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Packaging effects on highly nutritional value beverage obtained by a mix of typical sicilian fruits in accelerated storage

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ABSTRACT

Consumers' knowledge has been improved and now they are aware of the importance of consuming healthy food. They know how food and beverages are linked with the proper functioning of their body, so are always more drawn towards consuming healthy products with high nutritional value. The new challenge for soft drink producers is the increasing interest in high nutritive quality products. Therefore such high-quality fruit juice is required to meet consumer needs, but also to ensure safety aspects in order to prolong shelf life. The aim of this research was to understand the most suitable packaging through experimental research to preserve over time the chemical-physical characteristics of a beverage with a high nutritional value obtained by mixing blood orange, pomegranate, and prickly pear juices. The three types of stand-up pouches tested were respectively transparent packaging (PT) (PET/OPA/PE), metalized packaging (PM) (PET/ALU/PET/PE), and totally recyclable packaging (PR) (OPP/OPP/PP). On stand-up pouches, weight loss (%), color intensity, browning index (BI), anthocyanin content (mg/L), total phenols, antioxidant activity (%), and flavonoids were monitored during storage at +45 °C, 65 % RH for accelerated ageing. Results have shown that PR performances were good, it kept high qualitative characteristics among the packaging tested it is the advisable best packaging solution because it certainly offers a valid alternative in terms of performance and a lower environmental impact compared to PM or to traditional packaging (glass), thus representing a more sustainable, effective and innovative choice.

1. Introduction

The national and international market of functional soft drinks is growing rapidly. Consumers have rediscovered traditional fruit-based drinks, finding in them the positive and healthy characteristics of fruit and their antioxidant effects (Yildiz, Pokhrel, Unluturk, & Barbosa-Cánovas, 2020). Nowadays they are part of a balanced diet as well as a healthy lifestyle as they comprise health elements such as vitamins, dietary fiber, flavonoids, minerals, and in particular calcium, potassium and magnesium (Slavin & Lloyd, 2012).

The European Parliament and the Council of the European Union act in accordance with the ordinary legislative procedure, in order to protect the interests of consumers and to improve the free movement of fruit juices and other food products within the Union. The directives 2001/112/CE and 2012/12 UE are specific regulations regarding production, composition and labeling of fruit juice-based products (D.2001/112/CE; C.D. 2012/12).

The highest juice quality is required to meet consumer needs, also juice safety aspects are important considerations for prolonging shelf life. Soft drinks can be found in several container formats, such as cans, glass bottles, as well as plastic bottles. Each one is available in different sizes, varying from small bottles up to large containers.

Consumers demand functionality, like blood-pressure-reducing and also cholesterol-lowering drinks, so the new challenge for soft drinks producers is the increasing interest in high nutritive quality products (Baker, Lu, Parrella, & Leggette, 2022). The demand for higher quality and freshness in fruit juices without additives or preservatives has encouraged the use of mild technology in juice processing. Soft drinks must satisfy different characteristics such as healthy lifestyle, tastes, nutritional requirements, and physiological constraints.

This project starts from the production of a High Nutritional value Beverage (HNB), based on a mix of blood orange (*Citrus sinensis*), pomegranate (*Punica granatum*) and prickly pear (*Opuntia ficus-indica*) juices.

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The healthy and nutritional properties of such fruit are well known. Blood oranges are considered a health-promoting food owing to the large number of bioactive compounds that characterize their nutritional composition (Licciardello, Arena, Rizzo, & Fallico, 2018). As well as blood oranges, also pomegranates are rich in anthocyanins, the natural pigments responsible for the red color of fruits and vegetables, which have demonstrated their activity against various kinds of illnesses (Prior & Wu, 2006; Wang & Stoner, 2008) because they act as potent antioxidants in the human body (Riso et al., 2005).

To preserve over time the chemical-physical characteristics of HNB is necessary to identify the most suitable packaging through experimental research.

The purpose of this study was to evaluate the shelf life and to compare the performance of three types of innovative packaging with different polymeric compositions in order to meet the goals set by the UN Agenda 2030 in terms of producing a soft drink able to induce good health as well as pointing at a substantial waste reduction.

The marketing choice for HNB packaging is toward new stand-up flexible pouch, against classic glass bottles used for beverages, appreciated for its oxygen barrier clarity and inertness, but nowadays they are often replaced by plastics choices, considering weak points as price, fragility and weight (Rizzo, Torri, Licciardello, Piergiovanni, & Muratore, 2014)

The three types of stand-up pouches used were: transparent packaging (PT) (formed by polyethylene terephthalate PET/oriented polyamide OPA/ polyethylene PE), which allows the consumer to see the product, metalized packaging (PM) (PET/aluminium ALU/PET/PE) highly printable and brand-able, and totally recyclable packaging (PR) (formed by oriented polypropylene OPP/OPP/ polypropylene PP). Materials were tested and characterized according to international standards and then physicochemical analysis, on stand-up pouches filled with HNB, as weight loss (%), total soluble solids content (TSS), pH, color intensity, browning index (BI), anthocyanin content, total phenols (TP), antioxidant activity (AA) and flavonoids were monitored.

2. Materials and methods

2.1. Packaging materials

Three types of stand-up pouches were considered for our trial, in particular: a transparent multilayer pouch (PET/OPA/PE) called (PT), which has the benefit to allow the consumer to see the product inside; a metalized pouch highly printable and brand-able realized in (PET/ALU/PET/PE) identified as (PM), and a totally recyclable pouch (OPP/OPP/PP) from here identified as (PR). All the pouches, purchased by Gualapack Spa (Castellazzo Bormida, AL, Italy) have an 8.6 mm spout and a theoretical volume/capacity of 200 milliliters (Table 1), while a clear glass bottle of 300 mL was used as control (C). Ten pouches of each material were tested to assess the tensile properties and puncture resistance test of plastic films following the standards UNI EN ISO 527–3 (2019) and EN 14477 (ASTM F1306) respectively.

2.2. Sample preparation

The HNB was produced by Citrech Snc (Messina, Italy) and CNR-ITM (Rende, CS, Italy) involved in the same research project, mixing 70% of blood orange juice not from concentrate (NFC), 20% of pomegranate juice NFC and 10% of prickly pear juice NFC. In our laboratory, the HNB (60° Brix) was blended with water, sugar (112 g/L) and citric acid (2.8 g/L) to reach a final concentration of 12° Brix; ingredients were added according to volumetric proportion in order to prepare enough HNB to pack 15 pouches for each material and 15 glass bottles. Pouches were manually full-filled using a graduated cylinder with HNB in order to leave 10 % of free headspace calculated on total volume (Table 1); pouches were prepared to carry out analysis in triplicate. The samples were stored in constant temperature and humidity equipment at + 45 °C and 65 % of relative humidity, using a climate chamber Climacell 111 (MMM Medcenter Einrichtungen GmbH, München) for 50 days to accelerate storage. All samples for each packaging solution were analyzed in triplicate to perform analysis every 10 days after the starting day for the succeeding 20, 30, 40 and 50 days.

All the reagents and solvents for the chemical determinations were purchased from Sigma-Aldrich (Milan, Italy) and were of analytical or HPLC grade. Bi-distilled water was used throughout this analytical trial.

2.3. Physicochemical analysis of high nutritional value beverage

2.3.1. Weight loss

Each pouch was labeled and weighed at the beginning of the storage time (T0). Then, three replicate pouches for each packaging materials were selected at each sampling time and weighed before opening the pouches for further analysis. Starting weight loss (WL) was expressed as a percentage of the initial sample weight at day 0.

2.3.2. Total soluble solids contents and pH

The total soluble solids (TSS) contents of HNB were obtained with an Abbe refractometer (Sper Scientific, Scottsdale, Arizona, USA) at 20 °C. One drop was placed on the refractometer glass prism, and the TSS was obtained as Brix percentage (distilled water was used as a blank). The pH of the solutions was measured with a digital benchtop ionometer XS model PH80 with a DHS electrode (XS Instruments, Carpi (MO), Italy) at 20 °C.

2.3.3. Color and browning index

Color intensity (Hue) and browning index (BI) were determined spectrophotometrically reading the absorbance values. In particular, two milliliters of each sample were pipetted into a cuvette. A dual beam UV–vis spectrophotometer was used to measure absorbance values at 520 nm (A520), which is the maximum absorbance of red color from monomeric anthocyanins (Vegara, Marti, Mena, Saura, & Valero, 2013).

Table 1
Main physicochemical characteristic from technical draw; (PT) transparent packaging; (PM) metallized packaging and (PR) recyclable packaging. Glass was used as control (C).

	Material	Color	Width x height (cm)	Capacity (mL)	Total weight (g)	Thickness (µm)	OTR cm^3/(m^2*24 h)	Permeation cm^3* µm/(m^2*24 h)	Permeance [g/(m^2*24 h*mmHg)]	Permeability [(g*mm)/(m^2*24 h*mmHg)]	Cap
PT	PET/OPA/PE	clear	9,5 × 16,3	200	6.43	120	8,0 ± 0,7	1122 ± 48,08	0,34 ± 0,21	0,05 ± 0,03	HDPE
PM	PET/ALU/PET/PE	silver	9,4 × 16,5	215	6.55	120	-	-	-	-	HDPE
PR	OPP/OPP/PP	green	9,3 × 16,4	200	6.00	120/110	9,75 ± 0,35	1462,5 ± 53,03	0,27 ± 0,180	0,04 ± 0,01	PP
C	GLASS	clear	5,3[a] x 21,2	285	-	-	-	-	-	-	CROWN

OTR: Oxygen Transmission Rate; [a] diameter

The absorbance of juice was also measured at 430 nm (A430) and the browning index (BI) was expressed as a ratio of A430/A520 as reported by Roidoung, Dolan, and Siddiq (2016).

2.3.4. Anthocyanin content

The total anthocyanin content was determined by pH differential method of Meyers, Watkins, Pritts, and Liu (2003). HNB was opportunely diluted separately with pH 1.0 and pH 4.5 buffers. The absorbance values of the solution were determined spectrophotometrically at both 520 and 700 nm using UV–visible 2401 spectrophotometer (Shimadzu Corp. Tokyo, Japan). Anthocyanin content (AC) was calculated as reported by Roidoung et al. (2016).

2.3.5. Determination of total polyphenols

Total phenols (TP) were determined according to the Folin-Ciocalteu spectrophotometric method as reported by Tezcan, Gültekin-Özgüven, Diken, Özcelik, and Erim (2009). Briefly 0.3 mL of diluted HBN was mixed with 1.5 mL of 10-fold-diluted Folin–Ciocalteu reagent and 1.2 mL of 7.5 % of sodium carbonate; after 90 min the absorbance was measured at 760 nm. by a using the same spectrophotometer above mentioned. TP content was expressed as g gallic acid equivalents per L of juice sample (g GAE L^{-1}) using gallic acid standard curves obtained from serial dilution from a concentrate starting solution (1000 mg L^{-1}) (r^2 =0.9807).

2.3.6. Determination of antioxidant activity

The antioxidant activity (AA) was measured according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity method, following the methodology reported by Kalantari, Roufegarinejad, Pirsa, Gharekhani, and Tabibiazar (2021). The reduction of the violet DPPH radical by the antioxidant compounds to pink was measured at 515 nm against a blank solution (with no extracts) with the same spectrophotometer described above. The percentage inhibition of DPPH radical was calculated according to Pandino et al. (2017).

2.3.7. Determination of total flavonoids

The flavonoid content was determined referring to Zhishen, Mengcheng, and Jianming (1999) protocol. In particular, 1 mL of juice and 4 mL of distilled water were mixed, after 5 min were added 0.3 mL of 5 % sodium nitrite solution ($NaNO_2$), after 0.3 mL of 10 % $AlCl_3$ were mixed and the solution was left to rest for 6 min. Then 2 mL of NaOH (1 M) were added and the solution was brought to constant volume with distilled water. After vigorous stirring the absorbance was read at 430 nm against control. A calibration curve of quercetin ranging from 50 to 1000 mg L^{-1} (r^2 =0.991) was prepared and results were expressed as mg of quercetin.

All chemical analyses were performed on HNB collected from each pouch at each sampling times, samples were vortexed and then an aliquot was filtered through a 0.45 mm Millipore nylon filter.

2.4. Statistical analysis

First of all, a principal component analysis (PCA) was run on the following variables °Brix, color parameters (Abs420, Abs520, Abs620), anthocyanin, antioxidant activity, BI, Flavonoids, Hue, TP, total sugars, weight loss %measuring the desired characteristics of samples. Then, a two-way (packaging × storage time) mixed analysis of variance ANOVA was performed for each dependent variable. Means were compared using Duncan's multiple range test ($P \leq 0.05$), Tukey test was performed as a post-hoc analysis. IBM SPSS Statistics 27 version, was used to perform statistical analysis of the data.

3. Results and discussion

3.1. Packaging materials

Stand-up pouches were characterized in terms of tensile properties and puncture resistance test as required by official tests (UNI EN ISO and ASTM), and the results achieved as the stress (σ), defined as the force per unit cross-sectional area of material, and strain (Ɛ), defined as the fractional change in length of the material, are reported in Tables 2 and 3. As can be seen from the stress-strain analyses (Fig. 1), the three compounds have different trends since the PM and PT materials have similar initial behavior in which an accentuated effort is required to produce a small elongation of less than 3 % followed by an elongation of the order of 80 % in the case of PM and from 70 % to 120 % in the case of PT (with a marked variability of the tests) due to an increase in stress of about 20 Mpa. As far as the PR sample is concerned, the graph shows a more regular trend with an elongation of the order of 30 % consequent to a stress of 80 Mpa which is greater than that observed both in PM and in PT. Fig. 2 are reported the puncture test curves, they underline that PM thanks to the presence of the aluminum layer (9 μm) has good resistance, reaching the same F max used for PR (13.9), even if it has a smaller thickness 113 μm against 143 μm of PR, while the PT has got the lower resistance to the test Fmax (11.6). Better mechanical characteristics, greater resistance to both deformation and perforation are useful in the use of pouches as the filling is often performed at hot temperature and under pressure thus ensuring a lower probability of damage (during the all commercialization process) and therefore greater safety for the final consumer.

3.2. Physicochemical results of high nutritional value beverage

The PCA revealed two components that had eigenvalues greater than one and which explained 57.0 % and 13.0 % of the total variance, respectively. (Fig. 3). Visual inspection of the scree plot and the interpretability criterion confirm that two components should be retained. The two-component solution explained 70 % of the total variance. The interpretation of the data was consistent with the analytics measurements designed to measure changes in the beverage, with mass loss (%), browning index (Abs 430/520), Hue, Brix°, sugars (g L^{-1}), anthocyanins (mg L^{-1}) and total phenols (mg L^{-1}) explained by the Component 1, while the color coordinates (Abs) by the Component 2, data not shown. (Component loadings and communalities of the rotated solution are available, they could be presented on request).

The effect of packaging materials, storage time, and their interactions on the measured parameters in the stand-up pouches are presented in Table 4.

The analysis of variance (Table 4) showed that pH and flavonoids were not statistically different for packaging, storage and neither their interaction; hue and TP were significant ($P < 0.001$) only for the storage variable; 'Packaging' significantly ($P < 0.001$) affected WL, B.I. and AA.

Table 2
Determination of tensile properties of the tested pouches (n = 10): (PT) transparent packaging, (PM) metallized packaging and (PR) recyclable packaging, where σ is the stress defined as the force per unit cross-sectional area of material (σ = F/A); Ɛ is the strain defined as the fractional change in length of the material. Values are mean ± standard deviation on 10 replicates, data followed by different letter are significantly different at $P \leq 0.05$.

	Thickness (μm)	σ max MPa	Ɛ (σ max) %	σ at break MPa	Ɛ at break %
PT	132 ± 6b	50.5 ± 2.9a	83.5 ± 11.2bc	39.5 ± 7.5a	94.6 ± 12.8c
PM	116 ± 3a	60.2 ± 2.5b	77.7 ± 10.2b	58.4 ± 2.1b	78.0 ± 10.1b
PR	145 ± 4bc	79.9 ± 7.4c	26.5 ± 4.8a	78.9 ± 7.4c	26.5 ± 4.8a

σ = stress; Ɛ = strain

Table 3
Determination of puncture test of the tested pouches (n = 10): (PT) transparent packaging, (PM) metallized packaging and (PR) recyclable packaging, where F is the force (N) applied; Ɛ is the strain defined as the fractional change in length of the material. Values are mean ± standard deviation on 10 replicates, data followed by different letter are significantly different at P ≤ 0.05.

	Thickness µm	F max N	Ɛ (Fmax) mm	F at break N	Ɛ at break mm	E_B mJ
PT	130 ± 0.3b	11.6 ± 0.4a	1.78 ± 0.10b	11.5 ± 0.42a	1.79 ± 0.08b	10.2 ± 1.1ab
PM	113 ± 3.7a	13.9 ± 0.2b	1.76 ± 0.05b	13.6 ± 0.50b	1.78 ± 0.06b	11.5 ± 0.4c
PR	143 ± 3c	13.9 ± 0.4b	1.70 ± 0.08a	13.3 ± 0.08b	1.70 ± 0.08a	9.7 ± 0.6a

Ɛ = strain; F = force; E_B = elongation at break

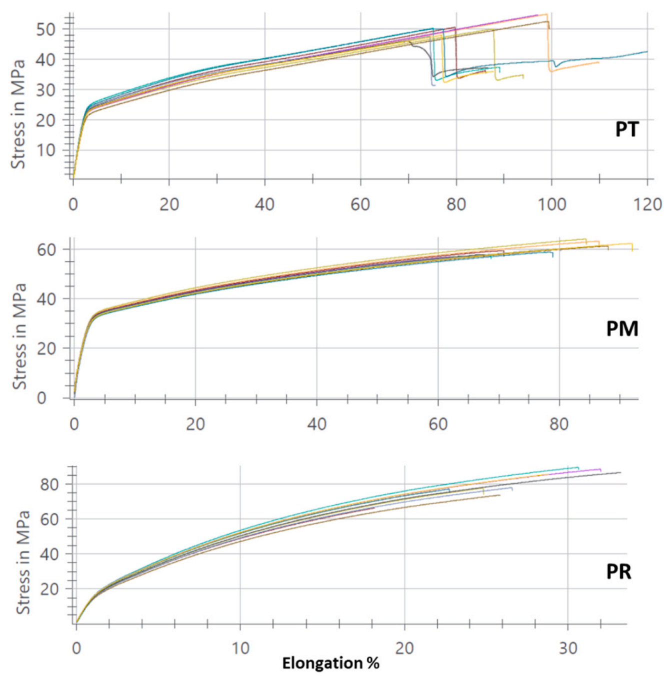

Fig. 1. Results of stress/strain curves obtained on (PT) transparent packaging, (PM) metallized packaging and (PR) recyclable packaging (UNI EN ISO 527–3:2019).

3.2.1. Weight loss

Weight loss (%) is influenced by packaging, storage and their interaction (Table 4); considering changes along the storage time, as expected, it increased and the PM had the lowest change thanks to the presence of the aluminum layer in its structure, so it has high barrier properties against the external environment. The PR performance was in the middle through the PM and the PT, which had the highest WL (%), even if the final results are around 0.3 % (Fig. 3).

3.2.2. Total soluble solids contents and pH

The starting value of TSS in HNB samples was 8 ± 1.84°Brix, and it was stable in the first 10 days for PR, while it significantly increased ($p < 0.001$) from 10 to 50 days of storage among PT, PM and C in all packaging and during storage. As expected control showed the highest increase, the value of PM was slightly changing during storage, while PT and PR behavior was similar (Fig. 4); trends observed in the different materials were statistical different (Table 4). Changes observed in all packaging suggest that this could be explained by the growth of microorganisms during the storage time, and so, as a consequence, linked with the production of organic acids and other metabolites. In this

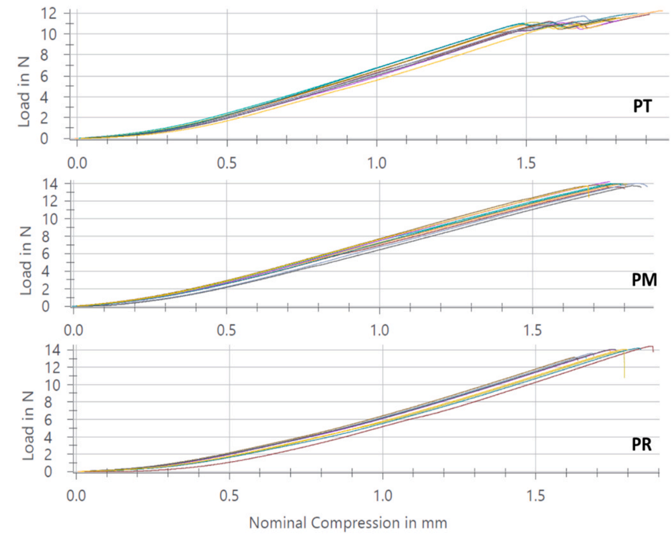

Fig. 2. Results of puncture resistance test according to EN 14477, ASTM F1306. in (PT) transparent packaging; (PM) metallized packaging and (PR) recyclable packaging.

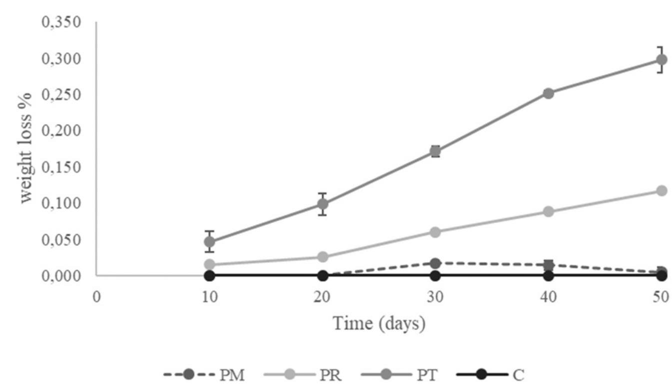

Fig. 3. Weight loss (%) calculated at 10, 20, 30, 40, and 50 days of storage on stand-up pouches in (PT) transparent packaging; (PM) metallized packaging and (PR) recyclable packaging. Glass was used as control (C) as affected by 'packaging×storage time' interaction. Bars indicate the standard deviation of the mean.

regard, Kalantari et al. (2021) and Alighourchi and Barzegar (2009) have reported the same results.

The pH is an effective parameter in the quality properties of fruit juice, however, remembering the addition of citric acid in the HNB formulation, no significant difference was observed among the different pouch samples neither during storage time from the values on the first day. Overall, all pH values of the juice samples, with no significant changes, were within the standard range (between 2.96 and 2.99) slightly lower than those reported by the previous studies (Kalantari et al., 2021; Alighourchi & Barzegar, 2009; Vegara et al., 2013).

3.2.3. Color and browning index

In the present study, the change in color during storage was characterized through Hue and browning index (BI) ratio. Packaging and storage conditions and their interaction (PxS) have a significant influence on the BI, while Hue was significantly different ($P < 0.001$) only for the time of storage of HNB. BI represents color changes from reddish to brownish and its increase is not acceptable, since it means brownish (A430) is predominant over reddish shades (Fig. 5). It's noteworthy how all materials follow the same path during storage, with a small difference after 30 days for PM; the medium value of increasing in all materials is

Table 4
Qualitative traits of High Nutritional value Beverage (HNB) packaged in (PT) transparent packaging; (PM) metallized packaging and (PR) recyclable packaging as affected by main factors. Glass was used as control (C). Different letters within the same parameter and main factor show significant differences (LSD test, P ≤ 0.05).

Main factor	Qualitative traits W L (%)	°Brix	pH	Hue	BI	Anthocyanins (mg/L)	Total Phenols (gGAE/L)	AA (%)	Flavonoids (mg/L)
PT	0.173 ± 0.012c	11.09 ± 1.41c	2.97	1.61	1.41 ± 0.15 a	2.70 ± 1.73 b	3.27	92.65 ± 1.73 b	1.73
PM	0.007 ± 0.003 a	10.86 ± 1.26 b	2.97	1.55	1.32 ± 0.05c	4.64 ± 2.51 a	3.3	91.064 ± 0.92 ab	1.81
PR	0.062 ± 0.009 b	10.57 ± 1.76 a	2.99	1.57	1.37 ± 0.07 ab	4.10 ± 1.91 ab	3.3	91.42 ± 2.21 ab	1.82
C	0.000 ± 0.000 d	11.57 ± 1.84 d	2.96	1.56	1.35 ± 0.01 bc	3.81 ± 2.07 ab	3.08	89.971 ± 2.40 a	1.84
Storage time (d)									
10	0.015 ± 0.006 a	10.11 ± 0.125 a	2.99	1.314 ± 0.04 a	1.14 ± 0.11 a	6.17 ± 3.3 a	2.6 ± 1.7 a	91.48 ± 1.85 ab	1.68
20	0.033 ± 0.009 b	11.10 ± 0.125 b	2.97	1.403 ± 0.51 a	1.23 ± 0.15 b	4.33 ± 2.06 ab	2.7 ± 0.5 a	91.30 ± 1.83 ab	1.79
30	0.062 ± 0.007c	11.08 ± 0.175 b	2.96	1.572 ± 0.51 b	1.32 ± 0.12c	4.58 ± 1.64 ab	3.5 ± 1.0 b	91.23 ± 3.12 ab	1.83
40	0.088 ± 0.004 d	11.15 ± 0.125 b	2.97	1.693 ± 0.42 b	1.45 ± 0.21 d	2.57 ± 2.80 bc	3.6 ± 2.0 b	90.20 ± 0.68 a	1.88
50	0.105 ± 0.014 e	11.67 ± 0.187c	2.98	1.903 ± 0.71c	1.65 ± 0.31 e	1.42 ± 2.07c	3.8 ± 0.7 b	92.16 ± 1.60 b	1.81
PACKAGING (P)	***	***	NS	NS	***	*	NS	***	NS
STORAGE TIME (S)	***	***	NS	***	***	***	***	*	NS
(P) X (S)	***	***	NS	NS	***	***	NS	***	NS

Note: WL: weight loss; B.I.: Browning Index; AA: Antioxidant Activity. ***, ** and * indicate significant at $P \leq 0.001$, $P \leq 0.01$ and $P \leq 0.05$, and NS, not significant.

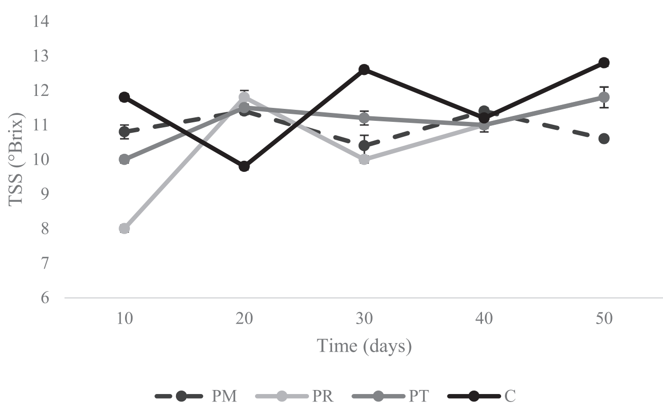

Fig. 4. Total soluble solids measured at 10, 20, 30, 40, and 50 days of storage on stand-up pouches in (PT) transparent packaging; (PM) metallized packaging and (PR) recyclable packaging. Glass was used as control (C) as affected by 'packaging×storage time' interaction. Bars indicate the standard deviation of the mean.

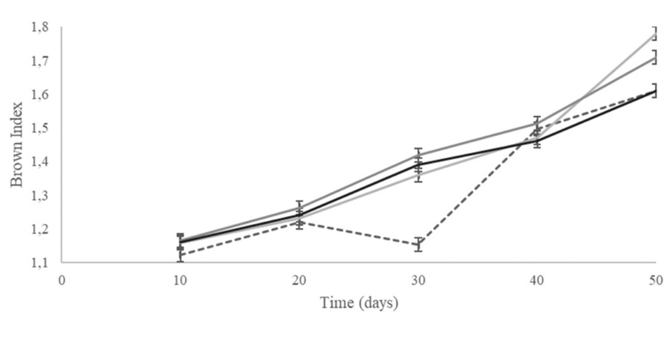

Fig. 5. Browning Index (B.I.) kinetics on High Nutritional value Beverage (HNB) packaged for 50 days in stand-up pouches (PT) transparent packaging; (PM) metallized packaging and (PR) recyclable packaging. Glass was used as control (C).

0.53 ± 0.07.

As said previously, the measurement of the red color in the beverage samples, is linked with the presence of total monomeric anthocyanins (Martin-Gomez, García-Martínez, Angeles Varo, Mérida, & Serratosa, 2021; Roidoung, Dolan, & Siddiq, 2017), and it is known that these compounds degrade during the storage period.

Prolonged storage (50 days) at stress temperature (+45 °C) is one of the causes of loss of compounds responsible also for the yellow color due to the prevalence of the brownish-brown color (Wibowo et al., 2015). According to previous work on clarified pomegranate juices BI increased rapidly with storage time, even at 25 °C (Vegara et al., 2013).

3.2.4. Anthocyanin content and total phenols

The total anthocyanins content was evaluated using the pH differential method, usually used for red fruit juices. They were quantified on the basis of their maximum absorption in the visible range (500–700 nm). Having in HNB the contribution of anthocyanin from red orange juice (65 %), represented mainly by cyanidin-3-glucoside (510 nm), and also from pomegranate and prickly pear, we need to consider also anthocyanins having a maximum of absorbance at 700 nm (Meyers et al., 2003). As expected storage time and packaging solutions were statistically significant ($P < 0.001$) and ($P < 0.05$) respectively.

Anthocyanin content results (Fig. 6) showed some difference during storage time, in particular the HNB packed in PR save more anthocyanins respect to the other packaging solutions for the first 10 days of storage, resulting similar to glass and PM after 20 days to decrease after as the other options.

It's well known as the anthocyanin content in juices with oranges is dependent on many factors as pH, and the association with phenolic compounds or metals. It was previously demonstrated as anthocyanin declined during the storage time, mainly due to temperature (Licciardello et al., 2018). It is known from the literature (Türkyılmaz, Hamzaoglu, Ünal, & Ozkan, 2022), that especially in the initial stages of storage there is a greater degradation of anthocyanins in the samples, in particular of cyanidin-3-glucoside (more present), since it is believed that at the beginning of storage, drinks have a higher amount of ascorbic acid (vitamin C). In fact, it has been seen how vitamin C and its degradation products can negatively influence the presence of these pigments over time and therefore progressively reduce their content. Other

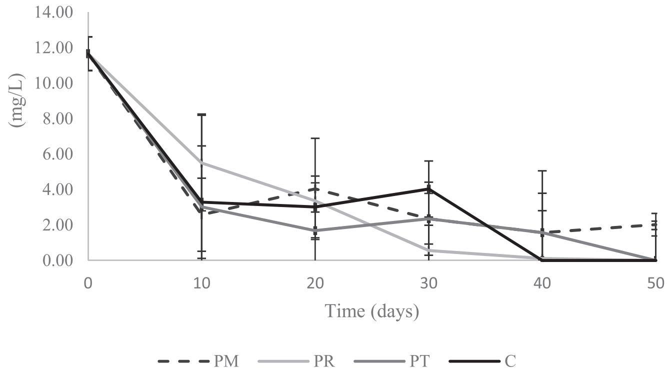

Fig. 6. Degradation kinetics of the anthocyanin content (mg/L) of High Nutritional value Beverage (HNB) packaged for 50 days for accelerated storage in stand-up pouches (PT) transparent packaging; (PM) metallized packaging and (PR) recyclable packaging. Glass was used as control (C).

studies performed on pomegranate-based drinks also suggest that the anthocyanin component degrades due to hydrolysis and polymerization reactions (Kalantari et al., 2021). Also, TP was influenced only by the storage ($P < 0.001$), showing and increasing trend after 20 days of storage to the end of the experimental trial (Table 4).

Subsequently, is noted an increase in these compounds, stabilizing around the 30th day of storage and undergoing small decreases in the final phase (50 days). It is important to note that TP remain more stable in the control for 10 days longer than in the polymeric materials, and then undergo an increase from the 30th day of storage, as well as the other materials tested. This trend is in line with what verified on orange and prickly pear juice respectively by Klimczak, Malecka, Szlachta, and Gliszczynska-Swigo (2007) and by Cruz-Cansino et al. (2015), according to them this could be explained in two way, one is for the method used, the Folin-Ciocalteau essay, since it is possible that during the storage period, some compounds are formed that can somehow interact with the method reagent, significantly increasing the content of TP. The second, supported by Cruz-Cansino et al. (2015), explain this trend over storage time possibly caused by the formation of phenolic acids and free amino acids, which contribute to this increase.

3.2.5. Antioxidant activity and total flavonoids

The changes in the AA were reported in Fig. 7. AA is statistically significant equally for 'Packaging' and for the interaction 'Packaging x Storage' ($P < 0.001$), less for 'Storage' ($P < 0.05$). Among materials, interestingly PR shows the same percentage value of PM (91 %), that as said offers a total barrier to the external environment; while during the storage time, it showed the higher variability among replicates, registering the highest standard deviation (Fig. 7). If the decrease in the antioxidant activity may be linked to a lower content of phenolic compounds and vitamin C in stored juice as compared to fresh, the increase in the AA is often attributed to Maillard's reaction products (Anese, Manzocco, Nicoli, & Lerici, 1999).

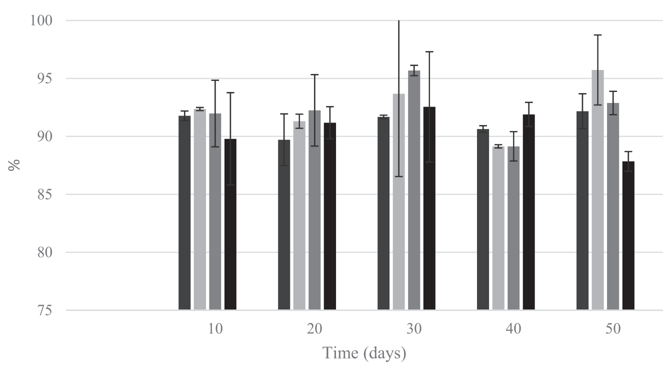

Fig. 7. Antioxidant Activity (%) on High Nutritional value Beverage (HNB) packaged for 50 days in stand-up pouches (PT) transparent packaging; (PM) metallized packaging and (PR) recyclable packaging. Glass was used as control (C).

In flavonoids, any statistical difference was observed. After 10 days of storage, there was a slight reduction in the flavonoid content in all the samples examined. These values tend to remain approximately constant on subsequent days of storage at 45 °C as confirmed by statistics results. On the other hand, it can be noted that in the control (glass) there is an initial decrease around the 10th day of storage. It has already been observed in the literature (Klimczak et al., 2007; Lu, Peng, Zhu, & Pan, 2018) that citrus fruits flavonoids are mainly represented by compounds such quite stable under storage conditions, like glycoside flavanones that are less resistant instead to the extended time of storage.

Among tested materials, PR is totally recyclable & mono-material pouch offers the excellent performance for monitored parameters. The PR "green" pouch certainly offers an excellent alternative to glass, representing a more sustainable, practical, and innovative choice. Specifically, it has a minimal variation in weight, maintains a longer (10 days) quantity of total anthocyanins comparable to those of the control (glass), and has a percentage antioxidant activity that is better maintained over time.

4. Conclusion

Beyond the packaging choice and the retention of the higher quality of HNB, it is mandatory to consider the importance of responsible consumption and production, looking at the environmental Sustainable Development Goals (SDGs) adopted by all United Nations Member States in 2015, to build a better world for people and our planet by 2030. The SDGs are a call for action by all countries to promote prosperity while protecting the environment. This study looks at the production of a high nutritional value beverage, a soft drink able to induce good health as required by the third point. At the same time, three kinds of polymeric pouches were evaluated to safely store the HNB and to guarantee consumers quality. The PR, the total recyclable and monomeric stand-up pouch would be in line with the object concerning responsible consumption, the substantial reduction of waste through activities, prevention, reduction, recycling, and reuse (n.12). The use of a sustainable and eco-friendly packaging, simple to use, practical and innovative, would be the recommended solution to launch on the market a healthy soft drink obtained exclusively processing Sicilian fruit, thus representing a potential opportunity to enhance the territory and the local economy.

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CRediT authorship contribution statement

Rizzo V.: Methodology, Investigation, Formal analysis; Writing original–draft; Dattilo S.: Conceptualization, Resources; Barbagallo S.: Conceptualization, Project administration; Puglisi C.: Conceptualization, Resources; Muratore G.: Supervision, Methodology, Review & editing.

Declaration of Competing Interest

The authors declare any competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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