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Research article

Investigating the potential of yacon (*Smallanthus sonchifolius*) juice in the development of organic apple-based snacks

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ABSTRACT

This study investigates the potential of yacon (Smallanthus sonchifolius) juice for the development of prebiotic-rich organic apple-based snacks. Yacon syrup, primarily composed of fructan, inulin, fructooligosaccharides (FOS), and free sugars, represents a promising nutraceutical product. Its great potential in food processing, particularly as an innovative source of prebiotics, has been demonstrated both *in vitro* and *in vivo* since it is fermented specifically by lactobacilli and bifidobacteria. Our objective was to explore the feasibility of employing vacuum impregnation process to incorporate yacon juice into organic apples, followed by hot air drying for the formulation of dried organic apple-based snacks with health-enhancing attributes. We assessed the prebiotic and physicochemical characteristics of the impregnated snacks, also considering 50 days of storage at room temperature. Vacuum impregnation and air drying produced dried apple slices impregnated with yacon juice with good quality and stability. Higher levels of fructan (16-fold difference compared to non-impregnated apples) in the apple slices increased their prebiotic potential, promoting the growth and viability of cells within simulated intestinal fluid, including strains of *Bifidobacterium animalis* subsp. *lactis* BB -12, *Bifidobacterium breve* DSM 20091, *Bifidobacterium longum* subsp. *infantis* DSM 20088, *Lacticaseibacillus rhamnosus* GG and *Lacticaseibacillus rhamnosus* C112, even after prolonged storage. Remarkably, the physicochemical parameters of the impregnated and dried apple slices remained nearly constant and akin to the control samples. Therefore, the combination of vacuum impregnation and air drying has the potential to be used to produce enriched prebiotic organic apple snacks, providing consumers with additional health benefits, including enhanced gut health, with its associated implications, and increased satiety. This innovation could contribute to the development of health-promoting food products with improved nutritional profiles.

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1. Introduction

Yacon syrup, extracted from the yacon root, a tuber indigenous to the Andean region of South America, is used as a popular sugar substitute due to its sweet taste and low-calorie attributes [1,2]. Comprising primarily fructans, notably inulin and fructooligosaccharides (FOS), along with a minor proportion of free sugars, yacon syrup can be considered a promising nutraceutical candidate [2]. The syrup can positively impact the nutritional values of food in which it is added and provide functional properties such as preventing certain chronic disease by providing prebiotics [3]. Indeed, the prebiotic effect of yacon's FOS has been demonstrated through *in vitro* and *in vivo* studies, where fermentation by bifidobacteria and lactobacilli was selectively promoted [4–6]. Furthermore, yacon consumption has shown a stimulatory effect on intestinal calcium absorption and bone mineral maintenance in Wistar rats consuming *ad libitum* yacon flour [7]. Recent research by Ref. [8] has highlighted that linear yacon FOS stimulate the immune system more efficiently than their branched counterparts. Additionally, inulin, another prebiotic polysaccharide present in yacon syrup, is resistant to the human digestive enzymes and can modulate the colonic region [9]. Both inulin and FOS possess immunostimulant properties, antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, promote the absorption of specific minerals and the production of B vitamins.

Despite its potential in the global prebiotic market, estimated at over US$3 billion, the application of yacon syrup remains limited [10]. Yacon represents a seasonal crop, and FOS tend to hydrolyze rapidly in post-harvest, impacting its functional properties [11]. Hence, identifying alternative food processing methods that can enhance the stability and accessibility of this food would be convenient. Yacon syrup could find a niche as a functional sweetener or as functional enhancer of other fruits and vegetables. Its incorporation into porous food matrices has shown promising results in improving the functional food characteristics [10]. For instance, apple, due to its high porosity [12] and phytochemical content [13], could serve as an ideal carrier for incorporation of yacon functional compounds, a process that could be facilitated by the vacuum impregnation (VI).

VI is a non-destructive technique employed to introduce water-soluble compounds into the porous matrix of fruits and vegetables. It encompasses two phases: the reduced pressure phase, inducing gas expansion, and the atmospheric pressure recovery phase, during which residual gas is compressed, allowing the impregnating solution to permeate free spaces and intracellular capillaries [14,15]. VI has been used to modify the texture, flavor, aroma, and color of porous fruits and vegetables while enhancing their physicochemical and sensory attributes [15]. Its most recent application extends to boosting the health-promoting qualities of foods by fortifying them with bioactive compounds, vitamins, minerals, prebiotics, and probiotics [16–18].

In earlier studies [10] prebiotic fructans deriving from yacon syrup were successfully impregnated into apple slices, demonstrating stability during storage. However, since lactobacilli and bifidobacteria metabolize FOS, inulin, and other fructans as carbon sources [19], our study focused also on the prebiotic effects of fructans and their stability over an extended storage period. Our study aims to overcome the limitations of yacon syrup by exploring the feasibility of incorporating yacon juice - rich in fructans (including inulin) - into apple slices through VI and subsequent dehydration processes, with the aim of producing functional snacks. The rationale for developing these functional apple snacks is underscored by the rising consumer demand for healthy and convenient snack options. By incorporating yacon juice into apple slices, we aim to meet this demand while providing additional health benefits associated with prebiotics. To achieve our objective, we conducted a comprehensive evaluation of various chemical, physical, and functional characteristics, including antioxidant capacity, total phenolic compounds, and prebiotic potential of the impregnated and dehydrated apple slices. Additionally, we investigated the stability of these characteristics over 50 days of storage at room temperature. The novelty of our study lies in its focus on examining the stability of prebiotic effects over an extended storage period, a factor that, to the best of our knowledge, has not been previously evaluated.

2. Materials and methods

2.1. Preparation of samples

2.1.1. Raw materials

Organic apples (*Malus domestica*, cv 'Golden Delicious') were purchased at a local market (Cesena, Italy) and stored at 4 ± 2 °C for a maximum of one week before the experiments. At the time of the experiments, the initial moisture content and soluble solids content of the apples were 87.7 ± 0.2 g/100g and 15.1 ± 0.4 °Brix, respectively. Moisture content was determined by drying approximately 3 g exactly weighted, fresh chopped apple tissue in a forced-air oven at 70 °C until a constant weight was reached. Total soluble solids content was determined at 20 °C by measuring the refractive index of three drops of filtered apple juice using a digital refractometer (A. Kruess Optronic, Germany). Apples were selected for similar size before the experiment and manually peeled, cored, and sliced into 0.5 ± 0.1 cm thick slices. Organic yacon root juice (*Smallanthus sonchifolius*) was purchased from a local producer (The Yancon Farm, Cesena, Italy) and had an initial soluble solids content of 13.8 ± 0.3 °Brix, and pH of 6.3 ± 0.02.

2.1.2. Vacuum impregnation of apple slices with yacon juice

Vacuum impregnation (VI) was performed at room temperature (20 ± 2 °C) in a treatment chamber connected to an automatic vacuum control system (AVCS, S.I.A., Italy) to control the pressure applied to the impregnating solution during the process, as described by Ref. [20]. Organic apple slices were immersed in the organic yacon juice at a ratio of 1:10 (w/v). According to preliminary tests, to achieve the maximum weight gain of the apples, the vacuum impregnation procedure was performed in two consecutive steps. In the first step, the pressure was reduced from atmospheric to a minimum absolute pressure of 200 mbar and maintained for 10 min. In the second step, the vacuum was removed, and the pressure was gradually returned to atmospheric pressure (1000 mbar) and

maintained for 10 min. At the end of the process, the apple slices were drained and immediately subjected to hot-air drying. The yield of vacuum impregnation was assessed comparing the difference in mass between the impregnated apples and the initial mass of the fresh apple slices, which is referred to as the weight gain (WG) of the vacuum impregnated apple slices. The calculated according to Eq. (1).

$$WG = \frac{m - m_0}{m_0} \bullet 100\% \quad (1)$$

where m is the mass of the impregnated apple slices and m_0 is the initial mass of the fresh apple slices.

The calculated WG resulted in 28.1 % ± 3.4 (average WG of 5 impregnation cycles).

2.1.3. Hot-air drying and storage

Control, non-impregnated dried apple slices (dA) and vacuum impregnated dried apple slices (dVA) were subjected to hot-air drying in a hot air cabinet dryer (POL-EKO-APARATURA SP.J., Poland) operating at a drying temperature of 70 °C, an air velocity of 2 m/s, and an air renewal rate of 50 %. Apple slices were dried until the water activity (a_w) reached 0.30 ± 0.03 (approximately after 8 h of drying). The dried samples were packed in high barrier high-density polypropylene bags, each containing 5 slices, and stored in the dark at room temperature (25 ± 2 °C) for shelf-life analyses up to 50 days.

2.2. Quantification of fructan

Fructan was determined in triplicate according to AOAC 997.08 1999 spectrophotometric methodology using Fructan Assay Kit (Megazyme, Milan, Italy). Fructan concentration was assessed after the vacuum impregnation and during the storage at room temperature (25th and 50th days of storage). The limit of detection was 0.1 g/100 g.

2.3. Survival of probiotic bacteria in simulated intestinal fluid (SIF)

The ability of vacuum impregnated apples to maintain the viability or promote the growth of probiotic strains was investigated upon incubation in simulated intestinal fluid (SIF: 0.1 % w/v pancreatin, 0.15 % w/v Oxgall bile salt, 100 mM phosphate salt buffer pH: 8) by plate counting, according to Ref. [21] with some modifications. The probiotic strains tested were: *Bifidobacterium animalis* subspecis lactis BB -12, *Bifidobacterium breve* DSM 20091, *Bifidobacterium longum* subsp. *infantis* DSM 20088, *Lacticaseibacillus rhamnosus* GG and *Lacticaseibacillus rhamnosus* C112. SIF fortified with 50 mg/ml vacuum-impregnated dried apple slices (dVA) or non-impregnated dried apple slices (dA) was inoculated with 6.5–7.5 log CFU/ml of selected commercial probiotic strains. Overnight anaerobically grown bacteria were centrifuged at 10,000 g for 2 min at 4 °C and the pellet was resuspended in an equal volume of saline solution (0.9 % NaCl) before inoculation. Survival in SIF was monitored in response to yacon juice (YJ), dVA or dA after 3, 6, and 24 h by plate counting on MRS with 0.05 % (p/v) cysteine-HCl. Plates were incubated anaerobically at 37 °C for at least 24 h. Analyses were performed on apples immediately after impregnation and at the end of storage period (50 days at room temperature).

2.4. Volatile organic compounds (VOCs) compositions

Volatile organic compounds (VOCs) of yacon juice (YJ), impregnated and not-impregnated apple splices were qualitatively and quantitatively evaluated using solid-phase microextraction (SPME) combined with gas chromatography–mass spectrometry (GC/MS). A Carboxen®/DVB/PDMS, 50/30 μm fiber (SUPELCO, Bellafonte, PA, USA) was used to perform the SPME. 3 g of sample were placed in a vial and incubated for 10 min at 45 °C. Then, the fiber was exposed to the vial headspace for 30 min at 45 °C. The volatile molecules adsorbed were transferred in the gas chromatograph (GC) injector port set in splitless mode at 250 °C for 10 min. The volatile compounds were analyzed using Gas-Chromatography (GC) 6890 N, Network GC System with mass spectrometry (MS) 5970 MSD (Aglient technologies, Milan, Italy). The column used was J&W CP-Wax 52 (50 m × 320 μm × 1.2 μm) (Aglient technologies, Milan, Italy). The initial temperature was 50 °C for 1 min and then increased by 4.5 °C/min up to 65 °C. After that, the temperature increased by 10 °C/min up to 230 °C and remained stable for 25 min. Gas-carrier was helium at 1.0 ml/min flow. Compounds were identified by comparison based on the NIST11 (National Institute of Standards and Technology) database, while the quantitative analysis were performed with the internal standard method, using 4-methyl-2-pentanol (6 mg/L), and expressed as equivalent ppm (*ppm eq*.). For each detected compound, the *ppm eq*. represents the amount of compound present in the headspace in dynamic equilibrium with the aqueous or solid phase depending on the kind of sample, yacon juice or dried apple slices. Analyses were performed in triplicate.

2.5. Total phenolic content and antioxidant activity

The antioxidant activities of apple extracts were analyzed by 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazin-1-yl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods, according to Refs. [22,23]. The Folin-Ciocalteu method [24] was used to determine the total phenolic content of yacon juice and impregnated and non-impregnated dried apples. Total phenolic content, ABTS and DPPH reagents as well as Trolox, gallic acid, and L-ascorbic acid were purchased from MERCK (Milan, Italy).

2.5.1. Samples extraction and preparation

Extracts were obtained by adding 20 ml of methanol 80 % (v/v) to 2 g of apple slices, previously reduced in powder, or 2 ml of yacon juice. Samples were stirred on orbital shaker for 30 min and then, centrifuged at 4000 g for 20 min. The supernatant was recovered and kept a 4 °C before performing ABTS, DPPH and Folin-Ciocalteu assay.

2.5.2. DPPH assay

Methanolic extracts (0.1 ml) of each sample were mixed with 2.9 ml of DPPH (0.1 mM) methanolic solution. After 30 min incubation at room temperature in the dark, the absorbance of the samples was measured at $\lambda = 517$ nm. Free radical scavenging activity was expressed as mg of ascorbic acid equivalent (AaE) per gram of initial sample (AaE mg/g) using the equation generated with a 6-points ascorbic acid standard curve (Eq. (2)).

$$\text{AaE (mg / g)} = \left(\frac{\text{Sample Abs}_{517\text{nm}} - \text{b}}{\text{a}}\right) * \text{sample dilution factor} \quad (2)$$

where a and b are the two coefficients (the slope and the Y-axis intercept) of the ascorbic acid standard curve. Results were reported as the mean of three independent reads (n = 3).

2.5.3. ABTS assay

ABTS radical scavenging activity was evaluated based on [25] with slight modifications. ABTS working solution was prepared by mixing equal amounts of 7 mM ABTS stock solution and 2.45 mM potassium persulfate solution, allowing them to react at room temperature in the dark overnight. Prior the analysis, the ABTS working solution was diluted with ethanol to reach an absorbance of 0.7 at $\lambda = 734$ nm. After that, 30 μL methanolic extracts of the samples were added to 30 ml of ABTS working solution and incubated at room temperature for 6 min in the dark. After incubation, absorbance at 734 nm was recorded. The antioxidant activity was expressed as Trolox equivalent (TxE) per gram of initial sample (TxE mg/g) using the equation generated with a 6-points Trolox standard curve (Eq. (3)).

$$\text{TxE (mg / g)} = \left(\frac{Sample\ Abs_{734nm} - b}{a}\right) * \text{sample dilution factor} \quad (3)$$

where a and b are the two coefficients (the slope and the Y-axis intercept) of Trolox standard curve. Results were reported as the mean of three independent reads (n = 3). Data were considered statistically significant ($p < 0.05$) on the base of ANOVA and Tukey HSD post-hoc tests.

2.5.4. Total phenolic content (TPC)

Apple slices and yacon juice methanolic extract (100 μL) were mixed with 500 μL of Folin-Ciocalteu reagent and 7.5 ml of distilled water. Then, 2 ml of 15 % (w/w) of sodium carbonate (Na_2CO_3) solution were added and again mixed. Samples mixtures were incubated for 120 min in the dark before reading the absorbances at $\lambda = 750$ nm. Phenol content was expressed as gallic acid equivalents (GaE) per gram of initial sample (GaE mg/g) using the equation generated with a 6-points Gallic acid standard curve (Eq. (4)).

$$\text{GaE (mg / g)} = \left(\frac{Sample\ Abs_{750nm} - b}{a}\right) * \text{sample dilution factor} \quad (4)$$

where a and b are the two coefficients (the slope and the Y-axis intercept) of the gallic acid standard curve. Results were reported as the mean of at least three independent reads (n = 3).

2.6. Technological parameters

2.6.1. Image analysis for color determination

The surface color of apple slices was measured using a Computer Vision System (CVS) consisting of an illumination source, a color digital camera (CDC), and an image processing software. Apple slices were placed inside a dark box to exclude external light, and RGB images were acquired by a CDC Mod. D7000 (Nikon, Japan), with a 105 mm lens Mod. AF-S Micro Nikkor (Nikon, Japan), located vertically over the sample at a distance of 35 cm and connected to a personal computer. The lighting system consisted of four daylight fluorescent lamps (60 cm in length) connected to an electronic ballast to ensure uniform illumination, with a colour temperature of 6500 K and sited at an angle of 45° with the CDC. For each dried sample, dV and dVA, 5 images were captured at each time point of storage. The pre-processing of RGB images, segmentation from background and color quantification were performed with ImageJ analysis software (NIH, USA). The average value of the segmented pixels in the CIE L* a* b* color space was registered as the colour of the sample. From numerical values of chromatic parameters, a* (green/red) and b* (yellow/blue), hue angle was calculated (Eq. (5)), and used to describe color variations between samples.

$$H^\circ = \tan^{-1}(b^* / a^*) \quad (5)$$

2.6.2. Texture analysis

Texture analysis of dried apple slices was performed at room temperature (20 ± 2 °C) using a Texture Analyser TA-XT2 Mod. HDi 500 (Stable Micro System, Surrey, UK) equipped with a 5 kg load cell. A puncture test was selected to evaluate firmness of samples. Selected samples with most uniformity, in terms of size and shape, were placed on a support rig and compressed for 100 % distance using a needle probe. Force *vs.* distance curves were obtained using a test speed of 1.0 mm s^{-1}, and results obtained from 10 samples were expressed as firmness, calculated by means of registered values of maximum force.

2.7. Statistical analysis

Significant differences among the results were evaluated by parametric analysis of variance (ANOVA) and Tukey multiple comparison, with a significance level of 95 % ($p < 0.05$) using R software (R Foundation for Statistical Computing, Austria). If Shapiro-Wilk test for normality and Levene's test for homoscedasticity of data results statistically significant ($p < 0.05$), non-parametric multiple range test Kruskal-Wallis and Holm stepwise adjustment were used, with significant level of 95 % ($p < 0.05$). To highlight differences among VOCs profiles, Principal Component Analysis (PCA) was performed using STATISTICA 8 (Statsoft, USA). Results are expressed as mean ± standard deviations of replications.

3. Results

3.1. Fructan content

The fructan content in both non-impregnated (dA), and vacuum-impregnated (dVA) dried apple slices is shown in Table 1. The yacon juice used for vacuum impregnation had a fructan content of 6.67 g/100 g. According to Table 1, the impregnation process significantly ($p < 0.05$) increased the fructan content in dVA compared to dA, resulting in a 16-fold difference (6.08 ± 0.47 g/100 g in dVA vs 0.38 ± 0.41 g/100 g in dA). Furthermore, the fructan content exhibited minimal variation over 50 days of storage, showing no statistical differences ($p < 0.05$) between 0, 25 and 50 days of storage. Please note that the fructan content in dA samples (at 0, 25, 50 days of storage) is close to the limit of quantification, explaining the higher standard deviation observed. Fructans, being plant-produced polymers comprising fructose molecules, have the potential to enhance both the nutritional and technological properties of foods, serving as prebiotic dietary fiber [26].

3.2. Survival of probiotic bacteria in simulated intestinal fluid (SIF)

The impact of apple slices fortified with yacon juice (dVA) in promoting probiotic viability and stimulating its growth was evaluated on five bacterial strains belonging to the genera of *Lacticaseibacillus* and *Bifidobacterium* (Fig. 1). The results showed different responses among the bacterial strains. Although *Lacticaseibacillus rhamonosus* C112 demonstrated robust adaptation to the challenging environment of SIF, the addition of dVA promoted the strain proliferation. In fact, after a 24-h incubation, the cell counts of *L. rhamnosus* C112 reached 8.9 log CFU/ml, while they remained nearly equivalent to the inoculum (7.1 log CFU/ml) with dA (Fig. 1A). YJ alone also enhanced the viability of *L. rhamonus* C112 in SIF (7.8 log CFU/ml), albeit to an intermediate degree compared to dA and dVA (Fig. 1A). *L. rhamnosus* GG was less resistant to SIF condition, in fact a reduction of about 3-log cell was observed after a 24-h incubation (Fig. 1C). However, the introduction of YJ, alone or as dVA, contributed to improve cell survival, exhibiting a trend similar to the one observed for *L. rhamonosus* C112. Following a 24-h incubation, *L. rhamonosus* GG cell counts reached 6.7 and 6.1 log CFU/ml in dVA and YJ, respectively (Fig. 1C).

Regarding bifidobacteria, the viability of *B. animalis* subsp. *lactis* BB-12 and *B. breve* DSM 20091 was not impacted by SIF. Instead, the addition of dVA strongly improved their growth, reaching 8.5 and 8.7 log CFU/ml, respectively, after 24 h (Fig. 1E–G), even when compared with the use of YJ or dA. On the other hand, *B. longum* subsp. *infantis* DSM 20088 was more sensitive to SIF (3 log reduction). However, the inclusion of dA, YJ, and especially dVA, counteracted this negative effect, maintaining the cell counts closer to the starting inoculum level (around 6.5 log CFU/ml) (Fig. 1I). Overall, the prebiotic attributes of apples following the proposed treatment (i.e. VI and hot-air drying) were confirmed across the strains, even after 50 days of storage at room temperature. As shown in Fig. 1B–D,

Table 1
Fructan content (g/100 g) in non-impregnated dried apple slices (dA), and vacuum-impregnated dried apple slices (dVA) during 50 days of storage at room temperature. Fructan levels were determined using the AOAC 997.08 (1999) methodology. Results represent the mean value of three replicates. The limit of detection was established at 0.1 g/100 g. Same letters within each row indicate that no statistically significant differences were found among days of storage ($p < 0.05$). Different numbers within each column indicate statistically significant differences among samples ($p < 0.05$) at specific time points.

	Fructan content (g/100 g)								
	Days of storage								
	0			25			50		
dA	$0.38^{a,1}$	±	0.41	$0.36^{a,1}$	±	0.41	$0.34^{a,1}$	±	0.42
dVA	$6.08^{a,2}$	±	0.47	$6.13^{a,2}$	±	0.43	$5.95^{a,2}$	±	0.43

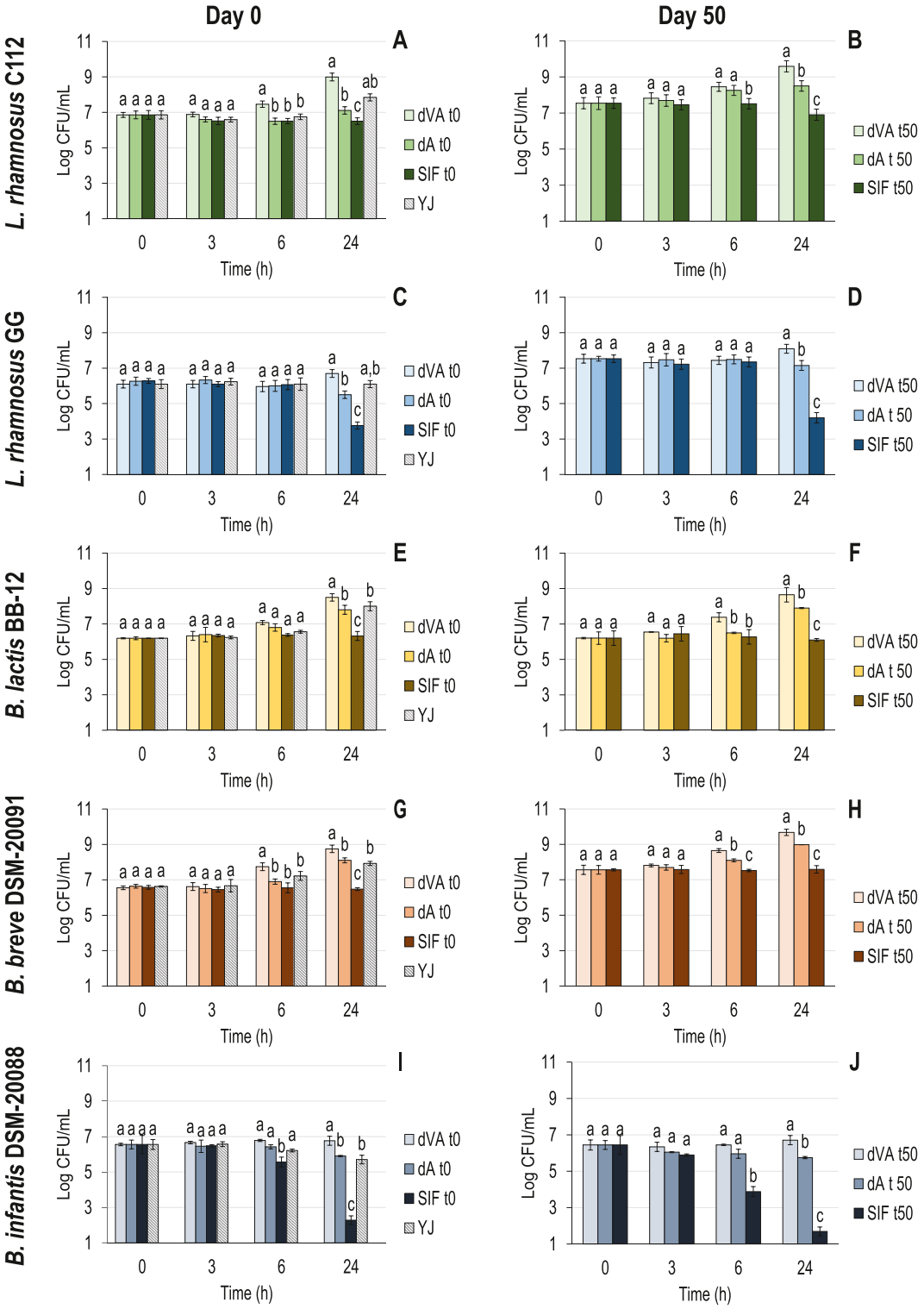

Fig. 1. Survival (Log CFU/ml) of Lacticaseibacillus rhamnosus C112, Lacticaseibacillus rhamnosus GG, Bifidobacterium animalis subsp. lactis BB-12, Bifidobacterium breve DSM 20091, and Bifidobacterium longum subsp. Infantis DSM-20088 in simulated intestinal fluid (SIF) with vacuum-impregnated dried apple slices (dVA), non-impregnated dried apple slices (dA), and yacon juice (YJ) supplementation. SIF without supplementation served as the control condition. Survival assessments were conducted at the initiation (A, C, E, G, I) and the conclusion (B, D, F, H, J) of 50 days of storage at room temperature. The detection limit was set at 1 Log CFU/ml. Data represent the mean value of three replicates. Different letters indicate significant differences among samples ($p < 0.05$) at specific time points.

F, H, J, the prebiotic strains in SIF displayed consistent growth patterns when supplemented with dA and dVA following the extended storage duration. However, the observed differences in cell counts were attributed to variations of the initial inoculum sizes (Fig. 1A–C, E, G, I). The prebiotic activity of apple impregnated with YJ represents a fundamental aspect for the development of apple-based snacks. These snacks can maintain their activity throughout the entire shelf-life of the product without the need for further treatments.

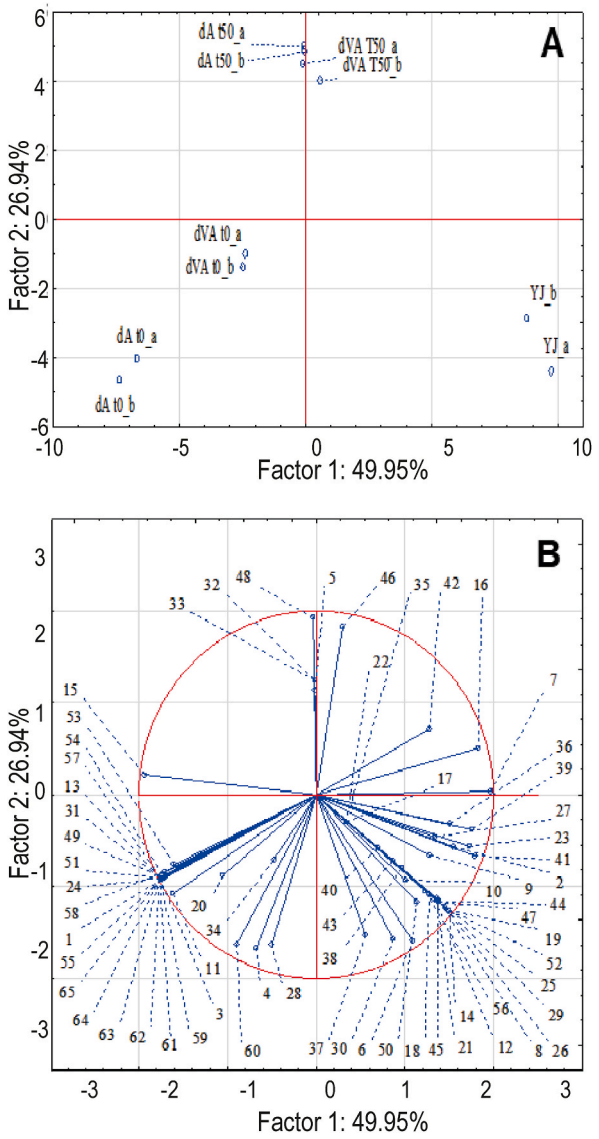

Fig. 2. Loading plot (A), and factor coordinates for the first two components (PC1 and PC2) (B) of principal component analysis (PCA) for volatile organic compounds (VOCs) in yacon juice (YJ), non-impregnated dried apple slices (dA), and vacuum-impregnated dried apple slices (dVA) during 50 days of storage at room temperature. Compound list: 1)3,5-Dimethylbenzaldehyde, 2)5-Methylfurfural, 3)Benzaldehyde, 4)Butanal, 3-methyl-, 5 Butanedial, 6)Decanal, 7)Furfural, 8)Heptanal, 9)Hexanal,10)Nonanal,11)Pentanal, 3-methyl-,12)2-Methylbutane-1,4-diol, 3-(1-ethoxyethoxy)-, 13)1-Octanol, 14)2-(Methylthio)ethanol, 15)2,4-di-*t*-Butylphenol, 16)2-Furanmethanol, 17)2-Hexanol, 18)2-Methyl-4-aminophenol, 19)3-(Methylthio)propanol, 20)3-Methyl-1-butanol, 21)5-Methyl-3-hexanol, 22)6-Methyl-1-heptanol, 23)Ethanol, 24)1-Pentanol, 4-methyl, 25)Phenylethyl Alcohol, 26)1-(2-Furanyl)ethanone, 27)2,3-Butanedione, 28)2,6-Dimethyl-4-heptanone, 29)3-Hydroxy-2-butanone, 30)4-Methyl-2-hexanone, 31)5-Hepten-2-one, 6-methyl, 32)Acetoin, 33)Butyrolactone, 34)5-Methyl-3-hexanone, 35)Hydroxypropanone, 36)Isobutenyl methyl ketone, 37)Methyl Isobutyl Ketone, 38)2,2-Dimethylpropionic acid, 39)Acetic acid, 40)Butanoic acid, 41)Hexanoic acid, 42)Nonanoic acid, 43)Propanoic acid, 44) 2,2,4,4-Tetramethyloctane, 45)Decane, 46)Dodecane, 47)Heptane, 2,2,4,6,6-pentamethyl-, 48)Hexadecane, 49)(+)-4-Carene, 50)1,3-Ditertiarybutylbenzene, 51)1-Verbenone, 52)α-Farnesene, 53)α-Pinene, 54)α-Fellandrene, 55)Benzene,1-methyl-4-(1-methylethenyl)-, 56)β-Cyclocitral, 57) Borneol, 58)Carvacrol, 59)ç-Terpinene, 60) D-Limonene, 61)Lemonol, 62)Linalol, 63)p-Cymol, 64)Vernol, 65)Z-Ocimene.

Table 2
Volatile organic compounds (VOCs) of yacon juice (YJ), non-impregnated dried apple slices (dA), and vacuum-impregnated dried apple slices (dVA) at the beginning and at the end of 50 days of storage at room temperature, expressed in equivalent parts per million (ppm eq.). Data represent the mean value of two replicates. Standard deviation observed ranged between 5 % and 7 %.

		YJ	Equivalent parts per million (ppm eq.)			
			Days of storage			
			0		50	
			dA	dVA	dA	dVA
Aldehydes	3,5-Dimethylbenzaldehyde	0.2	-[a]	–	–	–
	5-Methylfurfural	–	0.3	0.1	0.1	0.1
	Benzaldehyde	0.2	–	–	–	–
	Butanal, 3-methyl-	0.3	0.1	0.1	–	–
	Butanedial	–	–	–	0.2	–
	Decanal	–	0.1	–	–	–
	Furfural	–	15.1	11.4	12.1	9.6
	Heptanal	–	–	–	–	–
	Hexanal	–	0.3	–	0.1	–
	Nonanal	0.3	0.2	0.1	0.1	0.2
	Pentanal, 3-methyl-	0.1	–	–	–	–
Alcohols	1,4-Butanediol, 2-(1-ethoxyethoxy)-3-methyl-	–	0.1	–	–	–
	1-Octanol	0.3	–	–	–	–
	2-(Methylthio)ethanol	–	0.1	–	–	–
	2,4-di-*t*-Butylphenol	0.6	–	0.1	0.2	0.1
	2-Furanmethanol	–	0.1	0.1	0.1	0.1
	2-Hexanol	–	–	–	–	–
	2-Methyl-4-aminophenol	–	0.1	–	–	–
	3-(Methylthio)propanol	–	0.3	0.2	–	–
	3-Methyl-1-butanol	0.1	–	–	–	–
	5-Methyl-3-hexanol	–	–	–	–	–
	6-Methyl-1-heptanol	–	–	–	–	–
	Ethanol	–	0.3	0.3	0.1	0.2
	Isohexanol	0.3	–	–	–	–
	Phenylethyl Alcohol	–	0.1	–	–	–
Ketones	1-(2-Furanyl)ethanone	–	0.1	0.1	–	–
	2,3-Butanedione	–	0.1	–	0.1	–
	2,6-Dimethyl-4-heptanone	0.2	–	0.1	–	–
	3-Hydroxy-2-butanone	–	1.5	0.2	–	–
	4-Methyl-2-hexanone	0.1	0.1	0.1	–	–
	5-Hepten-2-one, 6-methyl	0.3	–	–	–	–
	Acetoin	–	–	–	0.9	–
	Butyrolactone	–	0.4	0.1	0.2	–
	Ethyl isobutyl ketone	–	–	–	–	–
	Hydroxypropanone	–	–	–	–	–
	Isobutenyl methyl ketone	0.1	0.2	0.2	0.1	0.2
	Methyl Isobutyl Ketone	0.2	0.1	0.2	0.1	–
Organic acids	2,2-Dimethylpropionic acid	–	–	0.1	–	–
	Acetic acid	–	1.8	0.6	1.1	0.3
	Butanoic acid	–	–	0.1	–	–
	Hexanoic acid	–	0.3	0.2	0.2	–
	Nonanoic acid	–	0.3	0.1	0.2	0.2
	Propanoic acid	–	–	0.2	–	–
Hydrocarbons	2,2,4,4-Tetramethyloctane	–	0.2	–	–	–
	Decane	–	–	–	–	–
	Dodecane	–	0.1	–	0.3	0.2
	Heptane, 2,2,4,6,6-pentamethyl-	–	1.7	0.2	–	–
	Hexadecane	0.1	–	–	0.6	0.3
Terpenes	(+)-4-Carene	0.2	–	–	–	–
	1,3-Ditertiarybutylbenzene	0.1	0.6	–	–	–
	1-Verbenone	0.3	–	–	–	–
	α-Farnesene	–	1.0	0.4	–	–
	α-Pinene	0.3	–	–	–	–
	α-Fellandrene	0.3	–	–	–	–
	Benzene, 1-methyl-4-(1-methylethenyl)-	0.5	–	–	–	–
	β-Cyclocitral	–	0.1	–	–	–
	Borneol	0.8	–	–	–	–
	Carvacrol	0.2	–	–	–	–
	ç-Terpinene	0.1	–	–	–	–
	D-Limonene	1.1	0.3	0.2	–	–
	Lemonol	0.8	–	–	–	–

(*continued on next page*)

Table 2 (*continued*)

	YJ	Equivalent parts per million (ppm eq.)			
		Days of storage			
		0		50	
		dA	dVA	dA	dVA
Linalol	2.2	–	–	–	–
p-Cymol	0.5	–	–	–	–
p-Menthan-4-ol	–	–	–	0.2	0.1
Vernol	0.8	–	–	–	–
Z-Ocimene	0.1	–	–	–	–

[a] under detection limit (<0.1 ppm eq.).

3.3. Volatile organic compounds (VOCs) compositions

Fig. 2 illustrates the principal component analysis (PCA) of volatile organic compounds (VOCs) for YJ, dA, and dVA samples, during 50 days of storage at room temperature. The PCA mapped these samples within the first two principal components, PC1 and PC2 (Fig. 2A). The analysis effectively explained 76.89 % of the total observed variability, with PC1 contributing for 49.95 % and PC2 accounting for 26.94 % of this variability. Notably, YJ samples formed a discernible cluster in the second quadrant, exhibiting a distinct separation from the rest of the samples (Fig. 2A). The behaviour of the apple samples was mainly influenced by the storage time. Initially, the dried apple samples (dA_t0 and dVA_t0) were situated in the third quadrant. However, after 50 days of storage, dVA_t50 and dA_t50 samples shifted to the first and forth quadrant, respectively (Fig. 2A).

The projection of variables onto the factorial plane defined by PC1 and PC2 (Fig. 2B and Table 2) highlights the differential accumulation of aldehydes, alcohols, ketones, organic acids, and terpenes in the samples. YJ samples were characterized by the presence of terpenes (D-limonene, linalol, borneol, vernol, and lemonol) and the absence of organic acids. Meanwhile, dA and dVA samples exhibited higher levels of furfural (15.1 and 11.3 ppm eq., respectively), albeit differing in the quantities of aldehydes, organic acids, and alcohols. These differences included hexanal (0.3 ppm eq. in dA only) α-farnesene (1 and 0.4 ppm eq.), and acetic acid (1.8 and 0.6 ppm eq.) (Fig. 2B and Table 2). The cumulative storage duration led to a stabilization of the volatile molecule profiles in the apple samples, regardless of the vacuum impregnation process. Both dA and dVA samples displayed reduced levels of characteristic compounds, including aldehydes, alcohol, ketones, and terpenes, which fall below the detection limit (<0.1 ppm eq.) (Table 2).

3.4. Antioxidant activity and total phenolic content

Antioxidant activity was quantified through radical scavenging assays using both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, with results expressed in terms of ascorbic acid equivalents (AaE) and Trolox equivalents (TxE) (Table 3). YJ samples exhibited the lowest radical scavenging activity, with values of 13.4 mg/g AaE and 36 mg/g TxE. Conversely, no significant differences ($p < 0.05$) in scavenging activity were observed between dVA and dA, even after 50 days of storage. Total phenolics, expressed as gallic acid equivalents (mg/g GaE), displayed slight but significant ($p < 0.05$) differences among apple samples over the course of storage. Initially, dA and dVA samples exhibited concentrations of 111.1 mg/g GaE and 105.0 mg/g GaE, respectively, which subsequently reduced to 101.2 mg/g GaE and 92.3 mg/g GaE at the end of storage. YJ, in contrast, possessed the lowest phenolic compound content, measuring 30.2 mg/g GaE, reflecting its lower scavenging activity.

3.5. Color and texture

Fig. 3 (left panel) displays images of non-impregnated (dA) and vacuum-impregnated (dVA) apple slices after drying process. The conversion of these images from RGB to Lab channels is illustrated on the right panel of Fig. 3. The computed values of L* and H° (as defined in Equation (5)) of the dried samples are presented in Fig. 4A and B, respectively. The values of lightness (L*) and the color (as indicated by the hue angle), demonstrated a consistent and stable trend in both the dA and dVA samples during 50 days of storage.

Table 3
Antioxidant activity expressed as ascorbic acid equivalents (AaE) and Trolox equivalents (TxE), and total phenol content expressed as gallic acid equivalents (GaE) in yacon juice (YJ), non-impregnated dried apple slices (dA), and vacuum-impregnated dried apple slices (dVA) at the beginning (T0) and at the end (T50) of 50 days of storage at room temperature. Data are presented as mg/g of initial sample. Different letters within each column indicate statistically significant differences among samples ($p < 0.05$).

	AaE mg/g		TxE mg/g		GaE mg/g<	
YJ	13.4^{a}	±1.0	36.0^{a}	±0.9	30.2^{a}	±1.2
dA T0	25.7^{c}	±1.3	73.0^{b}	±5.2	111.1^{b}	±2.4
dVA T0	22.0^{c}	±1.0	78.7^{b}	±1.9	105.0^{b}	±2.4
dA T50	23.3^{c}	±1.6	75.0^{b}	±3.3	101.2^{c}	±1.2
dVA T50	21.2^{c}	±0.2	73.3^{b}	±1.9	92.3^{c}	±2.4

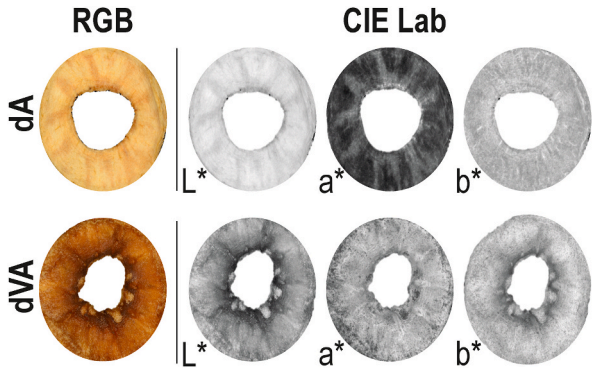

Fig. 3. Left: examples of RGB images of non-impregnated dried apple slices (dA) and vacuum-impregnated dried apple slices (dVA). Right: example of image conversion from RGB to Lab channels.

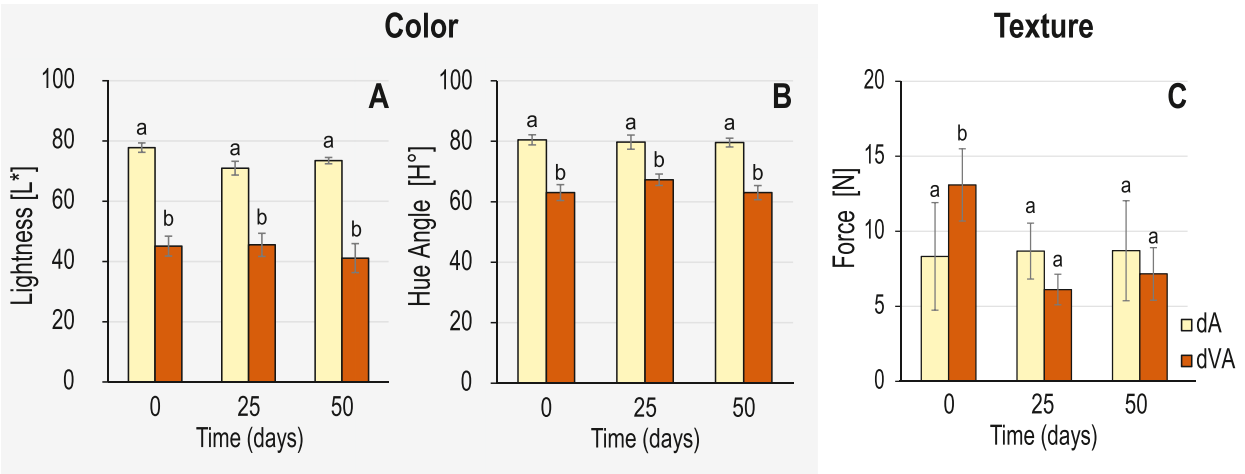

Fig. 4. Lightness (L*) (A), hue angle (H°) (B), and firmness (N) (C) of non-impregnated dried apple slices (dA), and vacuum-impregnated dried apple slices (dVA). Results are expressed as means ± standard deviations (error bars) of n = 5. Different letters indicate significant differences among samples ($p < 0.05$).

Fig. 4C presents the alterations in firmness observed in non-impregnated (dA) and vacuum-impregnated (dVA) apple slices following the drying process. Immediately after vacuum impregnation treatment, the dVA samples exhibited heightened initial firmness. Subsequently, over the storage period, a slight reduction in firmness was observed in the dVA samples; however, this reduction did not attain statistical significance. After 25 days of storage, the firmness of impregnated apple slices declined by approximately 50 % and maintained a consistent level until the 50th day of storage.

4. Discussion

The consumption of an adequate prebiotic diet is imperative for maintaining a healthy gut microbiota. The perennial daisy, *Smallanthus sonchifolius*, commonly known as yacon and belonging to the Asteraceae family, contains the prebiotic fructan inulin in its tubercles at concentrations ranging from 3 to 10 % (w/w) [27]. The scope of this study was to incorporate YJ into apple slices using VI followed by hot-air drying to develop organic apple-based snacks with enhanced prebiotic characteristics. The goal was to the increase the fructan content of organic apples, which could be preserved for up to 50 days. This research employed a multidisciplinary approach, incorporating both technological and microbiological analyses.

The VI process enabled the incorporation of YJ, containing 6.67 ± 0.45 g/100 g of fructan, into apple slices enriching their fructan content up to 6.08 ± 0.47 g/100 g of dried sample. These fructan levels remained constant during 50 days of storage (Table 1). Furthermore, minimal changes in technological and chemical properties were observed after 50 days storage at room temperature. The VI treatment combined with hot-air drying allowed to obtain apple snacks with low water activity ($a_w < 0.3$) and high microbiological stability throughout the storage duration (data not shown). The impregnation of apple slices with fructan increased the functional potential of the product due to the prompt availability of these molecules for the intestinal microbiota [10,28,29]. Moreover, the VI process coupled with hot-air drying led to the development of snacks showing a positive effect on the *in-vitro* survival and growth of

several commercial probiotic strains (*L. rhamnosus* GG, *L. rhamnosus* C112, *B. animalis* subsp. *lactis* BB-12, *B. breve* DSM 20091 and *B. longum* subsp. *infantis* DSM 20088) incubated in simulated intestinal fluid (SIF). Previous studies demonstrated that *Lactobacillus* probiotic strains can ferment FOS deriving from yacon juice [30], and this aspect can contribute to improve the gut health and support the immune system [31,32].

It is well documented that the ingestion of fructan inulin and FOS selectively stimulates the growth and colonization of various *L. rhamnosus* strains [6,33,34], including *L. rhamnosus* GG [35,36], and *Bifidobacteria* [4,5,37]. Furthermore, substantial evidence confirms that inulin-type fructans are well tolerated in the human diet. Multiple clinical studies have demonstrated that consuming up to 20 g/day of inulin and/or FOS is well tolerated [38]. Our investigation showed that apples slices enriched with yacon juice retained their prebiotic effect even after 50 days of storage.

The VI treatment combined with hot-air drying determined an overall reduction of the main volatile molecules of apples, especially esters [39] both in the sample with and without YJ. Despite this difference, mainly based on the treatment applied, samples containing YJ showed a slight reduction in hexanal and farnesene, also typical compounds of dried apples [40]. However, their threshold limits are typically lower (for example, the threshold limit for hexanal is 5 ppb; [41,42]) than what was observed in our study, suggesting that, from a sensory perspective, this reduction may not have a significant impact on the final aroma and taste of the snacks. An interesting aspect is also related to the fact that none of terpenes, which are known to have a strong sensorial impact, were not detected in dried apple with YJ even after 50 days of storage.

Certainly, conducting a panel test for consumer acceptance would offer a more definitive answer. Physicochemical properties of the apple snacks exhibited negligible changes during 50 days of storage. While certain studies have reported that vacuum impregnation could lead to the loss of phenolic compounds, resulting in decreased antioxidant activity [10], in our study, both Total Phenolic Content (TPC) and antioxidant activity did not change upon vacuum impregnation. The antioxidant compound content is closely associated with the composition of the impregnating solution. Solutions with high content of bioactive compounds have the potential to enhance antioxidant activity in impregnated samples, as previously reported in the literature. For instance, studies have demonstrated increased antioxidant activity of apples impregnated with lemon [43], tangerine juice solution [44], green tea polyphenols [45], and rapeseed juice concentrate [46,47]. During storage, antioxidant activity remained constant ($p < 0.05$), although a significant reduction in TPC was observed after 50 days of storage. This behaviour was similar to what reported for dried apple snacks enriched with chokeberry juice during storage [48]. Furthermore, the visual appearance of the snacks, as assessed by image analysis in terms of color, notably differed between non-impregnated and impregnated apple slices, particularly in lightness (L*) and hue angle. These differences were expected due to the dark brown color of yacon juice in contrast to the light yellow-green hue of fresh apples [49]. However, impregnated apples maintained their surface color throughout storage. On the other hand, the structural properties of the samples, in terms of firmness, displayed a significant decrease in impregnated apple snacks, albeit with similar values to control sample. In contrast, some authors have reported a reduction in the crispness of dried apple slices, which was reflected in an increase in firmness during the storage period [50–52].

5. Conclusion

The synergistic application of vacuum impregnation and air-drying processes enabled the production of apple slices impregnated with yacon juice, demonstrating adequate stability in technological and chemical properties during 50 days of storage at room temperature. These snacks exhibited low water activity and displayed high microbiological stability, showcasing their potential for long-term consumption. Impregnation of apple slices with yacon juice not only increased the fructan content but also enhanced the prebiotic attributes of the snacks, positively impacting the *in-vitro* survival or growth of various commercial prebiotic strains. Our investigation revealed significant benefits for *Lactobacillus* and *Bifidobacterium* strains, known for their association with improved gut health and immune system response. Moreover, our study confirmed that apples enriched with yacon juice retained their prebiotic effect even after an extended storage period of 50 days, reinforcing their potential as a stable, prebiotic-rich snack option. Given the similarity and near-constancy of physicochemical and technological parameters observed compared to the controls (non-impregnated dried apple slices), the vacuum impregnation process shows potential in producing enriched prebiotic apple snacks, offering significant advantages to consumers. Moreover, the flattening in volatile aroma compounds noted following the proposed vacuum treatment coupled with hot drying could positively contribute to consumer acceptance, leading to dried apples snacks with negligible yacon juice-specific aroma characterized by volatile terpenes. Nevertheless, it is imperative to conduct further trials to determine the specific prebiotic effects in the colon as well as to assess consumer acceptability comprehensively.

Data availability statement

Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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