as a standard, and the results determined from the calibration curve ($y = 0.00084$x-0.0024, $R^2 = 0.98$), and expressed as Trolox equivalents (mg TroloxEq/ mL).

Sensory Studies

More recently, the popularity of the consumer profiling approaches has increased for the evaluation of new products. According to this trend, the willingness to try of the proposed product was virtually evaluated (Spartano & Grasso, 2021). Additionally, a triangle test was performed to determine differences between E and EE. Finally, a temporal check-all-that-apply (T-CATA) question and a consumer field test were performed with a panel of consumers who tasted consecutive sips of the samples (UV-C/H, UV-C/H + E, and UV-C/H + EE) to assess the influence of extract encapsulation. For the tests, the panelists carried out their evaluations in individual booths under white light according to the ISO 8589 (ISO, 1988).

Willingness to Try Measurement

With the purpose of evaluating the consumers´ reaction to a non-traditional juice, a group of unpaid consumers of fruit juices (1039; 50.1% women, 49.9% men) was consulted through an online survey programmed and distributed by Google Forms about their *willingness to try* an orange-tangerine juice loaded with YM extract was determined, as described in Ares et al. (2009) and Spartano and Grasso (2021). The following claim was presented to the participants: *100% natural juice, with no preservatives nor sugar addition, rich in natural antioxidants, without severe thermal processing*. They were asked to score their willingness to try it using a probability 7-point scale, labelled on the left with ‘‘I would definitely not try it”, on the middle with ‘‘Maybe yes, maybe not” and on the right with ‘‘I would definitely try it” (Ares et al., 2009).

Triangle Test

Triangle test. A triangle test was carried out according to ISO 4120:2004 to evaluate if HPP treatments cause detectable sensory modifications on Alheira. Testing was made with 56 participants (60.7% male; 21 to 68 years old). Each panelist performed nine triangle tests, corresponding to the nine P ∗ t combinations in one single session, with 15 min of interval between the fifth and the sixth tests. Each test was composed of one piece for each of the triangle points, anonymously coded. Samples from the three production batches were mixed in the tests. The order of presentationof samples with different treatments was randomly defined. Spring water and unsalted crackers were available for mouth cleansing Triangle test. A triangle test was carried out according to ISO 4120:2004 to evaluate if HPP treatments cause detectable sensory modifications on Alheira. Testing was made with 56 participants (60.7% male; 21 to 68 years old). Each panelist performed nine

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A triangle test was performed with 60 (37 women, 23 men, aged 25–65 years old) frequent consumers of fruit juices to evaluate if panelists were able to detect differences between the encapsulated and non-encapsulated YM extracts that were incorporated to the juice. All the panelists received 3 red plastic cups containing 15 mL of the orange-tangerine juice loaded with the YM extract and the encapsulated YM extract, served at a temperature of consumption (7 °C).

Two of the samples were duplicate items and one was different. The order of presentation was balanced and the different sets of samples were randomly distributed among the panelists following the procedure describe in the ISO 4120:2021 (Sinkinson, 2017). Panelists were asked to choose the odd sample.

T-CATA and Consumer Field Test

A hundred and thirty unpaid volunteers (59 women and 71 men) recruited from personnel and students of Buenos Aires University, aged between 18 and 45 that were frequent consumers of fruit juices (consumption frequency: at least once a week), participated in the sensory test. Each panelist evaluated 15-mL of juice sample treated with UV-C/H, UV-C/H + E and UV-C/H + EE, answered the questionnaire, and repeated the intake two additional times. The samples were presented to the panelists at common serving temperature (5–7 °C) in white plastic cups, and water was available for rinsing between sips. For each sample, consumers firstly determined the overall acceptability and some relevant sensory attributes included in the consumer field test. Finally, they performed the T-CATA questionnaire, evaluating which attributes mentioned in the form were suitable for describing the different samples after each sip.

The consumer field test form was designed according to the general recommendations stated by Lawless and Heymann (2010) for this type of test. Firstly, all the panelists were instructed to judge the general acceptability of each sample on a 9-point hedonic scale (1-dislike extremely; 9-like extremely) which was immediately followed by open-ended questions for liking or disliking with an appropriate "skip pattern". The skip pattern dropped to reasons for liking if the response was positive, and then probed any dislikes, and vice versa. Additionally, some juice attributes were evaluated in 5-point acceptability and/or adequacy scales. Fruity taste, sour, herbal aroma, and juice body/viscosity were evaluated using just-about-right (JAR) scales. Previously to each session, each scale and all the terminology corresponding to the questionnaire were explained several times until panelists fully understood. Additionally, some demographic data regarding gender, frequency of fruit/vegetable consumption per day, intake of *maté* tea per week, physical activity, and age was requested to better characterise the consumers profile and enrich the statistical analysis.

Regarding the T-CATA question, all the panelists answered a questionnaire consisting of sensory descriptors, which were generated in a preliminary round table session. The selected sensory descriptors were: *weak*

colour, strong colour, natural taste, sweet, intense taste, artificial taste, refreshing, herbal taste, herbal aroma, fruity, not fruity, with aftertaste/taste persistence, pleasant juice body, bitter, sour, pleasant aroma, healthy. It was explained to the consumers that they had to taste three sips of the product, without drinking water in between sips to imitate the real way of consuming a juice, and immediately after each intake they had to choose as many terms mentioned in the checklist as they considered appropriate to describe each sip of the sample they were trying (Lawless, 2013). Therefore, the panelists tried 3 sips of 2 different samples, being the elapsed time between sips of 5 min. The T-CATA counts were totalled for each sip of a corresponding sample, and the resulting contingency table was used in subsequent analyses.

Statistical Analysis

A two-way analysis of variance (ANOVA) was used to evaluate the significance of the microbial strain and treatment effects on the log reductions achieved after the applied UV-C/H treatments. The significance level was set at $p < 0.05$. In case of finding significant differences, Tukey's test was performed. Homoscedasticity and normality assumptions were verified and outliers were removed, if necessary, from data set.

An analysis of covariance (ANCOVA) was applied to investigate differences in the *willingness to try* the product proposed, using *gender*, *frequency of fruit/vegetable consumption per day*, *intake of maté tea per week*, *physical activity*, and *age* as co-variables.

A multivariate analysis of variance (MANOVA) was performed to detect significant differences in the scores assigned by the panelists in the consumer field test, according to the independent factors "*treatment*" and "*sip*" and their interaction "*treatment*sip*", as described in Vásquez-Mazo et al. (2019).

Additionally, a principal component analysis (PCA) was performed to assess the relationship among the physicochemical parameters TPC, TAA, turbidity, °Brix and colour functions; L*, a*, b* of control and treated samples along storage (treatment/time). The Cophenetic Correlation Coefficient (*CCC*) was obtained as a measure of how faithfully the PCA analysis preserved the original Euclidean distances among data points. An adequate PCA analysis corresponded to a *CCC* value close to 1.0 (Everitt & Hothorn, 2011).

A correspondence analysis (CA), based on chi-square distances, was performed on the frequency tables for each sip and juice blend to obtain a bi-dimensional representation of samples and terms.

Statistical analyses were performed using InfoStat 2009 software (InfoStat Group, FCA-UNC, Córdoba, Argentina).

Results and Discussion

Microbial Challenge Tests

The log reductions of *E. coli, L. plantarum* and *S. cerevisiae* achieved in the juice blend samples processed by UV-C and UV-C/H are illustrated in Fig. 1. The use of mild heat (H, 50 ºC), as single treatment of the orange-tangerine juice blend, scarcely reduced less than 1 log-reduction the different assayed microbial populations (data not shown). *S. cerevisiae*, *L. plantarum*, and *E. coli*, were reduced by 1.6 or 4.9, 2.4, or 5.2 and 3.8 or 5.8 log cycles after single UV-C or UV-C/H treatments, respectively. Thus, synergistic inactivation effects were determined for the inactivation of all the microorganisms assayed. *E. coli* was the most sensitive one to UV-C and UV-C/H treatments, followed by *L. plantarum* and *S. cerevisiae* ($p < 0.05$, Fig. 1).

Gouma et al. (2020) evaluated the effect of UV-C (3.9 J/mL) assisted by mild heat (50 and 60 °C) for treating carrot juice (pH: 6.5) in an annular lab-scale reactor. In agreement, they concluded that for *E. coli* O157:H7 a synergistic effect occurred between the mild heat, at 50 °C or 60 °C, and the

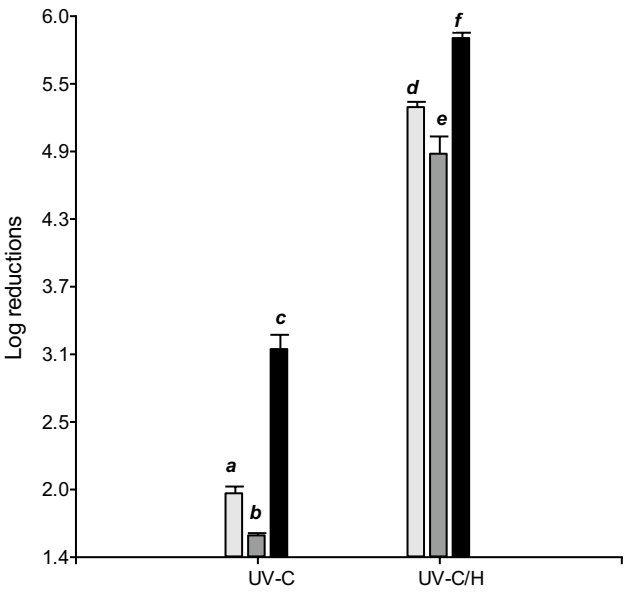

Fig. 1 Log reductions of *E. coli* ATCC 25922 (■), *L. plantarum* ATCC 8014 (■) and *S. cerevisiae* KE 162 (■) achieved in an orange-tangerine juice blend processed by single UV-C (390 mJ/cm^2, 20 °C) or assisted by mild heat (50 °C; UV-C/H). Different letters above the bars indicate statistically significant differences ($p < 0.05$) by two-way ANOVA. Standard deviation (I)

UV-C treatments, achieving a total inactivation of 4.3 and 5.3 log cycles of the pathogen, respectively. The yeast and moulds, total aerobics and acid lactic bacteria were inactivated by 4.5, 4.0, and 2.5 total log-reductions, respectively. On the same trend, García Carrillo et al. (2020) studied the inactivation of *Candida parapsilosis* ATCC 22019 in a turbid carrot-orange (pH: 3.8, 10.60 °Brix) juice processed by laminar thin-flow lab-scale UV-C (1720 mJ/cm^2) assisted by mild heat (50 °C) under laminar flow. The combined treatment highly inactivated the yeast population, achieving up to 5.5 log reduction, while single UV-C and heat treatments only reached 2.9 and 3.9 log reductions, respectively. In accordance with the present results, these authors also observed a more-than-additive effect in microbial inactivation. La Cava and Sgroppo (2019) compared the effect of single UV-C (39.6 J/L, laboratory-scale Teflon-coiled reactor), mild heat (65 °C) and their combination in the inactivation of total aerobic bacteria and yeast and moulds in grapefruit juice (pH 3.12, °Brix 9.6, 2500 NTU). Single UV-C led to 4.10 and 4.08 log reductions, while single heat decreased the loads by 2.28 and 2.24 log for total aerobic bacteria and yeast and moulds, respectively. They also found that when applying a consecutive combination of mild heat with UV-C, 4.99 and 5.24 log reductions were achieved, while the simultaneous treatment led to a total elimination of the microbes, which indicated that the order of the hurdles influenced the combined treatment efficacy. In this study, as the simultaneous treatment induced a complete microbial inactivation, it could not be concluded whether additive, more-than additive or synergistic effects were observed. As to pilot-scale experiences, Fenoglio et al. (2020) worked in a pilot-scale Dean flow UV-C reactor, with a coiled FEP tube, in turbulent flow conditions. They applied simultaneous mild heat (50 °C) and UV-C (390 mJ/cm^2) for the inactivation of *E. coli* ATCC 25922, *L. plantarum* ATCC 8014 and *S. cerevisiae* KE 162 in a turbid commercial orange-banana-mango-kiwi-strawberry, pH:3.7, 13.9 ºBrix) juice blend. They observed that mild heat (50 °C) used as single factor induced 1.0–1.3 microbial log reductions; whereas, single UV-C led up to 1.3, 3.6 and 3.7 log reductions of *S. cerevisiae*, *L. plantarum* and *E. coli*. Therefore, more than additive to synergistic effects were found when the UV-C treatment was assisted by H, as 4.6, 6.4 and 6.3 log-reductions were reported for *S. cerevisiae*, *E. coli*, and *L. plantarum* in the juice. In general, synergistic effects were found when mild heat was applied but the combination of hurdles became less effective at higher temperature. This was evaluated by Gayán et al. (2013) who assessed UV-C (0–27.1 J/mL) combined with heat in the range of 25 to 62.5 °C for the inactivation of *E. coli* STCC 4201 in apple juice (UV absorption coefficient: 25.54 cm^{-1}) to find the optimum treatment. The authors concluded that below 40 °C, the mild heat barely affected the microbial inactivation, but from 50 to 55 °C a synergistic effect was achieved between the UV and the temperature, which decreased at higher temperatures. The decrease in synergy above 55 °C could be attributed to the fact that thermal inactivation displays higher heat dependence than the UV inactivation. Therefore, at high temperatures, as thermal lethality increases faster (due to significant changes in the functionality and structure of the microbial cell) than UV-C lethality, the contribution of UV-C to microbial inactivation is almost negligible (Gayán et al., 2013).

Study of Some Physicochemical Parameters Along Storage

With the purpose of examining the influence of the treatments, the addition of the yerba mate extract and the effect of encapsulation, some physicochemical parameters were measured immediately after processing and along 20 days of refrigerated storage (4 ± 1 °C).

Antioxidant Activity and Polyphenol Content Evaluation

Figure 2 illustrates TPC and TAA values corresponding to untreated orange-tangerine juice (C), juice with YM extract (C + E), juice with encapsulated YM extract (C + EE), and processed by UV-C/H, UV-C/H + E and UV-C/H + EE treatments during 20-day refrigerated storage (4 ± 1 °C).

As to the YM extracts, the TPC values corresponding to E and EE were 57.0 and 87.6 mg GAE/g of dried extract powder, respectively. In reference to the treatment effect, UV-C/H (TPC: 0.028 ± 0.003 mg GAE/mL), did not modify the TPC of the untreated (C) juice sample (TPC: 0.028 ± 0.001 mg GAE/mL). On the other hand, the UV-C/H + EE treated juice samples were 2.1 times greater in TPC (0.059 ± 0.002 mg GAE/mL) compared to the C samples immediately after treatment. Whereas, the UV-C/H + E samples displayed a slightly lower increase in TPC (0.045 ± 0.020 mg GAE/mL) than the C samples.

With regard to the effect of UV-C/H on the TAA of the processed samples, the proposed treatment did not alter the activity of these biocompounds (TAA: 3.5 ± 0.6 mg Trolox Eq/mL) compared to C samples (3.9 ± 0.2 mg Trolox Eq/mL). Moreover, the UV-C/H + EE treated juice samples were 1.6 times greater in TAA (6.3 ± 0.2 mg Trolox Eq/mL) compared to the C sample immediately after treatment. Similarly, UV-C/H + E displayed a TAA value of (7.1 ± 0.3 mg Trolox Eq/mL).

In conclusion, the YM extract addition significantly increased the content of bioactive compounds in the UV-C/H treated juice blend. No significant differences were observed between C and UV-C/H treatments throughout storage for TPC and TAA values ($p < 0.05$). Additionally, the UV-C/H + E and UV-C/H + EE samples did not

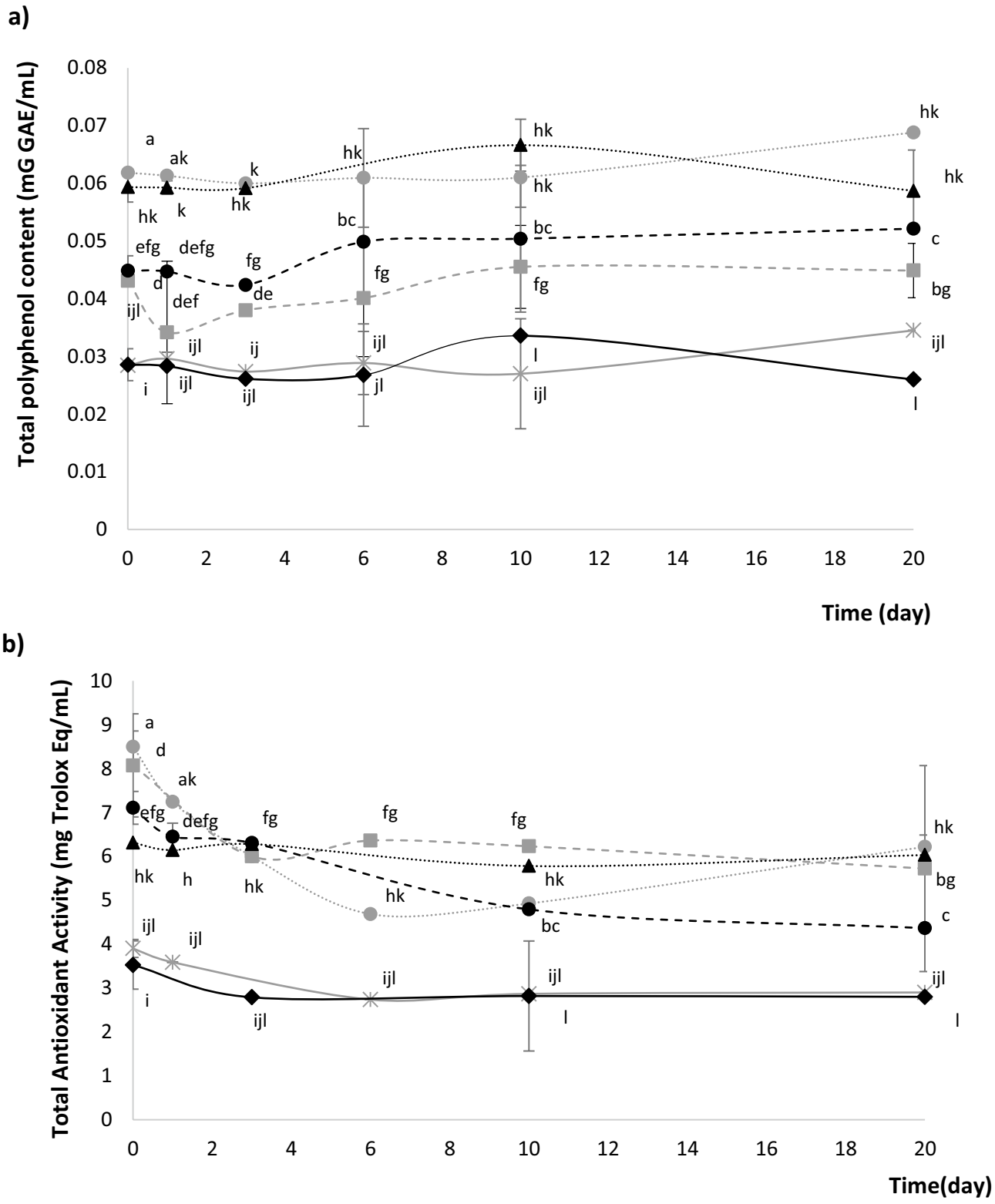

Fig. 2 Total polyphenol content (TPC, mg GAE Eq/mL) (**a**), antioxidant activity (TAA, mg Trolox Eq/mL) (**b**) determined in C (—✳—), C + E (- - ■ - -), C + EE (····●····), UV-C/H (—◆—), UV-C/H + E (- - ● - -) and UV-C/H + EE (····▲····) along storage at 4 ± 1 °C. Symbols and lines in grey were used for C samples (C; C + E; C + EE), symbols and lines in black were used for UV-C/H samples (UV-C/H, UV-C/H + E, UV-C + EE). Different letters above the bars indicate statistically significant differences (p <0.05) by two-way MANOVA. Standard deviation (I)

exhibit changes in TPC and TAA values along storage compared to day 0, thus remaining significantly higher than C or UV-C/H samples (p <0.05) (Fig. 2a, b). Consequently, the encapsulation of the extract prevented TPC from degradation during lyophilisation and storage. These results indicate that the consumption of the proposed herbal beverage may significantly contribute to the overall polyphenol and antioxidant intake with potentially beneficial biological effects for human health.

In agreement with the results reported in literature, the polyphenol content and antioxidant activity remained unaltered considering that the used temperature that assisted the UV treatment was lower than 55 °C. Ferrario et al. (2018) reported no changes in the total polyphenol content and antioxidant activity of a carrot-orange juice treated by an annular thin-flow UV-C reactor combined with mild heat (985 mJ/cm^2, 50 ºC). Similarly, Sew et al. (2014) observed no changes in the polyphenol content of pineapple juice subjected to UV-C assisted by mild heat (7.55 mJ/cm^2; 50 ºC). Outstandingly, Lemoine et al. (2010) applied a benchtop UV-C treatment (800 mJ/cm^2, 30-cm distance from lamp) to broccoli florets, and observed an increase of 10%, 13%, and 14% in total polyphenol content, total antioxidant activity and ascorbic acid, respectively. On the contrary, when UV-C was performed at temperatures equal or higher than 55 °C, a decrease in the bioactive compounds content was observed. For instance, La Cava and Sgroppo (2019) evaluated the effect of UV-C assisted by heat (39,600 mJ/L, 65 ºC) in a coiled-tube reactor for the treatment of grapefruit juice (9.6 °Brix, absorption coefficient ($\propto$): 49.5 cm^{-1}). They reported retention values of 86%, 84%, and 37% for total polyphenol content, antioxidant activity, and ascorbic acid, respectively. Similarly, a decrease of 17.62% in the antioxidant activity of a freshly squeezed apple juice after a UV-C (annular thin-film laboratory-scale reactor) treatment combined with mild heat (27.1 J/mL–55 °C) was detected by Gayán et al. (2013). As to the application of herbal extracts and their encapsulation, Savaghebi et al. (2021) encapsulated *Sargassum boveanum* Algae extract in nano-liposomes and used it to enrich a mayonnaise. The authors found that after four months of storage at room temperature, the samples that contained the encapsulated extracts displayed a lower lipid oxidation than the control samples. On the other hand, Baeza et al. (2018) determined the polyphenol content in some commonly consumed beverages in South America, like *maté* tea and chamomile tea. These authors determined that a cup (200 mL) of chamomile tea contained 11.2 mg GAE, while a cup of *maté* tea contained between 135.02 and 154.45 mg GAE, depending on the analysed commercial yerba mate brand. According to the results obtained in the present study, a cup (200 mL) of UV-C/H + E and of UV-C/H + EE treated juice would contain a total of 9 and 11.8 mg GAE, respectively. Therefore, the polyphenol contribution of a glass of the proposed functional juice blend would be similar to that one of chamomile tea.

Colour, °Brix, and Turbidity Evaluation

With regard to the colour assessment of the juice samples, no significant differences (p <0.05) were recorded for the juice colour parameters immediately after processing and throughout the refrigerated storage between C (L*: 43.2 ± 0.25, a*: −2.5 ± 0.1, b*: 40.8 ± 0.6) and the UV-C/H treated (L*: 42.9 ± 0.5, a*: −2.4 ± 0.3, b*: 39.9 ± 3.8) samples, which indicated that the treatment did not alter the colour parameter values. Moreover, the UV-C/H + E (L*: 44.1 ± 0.2, a*: -3.0 ± 0.4, b*: 39.7 ± 0.6) and UV-C/H + EE (L*: 44.7 ± 0.5, a*: −3.0 ± 0.1, b*: 40.8 ± 2.2) samples displayed a decrease in a* value with respect to C, immediately after YM extract addition, thus turning samples greener and less red. Additionally, a slight increase in L* was recorded when the encapsulated extract was added to the juice, probably attributed to the presence of maltodextrin. Accordingly, the controls C + E (L*: 43.39 ± 0.3, a*: −3.1 ± 0.01, b*: 42.2 ± 0.9) and C + EE (L*: 43.1 ± 0.2, a*: −3.1 ± 0.1, b*: 42.0 ± 0.6) were greener than the C sample. The colour parameters of all the samples remained unaltered throughout storage.

No differences in the °Brix values were observed between C and all the treated samples, which ranged from 12.6 to 13.1 °Brix. Moreover, these values did not change throughout storage. The turbidity was significantly increased due to the YM extract addition in the UV-C/H + E (6681 ± 986 NTU) compared to the C (3533 ± 229 NTU) samples. The increase in turbidity was more noticeable when the incorporated YM extract was encapsulated (UV-C/H + EE: 7416 ± 917 NTU), probably because of the presence of maltodextrin in this extract. Similarly, the C + E and C + EE samples showed turbidity values of 6209 ± 313 NTU and 7158 ± 37 NTU, respectively, which remained constant along 20-day refrigerated storage.

A PCA bi-plot was performed with the purpose of assessing the relationship between TPC, TAA, turbidity, °Brix and colour functions; L*, a*, b* of control and treated samples along storage (treatment/time). The two-dimensional representation is displayed in Fig. 3. The *CCC* value was 0.95, which means that an accurate dimension reduction was achieved with the analysis. The first two principal components (PC_1 and PC_2) explained the 80.0% of the total variance. The PC_1 separated L*, PC, and TAA which were associated positively, from a*, which was negatively associated. On the other hand, PC_2 was associated positively with °Brix and b*. This analysis showed that C and UV-C/H samples were associated to the highest a* and the lowest PC, TAA

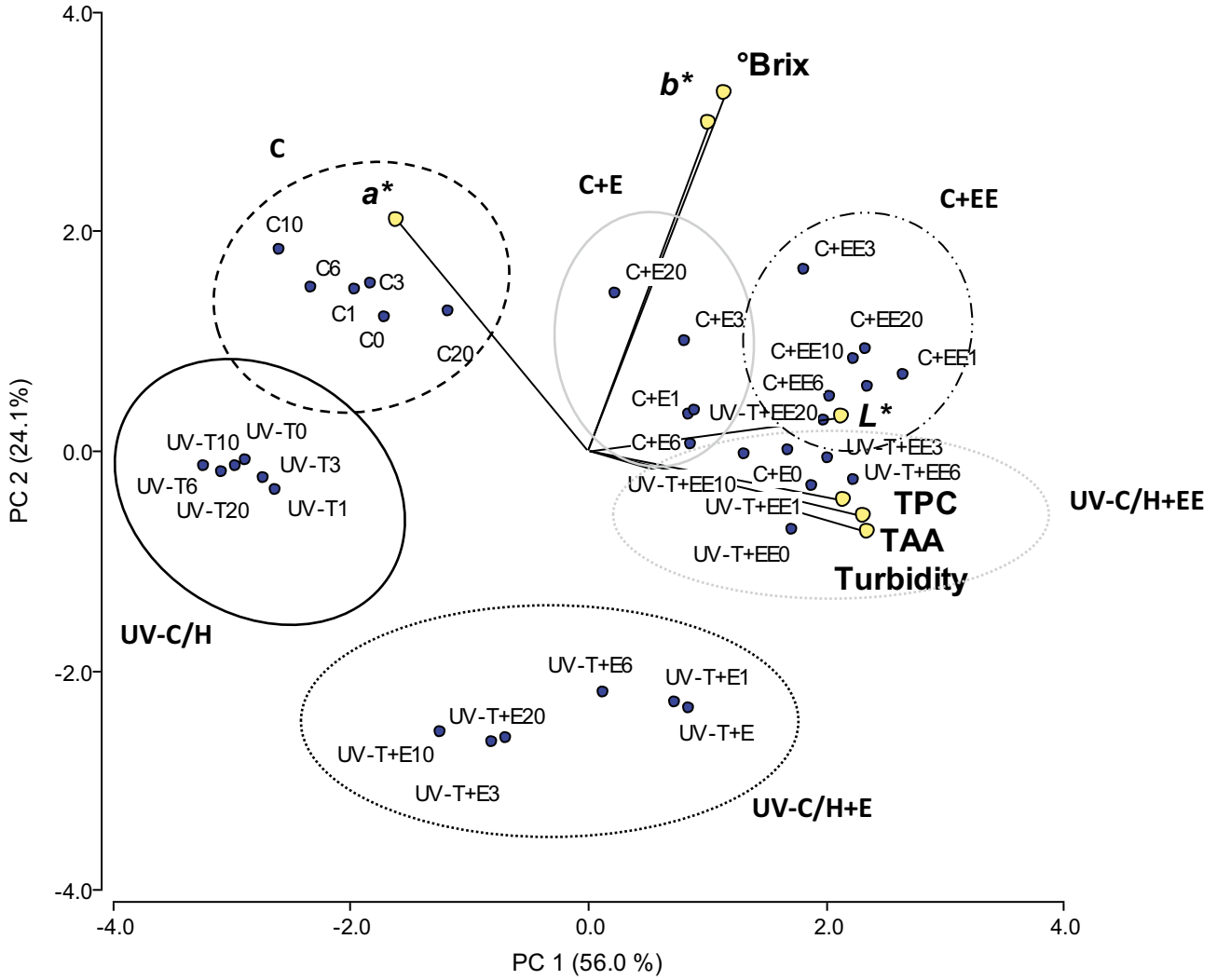

Fig. 3 Principal component analysis (PCA) bi-plot corresponding to the evolution of pH, turbidity, °Brix, colour functions, total polyphenol content, total antioxidant activity in the C, C + E, C + EE, UV-C/H, UV-C/H + E, and UV-C/H + EE samples along 20-day refrigerated (4 ± 1 °C). The number in the sample indicates the day of storage. The plotted ellipses indicate experimental data grouped by sample

and turbidity. In contrast, C + EE and UV-C/H + EE displayed the highest TPC, TAA, and turbidity and the lowest a* value. UV-C/H + E and C + E samples showed intermediate values of all the parameters. Therefore, the addition of the extract promoted an important rise in TPC and TAA values and increased the total number of suspended particles. This increase was higher when the YM was encapsulated as maltodextrin prevented YM bioactive compounds from degradation during the freeze-drying process. Moreover, the addition of YM provoked a colour shift in the samples, from red to green. An increase in L* was also observed in the case of the samples loaded with EE, compared to the C + E and UV-C/H + E samples, due to the presence of maltodextrin.

There is significant evidence that the effect of UV-C combined with other technologies scarcely affects or does not modify the °Brix, colour, and turbidity values of treated samples. For instance, Gouma et al. (2020) assessed the effect of UV-C (3.9 J/mL, annular thin-film lab-scale device) assisted by mild heat (60 °C) on the °Brix values of a carrot juice (pH: 6.5). They did not observe differences between the control and the treated samples. Gayán et al. (2013) determined that some physicochemical characteristics (°Brix, pH, and titratable acidity) of an apple juice remained constant after 3.58 min of UV-C (27.J/mL, annular thin-film lab-scale device) assisted by mild heat (55 °C). Similarly, Kaya and Unluturk (2015) examined the °Brix, turbidity, and colour values of a melon-lemon juice treated by a continuous-flow annular UV (205 mJ/cm^2) reactor combined with heat (72 °C). These authors reported a slight decrease on the °Brix and b* values of the treated samples, probably due to the

high temperatures applied which may have degraded the carotenoids. With regards to the addition of herbal extracts to juices, Ferrario et al. (2018) developed a carrot-orange juice blend processed in lab-scale UV-C unit under laminar flow (1060 mJ/cm^2) and subsequently loaded with a non-encapsulated yerba mate extract. They also reported that the UV-C treatment did not induce changes in °Brix, colour, and turbidity values compared to the control samples, and observed an increase in the turbidity and a decrease in the a* values of the juice enriched with the yerba mate extract.

Sensory Studies

Triangle Test

A triangle test was performed to evaluate if consumers were able to discriminate two juice samples loaded with the non-encapsulated or the encapsulated YM extracts. A significant difference between the YM extracts was determined as the number of correct responses (29) was higher than the critical number (28) expected for 60 panelists with a p value of 0.05.

Willingness to Try the Product

The *willingness to try* the orange-tangerine juice blend loaded with YM extract was assessed as previously explained in the "Materials and Methods" section. W*illingness to try* averaged 6.0 ± 1.3 points in a 7-point scale, close to the category "I would definitely try it".

The ANCOVA revealed that *willingness to try* perception was significantly higher when the consulted consumers declared to eat more than 2 or 3 daily servings of fruits (willingness to try: 6.05 ± 1.45), compared to the ones who only consumed 1 or less daily servings (willingness to try: 5.55 ± 1.66). Moreover, consumers of *maté* daily exhibited significantly higher *willingness to try* (6.10 ± 1.20) than non-consumers (5.85 ± 1.56). No differences regarding gender, age or frequency of practicing physical activity were observed for the *willingness to try* ($p > 0.05$). In contrast, Ares et al. (2009) observed gender differences in the perception of a functional yogurt. Compared to men, women perceived it healthier and were more prone to try it. García Carrillo et al. (2020) evaluated the influence of the product information provided to the consumers before trying a carrot-orange juice processed by UV-C (1720 mJ/cm^2) assisted by mild heat (50 °C) on a laboratory scale. The authors determined that the willingness to try was close to the "I would definitely try it" category. They also reported that women expressed a higher willingness to try the product than men.

Consumer Field Test

A dynamic consumer field test was applied to determine if there were changes in the overall liking and adequacy of some relevant attributes of UV-C/H + E and UV-C/H + EE treated juice samples as the intake progressed. For this purpose, the consumption of three consecutive sips of the samples were evaluated. Additionally, a three-way MANOVA was performed, which revealed that, in the case of cluster 2, no differences were determined, independently from the considered sample (UV-C/H + E or UV-C/H + EE) or sip (1, 2, or 3) (Table 1). These findings could be attributed to the fact that the panelists encompassing cluster 2 were frequent consumers of yerba *maté*, as a significant increase in the willingness to try the product was observed among daily consumers of yerba *maté* compared to those who did not consume this infusion at all (Statistical Analysis). Thus,

Table 1 Mean values and standard deviations corresponding to the overall liking, and JAR evaluation of sour, herbal aroma juice body/viscosity and fruity taste of the UV-C/H + E and UV-C/H + EE samples during three consecutive sips (S1, S2, and S3)

Sample	Cluster	Sip	Overall liking	Sour	Herbal aroma	Juice body/viscosity	Fruity taste
UV-C/H + E	1	1	3.62 ± 1.35^{a}	3.17 ± 0.96^{a}	3.14 ± 0.98^{a}	2.47 ± 0.98^{a}	2.63 ± 0.60^{a}
UV-C/H + E	1	2	3.79 ± 0.87^{bc}	3.24 ± 0.97^{bc}	2.99 ± 0.88^{bc}	2.99 ± 1.97^{bc}	2.64 ± 0.87^{bc}
UV-C/H + E	1	3	3.94 ± 0.60^{ab}	3.28 ± 0.95^{ab}	3.06 ± 0.80^{ab}	2.57 ± 0.86^{ab}	2.64 ± 0.64^{ab}
UV-C/H + EE	1	1	3.54 ± 0.54^{c}	3.11 ± 0.72^{c}	2.98 ± 0.79^{c}	3.06 ± 0.83^{c}	2.59 ± 0.76^{c}
UV-C/H + EE	1	2	3.58 ± 0.80^{c}	3.22 ± 1.02^{c}	3.04 ± 1.15^{c}	3.18 ± 0.94^{c}	2.75 ± 0.90^{c}
UV-C/H + EE	1	3	3.67 ± 0.77^{c}	3.27 ± 1.05^{c}	2.84 ± 0.76^{c}	3.07 ± 0.80^{c}	2.76 ± 0.84^{c}
UV-C/H + EE	2	1	6.81 ± 0.35^{d}	3.00 ± 0.63^{d}	2.81 ± 0.97^{d}	2.98 ± 0.86^{d}	2.73 ± 0.98^{d}
UV-C/H + EE	2	2	6.62 ± 0.72^{d}	3.00 ± 1.01^{d}	2.78 ± 1.01^{d}	2.92 ± 0.87^{d}	2.77 ± 0.88^{d}
UV-C/H + EE	2	3	7.33 ± 1.01^{d}	2.50 ± 0.74^{d}	2.83 ± 0.74^{d}	2.86 ± 0.53^{d}	2.74 ± 0.81^{d}
UV-C/H + E	2	1	6.97 ± 1.13^{d}	2.58 ± 0.87^{d}	2.78 ± 0.87^{d}	2.83 ± 0.81^{d}	2.53 ± 0.91^{d}
UV-C/H + E	2	2	7.10 ± 0.68^{d}	2.54 ± 1.01^{d}	2.80 ± 0.76^{d}	2.90 ± 0.87^{d}	2.68 ± 0.87^{d}
UV-C/H + E	2	3	7.33 ± 0.44^{d}	2.50 ± 0.61^{d}	2.83 ± 0.80^{d}	2.86 ± 0.78^{d}	2.74 ± 0.81^{d}

Different superscript letters in each column represent significant differences according to the three-way MANOVA test

the proposed product would be specifically targeted to this group of consumers of yerba *maté* tea, whose juice attribute perception did not vary along juice intake or with the type or yerba mate extract that was added to the juice.

In the case of cluster 1, the same behaviour was observed only in the case of the UV-CH + EE samples (probably since the encapsulation could have reduced the perception of certain yerba flavour notes that vary throughout consumption) while significant differences were found in the perception of the herbal aroma of the UV-C/H + E samples between sip 1 (3.14) and 2 (2.99). Significant differences were recorded by the consumers of this cluster between the UV-C/H + E (herbal aroma: 2.99–3.14, juice body: 2.47–2.99) and UV-C/H + EE samples as they perceived lower herbal aroma (2.84–3.04) and higher juice body (2.59–2.76) when the yerba mate extract was encapsulated (UV-C/H + EE).

Despite all the samples averaged the category "*neither like or dislike*" when a cluster analysis was applied to obtain a segmentation of the consumer groups' preferences, two clusters emerged for the UV-C/H + E and UV-C/H + EE samples. The cluster 1 (C1), with 79 and 42 consumers, for UV-C/H + E and UV-C/H + EE respectively, encompassed the categories 1 to 4 in the 9-point hedonic scale, and the cluster 2 (C2), with 37 and 42 consumers, for UV-C/H + E and UV-C/H + EE, respectively, included the higher categories, from 5 to 9 points (data not shown). The analysis had an adequate fit, as a *CCC* of 0.80 was obtained. The cluster that exhibited a marked interest in the product (C2) scored an averaged overall liking of 7.0, 7.1 and 7.3 or 7.0, 7.0, and 7.0 in the 9-point hedonic scale (which corresponded to the category "like it moderate") for UV-C/H + E or UV-C/H + EE after each sip, respectively. Thus, a slight increase in the overall liking throughout the intake was only observed when the extract was not encapsulated, suggesting that the panelists got used to the bitter taste of yerba mate after consuming the juice repeatedly. Likewise, Orjuela-Palacio et al. (2014) examined the effect of replicate sessions to measure consumer´s acceptability of a yerba mate infusion blended with black currant (50:30) with 15% maltodextrin and 5% sucrose. They observed that the overall acceptability of the samples significantly increased from 5.0 to 5.5 after the fourth session, suggesting that the panelists got used to the unusual taste of the product.

Gómez-Mascaraque et al. (2017) examined the overall impression of biscuits loaded with encapsulated green tea extract using gelatine. In agreement with the present study, these authors reported no significant differences in the liking values of the biscuits elaborated with encapsulated and non-encapsulated green tea extract. In contrast, Pasrija et al. (2015) evaluated the overall acceptability of bread loaded with green tea extract encapsulated or not with maltodextrin, β-cyclodextrin, and a combination of both substances. They observed the lowest overall sensory scores in breads with non-encapsulated extract, and they attributed this difference to the presence of a slight tea taste, and astringency. As to the influence of the UV-C treatment on some sensory attributes, Gök (2021) assayed the overall acceptability of a single intake of apple and grape juices treated in a lab-scale, Dean-vortex based UV-C reactor (1668 and 1232 mJ/cm^2, respectively) and concluded that no significant differences were perceived between the control and the treated samples.

A principal component analysis (PCA) was performed to assess the relationship among the scores corresponding to the hedonic scale and the % of responses in *sour*, *herbal aroma*, *fruity taste*, and *juice body*. The two-dimensional representation (PCA bi-plot) is displayed in Fig. 4. The *CCC* value was 0.98, which indicates that an accurate dimension reduction was achieved with the analysis. The first two principal components explained the 84.4% of the total variance. The PC_1 was positively associated to sour and herbal aroma. On the other hand, PC_2 showed positive association with juice body and fruity taste. This analysis showed that UV-C/H + EE samples were associated to higher juice body and fruity taste than UV-C/H + E samples, suggesting that the presence of maltodextrin increased the viscosity of the juice while partially masking the bitter taste of yerba mate, which allowed to better perceive the fruity taste. On the other hand, all the samples corresponding to C2 were associated to higher hedonic scores and lower sour and herbal aroma than C1. This indicated that this group was interested in the product perceived sour (2.6–2.9) nearer to the just right point than the other group (sour: 3.3–3.3), and lower herbal aroma intensity (2.78–2.83) than C1 (herbal aroma: 2.84–3.14).

Orjuela-Palacio et al. (2014) evaluated the overall liking of a high-polyphenol yerba mate/black currant beverage, prepared by mixing ripe black currant berries juice with yerba mate extract obtained by a traditional extraction (100 °C, 15 min) (60:20), with or without the addition of sweeteners. In agreement with the present study, they observed that the acceptability was 5.6 when considering all the panelists. However, 57% of the consumers evaluated the beverage with scores in the range from 6 to 9 on the 9-point hedonic scale.

Temporal Check-All-That-Apply (T-CATA) Question

The most frequently used terms by the panelists to describe the UV-C/H + E and UV-C/H + EE treated juice samples were *herbal taste, strong colour, natural taste, intense taste*, *with aftertaste/taste persistence* and *bitter.* On the other hand, the least used terms were *weak colour, artificial taste*, *and sweet.*

A correspondence analysis (CA) was performed for the sensory descriptors used in this study, as explained in the "T-CATA and Consumer Field Test" section and displayed in Fig. 5. The first and second dimensions of the CA calculated from T-CATA counts represented 56.7% and 30.9% of the

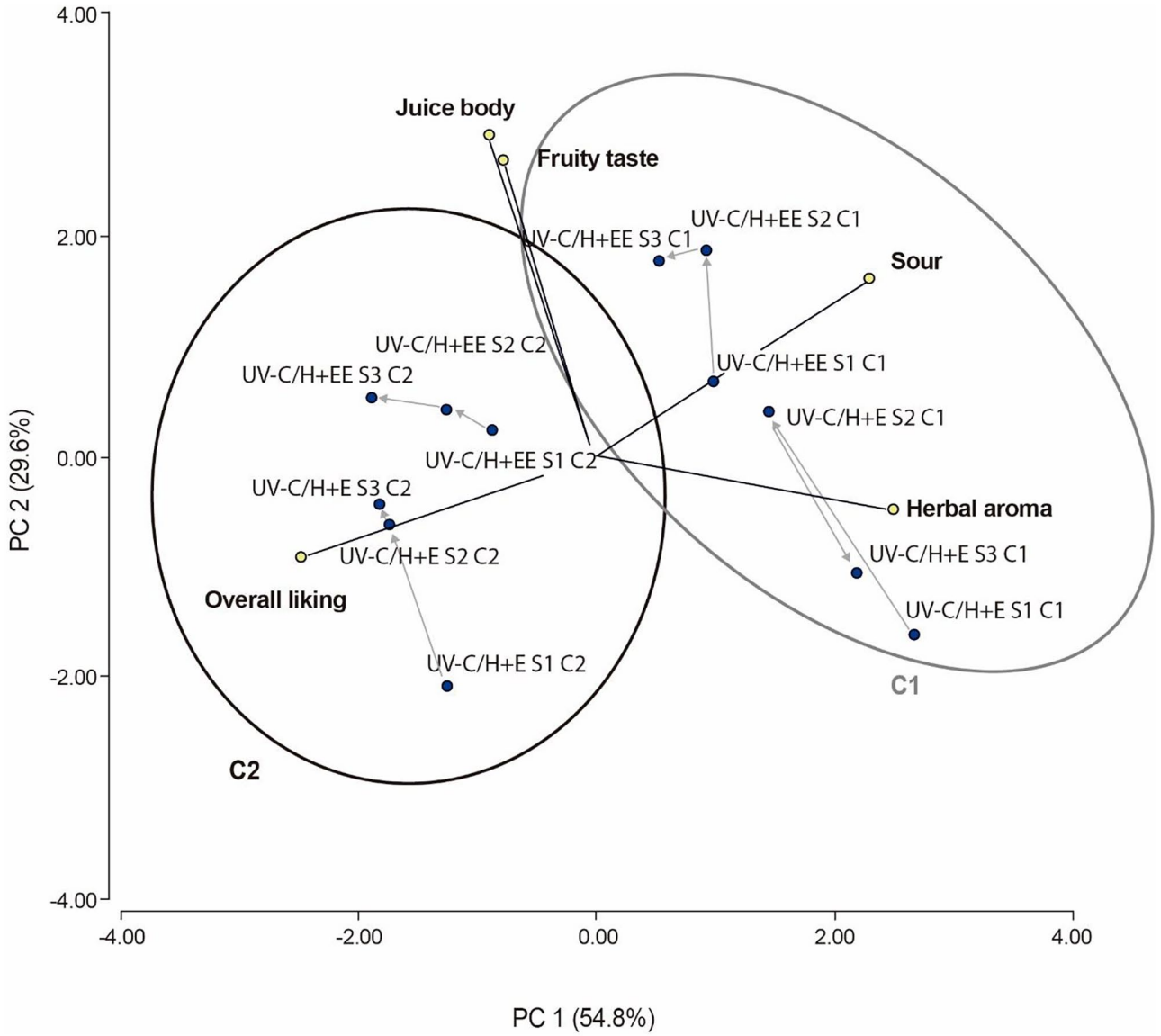

Fig. 4 Principal component analysis (PCA) bi-plot of scores assigned by the panelists in the overall impression test, herbal aroma, sour, fruity taste and juice body of the three consecutive sips (S1, S2 and S3) of the UV-C/H + E and UV-C/H + EE samples. The plotted circles encompass scores corresponding to clusters C1 and C2. The arrows indicate the perception trajectory with intake

experimental data variance, respectively. The UV-C/H + E juice samples exhibited negative values for axes 1 and 2 for the three sips, and were described by consumers as *healthy, with aftertaste/taste persistence*, *weak colour, fruity, herbal aroma, and sweet*. After the third sip, the UV-C/H + E sample was evaluated closer to the *sweet* and *herbal aroma* attributes compared to the previous sips, suggesting that the panel of consumers started to perceive a sweet taste that had been previously masked by a bitter taste, while the citation of the term *herbal aroma* became more relevant as consumption proceeded. Conversely, the UV-C/H + EE sample perception featured a scattered pattern, depending on the sip. In the bi-plot, the UV-C/H + EE sample/sip 1 was located close to all the UV-C/H + E samples, being described as *healthy, taste persistence*, *weak colour, fruity taste, herbal aroma*, *and sweet*. Otherwise, when evaluating the UV-C/H + EE sample, the sips 2 and 3 were associated to the attributes*: strong colour* and *intense taste or sour, pleasant body, bitterness,* and *pleasant aroma*, respectively. This shift suggested that the encapsulation procedure could have masked the bitter taste in the first sip, thus highlighting the *sweetness* perception. However, as the juice intake proceeded, consumers started to perceive the juice bitterness more intensely, probably due to the solubilization of maltodextrin in the water and/or saliva. Even though juice bitterness increased with sips,

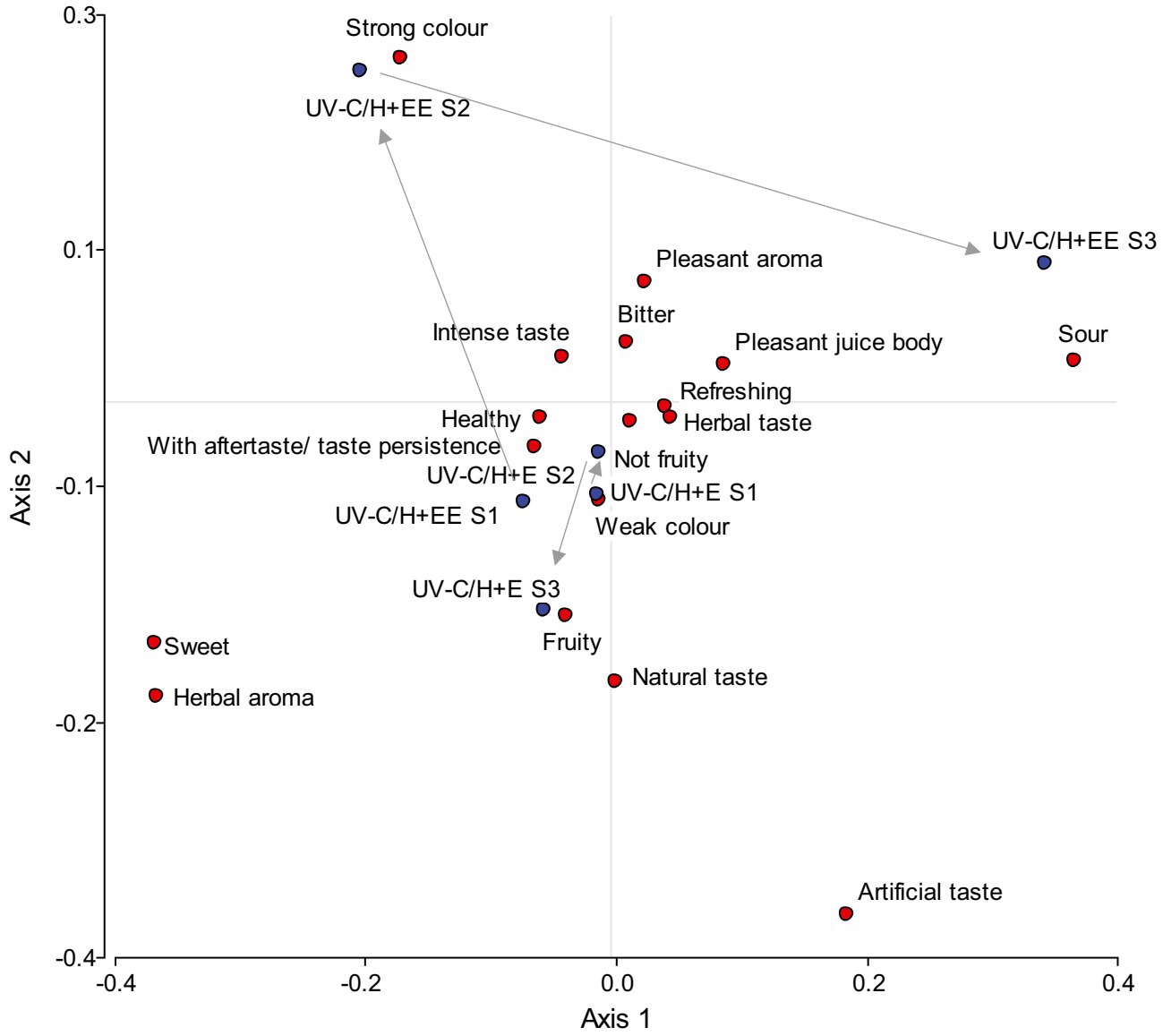

Fig. 5 Correspondence analysis bi-plot representing term citation frequency according to the T-CATA questionnaire for the sensory evaluation of three consecutive sips (S1, S2 and S3) of UV-C/H + E and UV-C/H + EE juice blend samples defined by consumers from a checklist included in the T-CATA question. The arrows indicate the perception trajectory with intake

frequent consumers of yerba *maté* tea expressed a strong interest in this product according to the results of the cluster analysis previously described.

Conclusions

The proposed functional orange-tangerine juice processed by UV-C light and assisted by mild heat at pilot-scale and loaded with yerba mate extract may represent a relevant contribution to the diary polyphenol and antioxidant intake. The use of assisted UV-C may represent a promising alternative to the processing of complex juices and blends as it has shown a high efficiency in the inactivation of some relevant microorganisms while well-preserving colour, turbidity, °Brix, polyphenol content, and antioxidant activity in the product. UV-C processing, conducted on a pilot scale, may also promote a rapid adoption of this technology by the food industry.

The addition of yerba mate induced a significant increase in total polyphenol content and total antioxidant activity of the juice blend, being higher when the extract was encapsulated. Yerba mate incorporation produced a slight change in colour of the juice, turning samples less red and greener. Moreover, a slight increase in turbidity was also observed. The segmentation analysis revealed that the herbal aroma

and bitterness of the product were appreciated by a group of consumers.

On the other hand, some sensory aspects have been studied along juice, which provides a more complete overview of the matter. This study evidenced that some attributes shifted as consumption proceeded: *sweet* and *herbal aroma* were perceived when consuming UV-C/H + E samples. Whereas, the UV-C/H + EE samples shifted from *fruity taste*, *herbal aroma* and *sweet* to *intense taste*, *bitterness* and *pleasant aroma.* Thus, the encapsulation could have masked the bitter taste and highlighted the sweetness in the first sip, but consumers started to perceive bitterness more intensely throughout the consumption of the juice.

Therefore, further studies should be conducted with the aim of improving the encapsulated extract performance, to better mask the bitterness perception of the yerba mate extract, which would contribute to expand its application to other matrixes. Additionally, new flavours combination should be tried to broaden the offer of enriched fruit juices.

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Data Availability The data and material presented in this study will be made available on reasonable request.

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