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In vitro fermentability of globe artichoke by-product by *Lactobacillus acidophilus* and *Bifidobacterium bifidum*

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ABSTRACT

Potential prebiotic effect of globe artichoke by-product (*Cynara cardunculus* L.) was evaluated *in vitro*. Representative lactic acid bacteria from human colon, like *Lactobacillus acidophilus* and *Bifidobacterium bifidum,* were incubated up to 96 h with artichoke by-product *versus* sucrose control. Low molecular weight carbohydrates (6.14% dw) in raw artichoke by-product were extracted with ethanol. Fermentability was evaluated by monitoring growth, pH, short chain fatty acids (SCFAs) and lactic acid production. Maximum growth of *L. acidophilus* differed on both substrates but *B. bifidum* exhibited a similar growth pattern. Artichoke by-product provided a higher growth than control for both bacteria. Release of total SCFAs was also significantly higher on artichoke by-product than on the control in both bacterial cultures. Nonetheless, no clear pH reduction was found in culture medium for artichoke by-product batches. Lactic acid content in *L. acidophilus* culture was remarkably higher on control (6.6-fold) than on artichoke by-product. Non-fermented residue of artichoke by-product was 56.2% and 41.8% for *L. acidophilus* and *B. bifidum*, respectively. Artichoke by-product shows a potential prebiotic effect under the assayed conditions. The use of valuable bio-resources, like artichoke by-product, is another step ahead on waste management and reduction of the main residues from the agro-food industry.

1. Introduction

Nowadays, in a growing world population, there is an increase in the demand for food, together with a change in eating habits and specifically of healthy foods. Therefore, it is necessary to get the most out of food. Zero hunger and importance of reducing food loss and waste are reflected in the goals 2 and 12 of the United Nations 2030 Agenda for Sustainable Development (UN, 2015). Losses in fruits and vegetables are greater than in other foods as cereal and legumes (FAO, 2019).

Globe artichoke (*Cynara cardunculus* L. (formerly *Cynara scolymus* L.) is a Mediterranean area plant, Italy and Spain being the main producing countries. In Spain, Murcia in the southeast is the main producing region where the predominant variety is "Blanca de Tudela".

The positive medicinal effects attributed to artichoke such as hepatoprotection, antioxidant, antimicrobial and anticholesterol among others are well known (Gebhardt, 2001; Gostin & Waisundara, 2019; Lattanzio, Kroon, Linsalata, & Cardinali, 2009). Except water, the main components of artichokes are carbohydrates (inulin, FOS and dietary fibre). Artichoke is widely used as dietary food due to its high fibre content, low calorie and minimal fat content. In addition, its important levels of essential minerals (potassium, sodium and magnesium), the presence of vitamins (C, B3 and E) and minor compounds (sterols and cynarin) provide beneficial health properties (FEN, 2015).

The edible part of artichoke plant is the capitulum or head, which approximately accounts for the 30% of its fresh weight (Lattanzio et al., 2009). The central part of the capitulum, the heart or core, is the most consumed of the artichoke. Thus, artichoke by-products are mainly composed by outer bracts, stalks and leaves (approx. 70% of its fresh weight).

The artichoke is destined for the fresh market, although it is mainly processed industrially (frozen, cooked and canned). The canning industry represents 70% of artichoke processed in Spain. This industry generates large amounts of artichoke by-products (bracts, stalks and leaves). Moreover, these by-products are perishable due to their high water content. Part of this waste is used for animal feeding after ensilage, but the remainder is generally discarded.

Nevertheless, artichoke by-products can be a valuable source of bioactive compounds with nutraceuticals properties. In the same way as heart of artichoke does, its by-products can provide inulin, phenolic compounds and pectins among other beneficial compounds (Llorach,

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Espín, Tomás-Barberán, & Ferreres, 2002; Sabater, Sabater, Olano, Montilla, & Corzo, 2020). For this reason, artichoke by-products could be used as "ingredient" in food additives, food supplements and nutraceuticals.

Other vegetable by-products from onion (Campone et al., 2018) and olive oil production (Gullón et al., 2020) are already used to obtain bioactive compounds.

The edible part of artichoke is well physico-chemically characterized. Several studies show the antioxidant capacity due to phenolic compounds such as cynarin (1,5-dicaffeoylquinic acid) and chlorogenic acid (5-caffeoylquinic acid) (Claus et al., 2015; Llorach et al., 2002; Moglia et al., 2008).

There are few studies related to artichoke waste, focusing mainly on phenolic compounds. Phenolic contents in bracts have been reported (Llorach et al., 2002; Negro et al., 2012; Pandino, Lombardo, Mauromicale, & Williamson, 2011; Zuorro, Maffei, & Lavecchia, 2016), in whole artichoke waste and its antioxidant capacity on Caco2-cells (Jiménez-Moreno et al., 2019); caffeoylquinic acid and inositols (Mena-García, Rodríguez-Sánchez, Ruiz-Matute, & Sanz, 2020). In addition, studies on artichoke by-products used as a source of dietary fibre (Femenia, Robertson, Waldron, & Selvendran, 1998) and specifically due to its inulin content (Castellino et al., 2020; Cavini et al., 2020; López-Molina et al., 2005; Ruiz-Cano et al., 2014) have been carried out.

Inulin is after starch, the most abundant storage carbohydrate in plants. It is classified as fructans, and it is formed by chains of D-fructose units linked via β-(2,1) glycosidic bonds with a terminal glucose. Inulin is indigestible by enzymes due to the β-(2,1) linkage which gives its prebiotic character. Therefore, it reaches the colon without alteration where the microbiota can ferment it (Apolinário et al., 2014). Colon metabolism is of great importance in human physiology (Meyer & Stasse-Wolthuis, 2009). Moreover, the colon microbiota is very heterogeneous, with more than 500 different species and a great interpersonal variability (Pham, Lemberg, & Day, 2008). However, it is assumed that the predominance of lactobacilli and bifidobacteria is related with a good health. Inulin and fructo-oligosaccharides (FOS, prebiotics) are preferred fermentation substrates for these beneficial bacteria. Positive effects of their growth are pH reduction and release of short chain fatty acids (SCFAs) in the gut. Acetic, propionic and butyric are the most common SCFAs produced but there are also other minor SCFAs released during fermentation; lactic acid bacteria also produce lactic acid.

Beneficial properties are attributed to SFCAs such as antioxidant, anti-inflammatory, antimicrobial, antiallergic, and inhibition of the growth of cancerous colonocytes (Tingirikari, 2018). Among other parts of artichoke waste, the outer bracts contain inulin (Lattanzio et al., 2009).

Briefly, there are very few studies investigating about the fermentability of artichoke by-products (López-Molina et al., 2005; Van den Abbeele et al., 2020; Zeaiter et al., 2019). For this reason, the main objective of this work was to evaluate the potential prebiotic effect of globe artichoke by-product by *in vitro* fermentability studies with pure cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. The use of artichoke by-products can also add value to the production of this vegetable and reduce the environmental impact of waste accumulation.

2. Material and methods

2.1. Raw material

Globe artichoke (*Cynara cardunculus*) var. Blanca de Tudela (formerly *Cynara scolymus* L.) was kindly provided by "Alcachofas de España" (Murcia, Spain). Raw artichoke by-product was composed by the outer bracts, stalks, leaves and the upper part of inflorescence (approximately 3 cm). After cutting in small pieces and freeze-drying, the artichoke by-product was milled to a particle size of less than 0.5 mm and stored for further use in a dry container at room temperature and protected from direct light.

2.2. Alcohol insoluble residue preparation

The powder of artichoke by-product was extracted (3% w/v) with ethanol (85% v/v) (Panreac, Darmstadt, Germany). Ten milliliter was collected (in triplicate), supernatant and residue were separated by centrifugation at 2,300×*g*, for 10 min (Centronic-S-577, PSelecta). The alcohol insoluble residue was oven dried at 60 °C and stored until analysis at room temperature. Powdered alcohol insoluble residue (AIR) from artichoke by-product was used for the fermentability assays.

2.3. Low molecular weight carbohydrates

The supernatant (alcohol soluble fraction) described above was collected, rotatory evaporated at 50 °C to dryness to remove ethanol and then suspended in ultrapure water (MilliQ Millipore Corp., Bedford, MA) to give a final concentration of 2 mg/mL. The water-soluble extract was filtered through 0.45 μm cellulose acetate syringe filters. Afterwards, low molecular weight carbohydrates (LMWC) were identified and quantified by HPLC-ELSD (Condezo-Hoyos, Pérez-López, & Rupérez, 2015). Commercial standard of inulin from chicory root was obtained from Sigma-Aldrich Chemicals (Alcobendas, Madrid, Spain), glucose, fructose and sucrose were of analytical grade (Panreac, Darmstadt, Germany).

2.4. Fermentation of artichoke-by product

2.4.1. Bacterial strains

Lactobacillus acidophilus (CECT 903) (Colección Española de Cultivos Tipo, Universidad de Valencia, Valencia, Spain, http://www.uv.es/cect/) and *Bifidobacterium bifidum* (DSMZ 20215) (Deutsche Sammlung von Mikroorganismen und Zeellkulturen, Braunschweig, Germany) were maintained as stock cultures on Man Rogosa and Sharpe (MRS) broth (Merck-Millipore, Darmstadt, Germany) on 20% of glycerol (Panreac, Darmstadt, Germany) and MRS broth with 20% of glycerol and 0.05% HCl-cysteine (Panreac, Darmstadt, Germany) respectively, at −80 °C for long-term storage.

2.4.2. Preparation of inoculum

An aliquot of the strain stock cultures was grown in 10 mL of MRS broth for *L. acidophilus* and 10 mL of MRS supplemented with 0.05% of cysteine for *B. bifidum*. Both bacteria were incubated at 37 °C for 48 h. Anaerobic jars with Anaerocult®A mini system (Merck Millipore, Darmstadt, Germany) were used for *B. bifidum*.

To standardize the inoculum, serial dilutions were made in 9 mL of Buffered Peptone Water, BPW (Panreac, Darmstadt, Germany), sowing in MRS Agar (*L. acidophilus*) and MRS cysteine-Agar (*B. bifidum*).

2.4.3. Culture medium

The culture medium for both bacteria was *Bifidobacterium* sp. medium (152 CECT). It was composed by casein peptone, meat extract, yeast extract, Tween® 80, sodium ascorbate, K_2HPO_4 and HCl-cysteine. All chemicals were purchased from Panreac (Darmstadt, Germany). The carbon source was 1% of sucrose in control batch and 1% of globe artichoke by-product in artichoke batch.

Five flasks for each batch were prepared with a culture medium volume of 40 mL and autoclaved at 121 °C for 15 min. After cooling to room temperature, a sterilized solution of 1% sodium ascorbate and 0.05% HCl-cysteine was added to the medium under sterile conditions and the inocula (with a final concentration of 10^4 colony-forming units, CFU/mL), being 50 mL the final volume in each flask. Two blank flasks were prepared under the same conditions without addition of inocula.

2.4.4. Fermentability assay

The incubation was carried out at 37 °C, in aerobic conditions for

L. acidophilus and in anaerobic jars with Anaerocult® A mini system for *B. bifidum*. Incubation was kept for 12, 24, 48, 72 and 96 h for both microorganisms.

One mL-aliquot from each flask was collected aseptically in duplicate for each incubation time.

Serial dilutions of aliquots were prepared in BPW and spread onto the surface of a MRS-agar Petri dish for *L. acidophilus* and a MRS-agar with HCl-cysteine Petri dish for *B. bifidum*. These Petri dishes were incubated under the same conditions used for inoculum. Then, the colonies were counted to determine the number of CFU/mL.

The flasks of artichoke by-product corresponding to 72 and 96 h were sown on the surface in agar *Bifidobacterium* sp. modified where the carbon source was replaced by the artichoke by-product. After that, they were incubated under the conditions described above to confirm the degree of growth and morphology of the colonies.

After incubation, pictures of individual colonies were taken with an Optika camera model Optikam B5 coupled to an optical stereomicroscope Leica S8APO.

The fermentation was followed by measuring: pH of culture medium, substrate consumption, short-chain fatty acids (SCFAs) production by gas chromatography (GC) and lactic acid production by ion chromatography (IC). Samples were kept at −20 °C until analysis.

2.5. Measurement of pH

The pH was measured in culture medium with pH test strips (Panreac, Darmstadt, Germany).

2.6. Determination of short chain fatty acids

An aliquot of sample (0.4 mL) with 0.1 mL of methyl valeric acid (Sigma Aldrich, St. Louis, USA) as internal standard (394 μM) was acidified with 0.3 mL of phosphoric acid (0.5%). Fatty acid standards were prepared under the same conditions and the extraction of SFCA was carried out with 0.7 mL of n-butanol (García-Villalba et al., 2012).

One microlitre of organic phase was injected in split less mode in a gas chromatograph Agilent 6890A (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector. SCFAs were separated using a DB-WAXtr polyethylene glycol capillary column (60 m length, 0.325 mm i.d. x 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA, USA). The injector and detector temperatures were 250 °C and 260 °C respectively. The oven temperature was set initially at 50 °C and held for 2 min, then raised to 150 °C at a rate of 15 °C/min, to 200 °C at a rate of 5 °C/min, to 240 °C at a rate of 15 °C/min and held for 20 min. Helium was the carrier gas at a flow rate of 1.5 mL/min. Individual short chain fatty acids were identified on the basis of their retention times using FAMQ-4 Certified Reference Material (AccuStandard Inc, New Haven, USA) and quantified with WSFA-2 Mix Supelco Standards of fatty acids (Sigma Aldrich, St. Louis, USA).

2.7. Determination of lactic acid

Samples from L. *acidophilus* and *B. bifidum* culture media were diluted with ultrapure H_2O (MilliQ). Lactic acid (as lactate anion) was analyzed by ion chromatography on a Metrohm Advanced Compact equipment with a conductivity detector (Metrohm AG, Switzerland). Sample (20 μL) was injected and the separation was performed on a Metrosep Organic Acids column (250 mm length, 4 mm i.d., 5 μm particle size; Metrohm AG, Switzerland) with 0.5 mM sulphuric acid/15% acetone as mobile phase at a flow rate of 0.5 mL/min. Quantification of lactate was carried out with a calibration curve (5–500 μg/mL) of standard Sodium L-Lactate (Sigma Aldrich, St. Louis, USA).

2.8. Substrate consumption

The content of each flask was centrifuged at 2,300×*g* for 15 min at 4 °C (Centronic-S-577, PSelecta). After removing of supernatant, the pellet was freeze-dried and determined gravimetrically.

Table 1
Low molecular weight carbohydrates determined by HPLC in aqueous-soluble extracts of artichoke by-product.

AIR (3% w:v) aqueous extracts	LMWC (g/100 g dw)				
	Glucose	Fructose	Sucrose	Inulin	Total (%)
	2.87 ± 0.16	2.23 ± 0.09	0.98 ± 0.05	0.05 ± 0.01	6.14

Mean values ± SD (n = 3).
AIR: alcohol insoluble residue.
LMWC: Low molecular weight carbohydrates.

2.9. Statistical treatment

Results were expressed as the mean values followed by the standard deviation (SD) of at least three replicates. Statistical analyses were performed using Statgraphics Centurion XVIII (Herndon, VA, USA). Analysis of variance (ANOVA) and the least significant difference (LSD) test were applied to determine differences between means. Differences among samples throughout incubation time were evaluated. Differences were considered to be significant at $p < 0.05$.

3. Results and discussion

Raw by-product was formed by the outer bracts and leaves of globe artichoke, which major component is dietary fibre (63.19 ± 0.78 g/100 g, dry matter), and more specifically insoluble dietary fibre (51.59 ± 2.59 g/100 g, d.m.) (Giambra, 2017).

Our preliminary results showed that there was no microbial growth when raw globe artichoke by-product was used as substrate for *Lactobacillus acidophilus*. This fact could be possibly attributed to antimicrobial compounds present in raw artichoke material (El Sohaimy, 2014; Falleh et al., 2008). Furthermore, there are also studies that attribute to artichoke extracts antifungal, anti-VIH and antigenotoxic effects (Miadokova et al., 2008; Zhu, Zhang, & Lo, 2005).

Therefore, to study the potential prebiotic effect of artichoke by-product, soluble sugars, polyphenols and pigments were first removed by ethanolic extraction to obtain the alcohol insoluble residue. It is well known that the alcohol insoluble residue from agro-food waste is rich in dietary fibre. The supernatant of AIR from artichoke by-product contained a 6.14% of low molecular weight carbohydrates (LMWC) glucose and fructose being the main carbohydrates (Table 1). Regarding glucose, levels are in agreement with the characterization of LMWC in artichoke by-product obtained by Villanueva-Suárez and co-workers (Villanueva-Suárez, Mateos-Aparicio, Pérez-Cózar, Yokoyama, & Redondo-Cuenca, 2019). Furthermore, similar contents of reducing sugars were found by Zeaiter and colleagues with an ultrasound extraction combined with ethanol precipitation of artichoke by-product from *Cynara scolymus*, although reported inulin content was higher than in the present study (Zeaiter et al., 2019). This difference could be attributed to different subspecies of *Cynara* artichoke used, plant parts composing artichoke by-product, harvest season and geographical origin of artichoke, among others.

3.1. In vitro fermentability assay

Regarding *in vitro* fermentability assays, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* showed a different behaviour in both, control and sample batches (Fig. 1).

In sucrose control batch, colony formation units of *L. acidophilus* showed a sharp increase with a maximum at 48 h and, and then a decrease at the same rate. However, *L. acidophilus* grew gradually on artichoke by-product culture from the beginning, without showing a

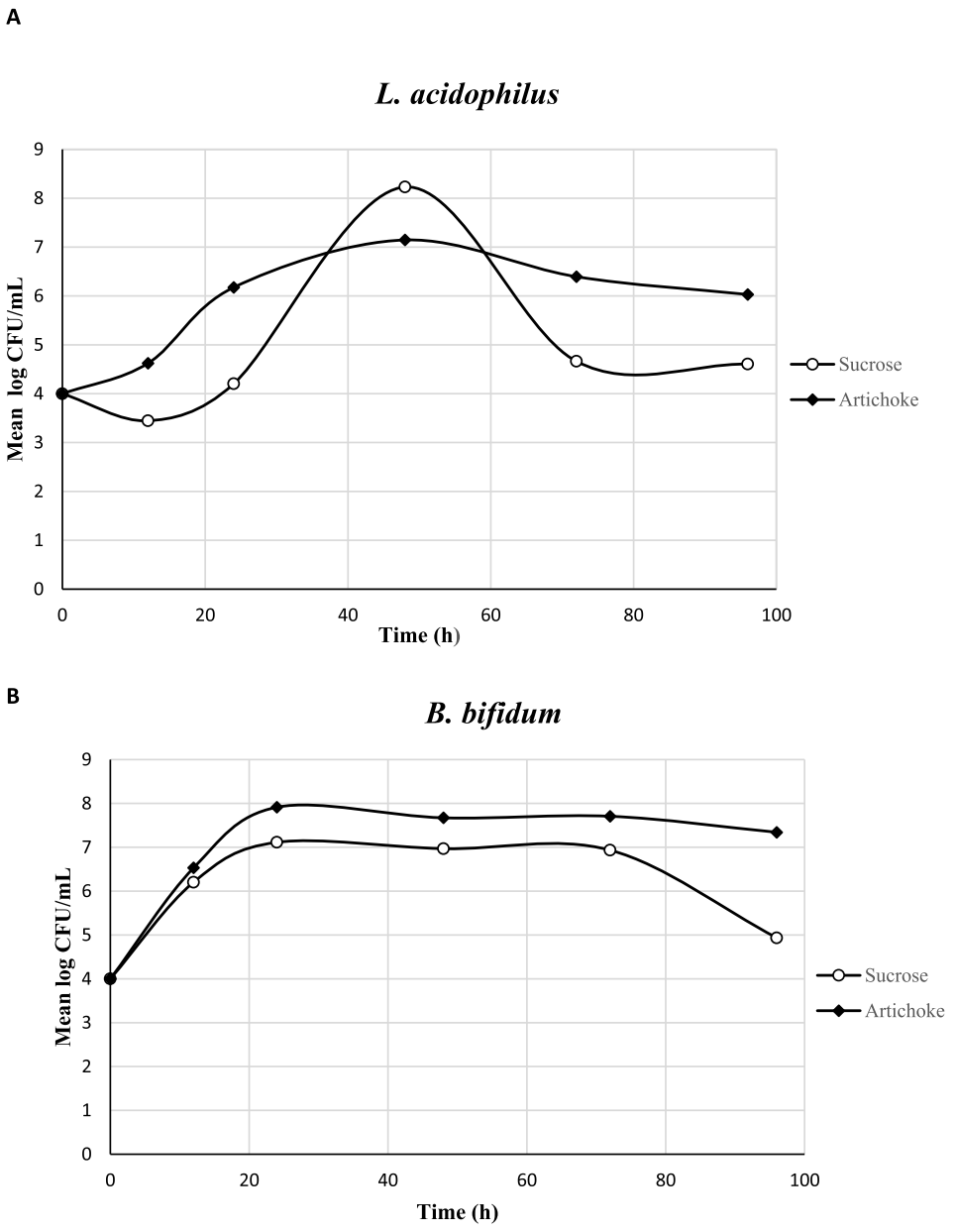

Fig. 1. *In vitro* fermentability of sucrose and globe artichoke by-product by pure cultures of *Lactobacillus acidophilus* (1A) and *Bifidobacterium bifidum* (1B).

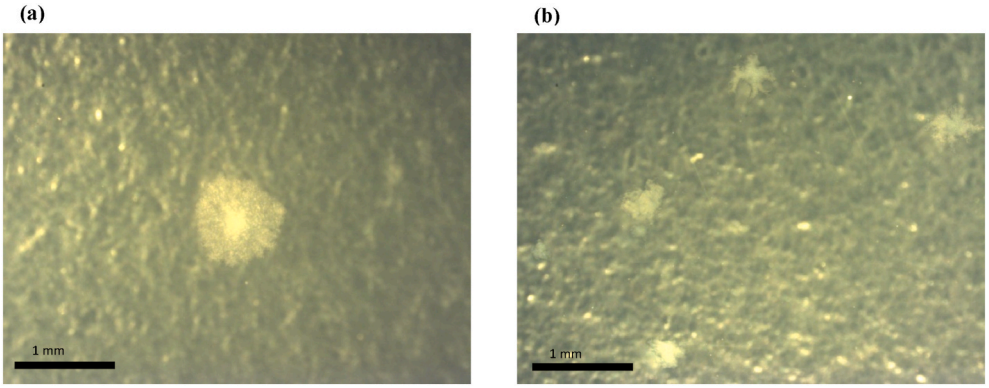

Fig. 2. Pictures of *Lactobacillus acidophilus* colonies grown on MRS for 72 h. (a) MRS with *L.a* grown on sucrose (magnification 1.25x). (b) MRS with *L.a* grown on artichoke by-product (magnification 1.25x).

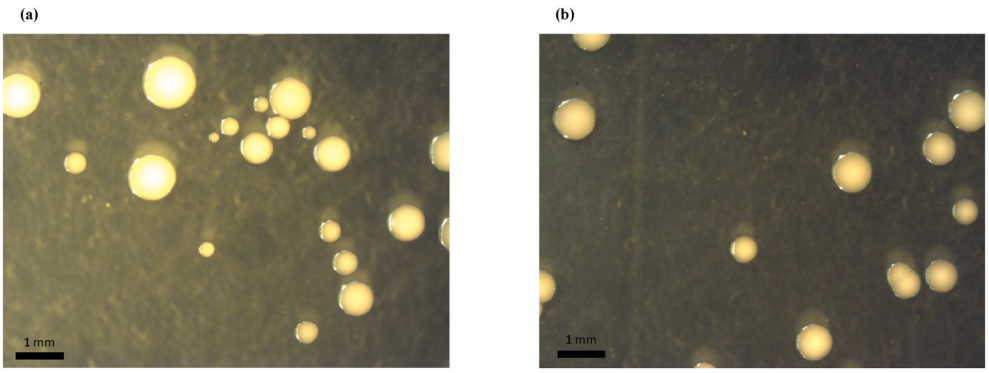

Fig. 3. Pictures of *Bifidobacterium bifidum* colonies grown on MRS for 96 h. (a) MRS with *B.b* grown on sucrose (magnification 1x). (b) MRS with *B.b* grown on artichoke by-product (magnification 1x).

pronounced maximum over the incubation times studied (Fig. 1A). Sucrose and higher molecular weight FOS are preferred substrates for lactobacilli genera (Gänzle & Follador, 2012).

The growth of *B. bifidum* was similar in both control and sample batches (Fig. 1B). Colonies developed gradually until 24 h and reached a plateau, in artichoke by-product being higher throughout all assay. However, at the end of incubation (96 h) there was a pronounced decrease in sucrose batch, approximately two log CFU/mL lower than in artichoke by-product.

Both bacteria grew on artichoke by-product as carbon source. The difference in growth pattern can be attributed to different carbohydrate metabolism by both microorganisms.

Meanwhile sugar metabolism in *Lactobacillus acidophilus* is mainly carried out by intracellular enzymes, in *Bifidobacterium bifidum* this metabolism is related with an extracellular hydrolysis linked to transport of complex carbohydrates (Gänzle & Follador, 2012).

The present study over 96 h allowed verifying the growth of each bacterium under its optimal conditions (aerobic or anaerobic). Despite not having excessively high inulin content, inulin and the contribution of FOS confer prebiotic character to artichoke by-product. These results agree with the study commented above, after incubation of *L. acidophilus* and *B. bifidum* with artichoke by-product for 48, the last one grown under anaerobic conditions (Zeaiter et al., 2019). The bifidogenic effect of aqueous extracts from the edible part of artichoke has also been found on an *in vitro* gut model based on the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) (Van den Abbeele et al., 2020). The bifidogenic effect of very-long-chain inulin extracted from globe artichoke has been shown *in vivo* (Costabile et al., 2010).

The morphology of both bacteria was also different on agar plates. Colonies of L. *acidophilus* were flat, rough, with irregular edges, star-like, cotton-fluffy appearance and white colour (Fig. 2A and B). *B. bifidum* developed smooth, convex, glistening colonies with entire edges, soft consistency and cream to white colour (Fig. 3A and B).

For each particular bacterium, *L. acidophilus* or *B. bifidum*, no differences were found in their colony morphology or size when they were grown either on sucrose or artichoke - MRS media on agar plates.

3.2. Short chain fatty acids

Acetic, butyric and propionic acids are the most abundant SCFAs in the gut (Cummings, 1981). Tables 2 and 3 show the contents of these end-products of fermentation for each bacterium.

Regarding L. *acidophilus*, in sucrose control batch the content of SCFAs increased significantly over incubation time, being at 12 h 2-fold higher than initial content. The production of SCFAs increased continuously up to 48 h, thus a marked pH reduction was observed. The release of SFCAs reduces pH inside the colon; therefore, it improves the solubility of ions as calcium which reabsorption is related with bone benefit (Flint, Scott, Louis, & Duncan, 2012).

This increase in total SFCAs was exclusively due to significant formation of acetic acid. As it was expected, acetic acid was the main SCFA produced in *L. acidophilus* fermentation. Therefore, the molar ratio of acetic and propionic acids increased throughout the experiment, especially every 24 h (Table 2).

The artichoke batch initially showed a significantly higher SCFAs content than sucrose batch. The development of SCFAs was also significantly higher, although it was not reflected in a drastic pH reduction. The ratio of acetic and butyric acid increased 25-fold at the end of experiment.

The formation of SFCAs in *L. acidophilus* culture was evaluated up to 72 h, because at this time a high bacterial growth was not observed in both batches. The pictures of *L. acidophilus* colonies shown in Fig. 2 correspond to this incubation time.

The development of SCFAs in *B. bifidum* cultured on sucrose was higher than *L. acidophilus* from 24 h (being at 72 h the ratio of acetic: propionic: butyric acid 37:1:3 *vs* 71:1:3 in *L. acidophilus* and *B. bifidum* sucrose batches, respectively). As commented above, acetic acid was the major SCFA, although in this sample butyric and propionic acids also increased significantly. In spite of a marked increase of acetic acid at 96 h, it was not reflected in a strong reduction of culture pH. Regarding artichoke batch, it was also an excellent substrate for *in vitro* fermentation by *B. bifidum*. Total SCFAs content was higher in artichoke batch than in sucrose batch for *B. bifidum* as observed for *L. acidophilus* culture, except at 96 h. However, a similar ratio of acetic and butyric acid was reached at the end of the experiment, regarding that observed for *L. acidophilus* (26-fold).

The production of SCFAs by intestinal bacteria is desirable to maintain an adequate colon function and they are a source of energy for the host supporting approximately 10% of energy requirement when they are absorbed in the colon (Flint et al., 2012). Moreover, these organic acids that connect the microbiome with the immune system to maintain homeostasis and their production are also related to the expression of peptides and hormones (Sleeth, Thompson, Ford, Varghese, & Frost, 2010; Tingirikari, 2018).

3.3. pH

Artichoke batches of both bacteria did not show a significant decrease of pH (Tables 2 and 3). This behaviour is also found in a commercial extract from artichoke inflorescences (Van den Abbeele et al., 2020). Although there was only a slight reduction of pH by the production of SCFAs, it could limit the development of potentially pathogenic microorganisms. Previous results of our group with soybean Okara showed that substrate fermentability is not always accompanied by pH decrease (Espinosa-Martos & Rupérez, 2009a). Furthermore, the reduction of pH is a good fermentability marker when it is associated with the SCFAs production, but in some plant fibres (for example soybean fibre), both parameters are not apparently related; therefore,

Table 2
pH and short chain fatty acids released by *in vitro* fermentation of sucrose and globe artichoke by-product by pure cultures of *Lactobacillus acidophilus*.

Fermentation time (h)		Concentration (μM)							Acetic:propionic:butyric
Sucrose	pH	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Caproic	Total	
0	7.5	626.08 ± 8.8 aA	58.50 ± 0.84 cA	16.46 ± 1.27 cA	118.75 ± 10.11 aA	14.01 ± 0.07 bA	23.06 ± 0.49 aA	**856.86 ± 13.48 aA**	11:1:2
12	7.5	1339.38 ± 23.7 bA	54.16 ± 0.28 bA	11.60 ± 1.16 bA	131.62 ± 13.10 aA	14.67 ± 0.32 bA	26.47 ± 1.82 bcA	**1577.91 ± 27.13 bA**	25:1:2
24	7	1340.08 ± 30.6 bA	55.30 ± 0.61bcA	10.74 ± 0.99 bA	136.84 ± 13.75 aA	14.39 ± 0.40 bA	26.71 ± 1.38 cA	**1584.06 ± 33.56 bA**	24:1:2
48	4.5	1890.60 ± 44.8 cA	51.43 ± 2.12 bA	9.60 ± 0.91 abA	137.02 ± 13.64 aA	14.91 ± 1.19 bA	21.86 ± 0.04 aA	**2125.43 ± 46.93 dA**	37:1:3
72	4.5	1734.99 ± 52.3 dA	47.11 ± 2.45 aA	6.34 ± 0.41 aA	120.73 ± 0.68 aA	11.79 ± 0.14 aA	23.81 ± 0.36 abA	**1944.77 ± 52.41 cA**	37:1:3
Artichoke									
0	7	1344.25 ± 11.2 aB	60.76 ± 4.09 aA	15.12 ± 1.00 cA	106.36 ± 8.05 aA	21.46 ± 0.46 dB	22.65 ± 0.31 aA	**1570.60 ± 14.42 aB**	22:1:2
12	7	2019.36 ± 10.5 bB	55.10 ± 1.28abA	11.38 ± 1.14 bA	104.27 ± 6.34 aA	22.27 ± 0.32 eB	22.95 ± 0.07 aA	**2235.33 ± 12.38 bB**	37:1:2
24	6.5	2154.26 ± 24.5 cB	57.17 ± 3.17abA	10.31 ± 0.22 bA	123.67 ± 12.17 aA	19.67 ± 0.40 cB	22.84 ± 0.43 aB	**2387.91 ± 27.59 cB**	38:1:2
48	6	2368.10 ± 34.3 dB	54.25 ± 0.46 bA	6.77 ± 0.64 aB	107.77 ± 1.87 aA	15.60 ± 0.13 aA	26.76 ± 0.06 bB	**2579.26 ± 34.31 dB**	44:1:2
72	6	2924.32 ± 47.1 eB	58.70 ± 0.48 abB	10.05 ± 0.90 bB	126.51 ± 10.39 aA	17.78 ± 0.05 bB	26.09 ± 0.39 bB	**3163.44 ± 48.29 eB**	50:1:2

Mean values ± SD (n = 3).
Lowercase letters mean significant differences in each SCFA along time in each type of sample.
Uppercase letters mean significant differences among samples for each SCFA at the same fermentation time.
SCFA determined by gas-liquid chromatography.

Table 3
pH and short chain fatty acids released by *in vitro* fermentation of sucrose and globe artichoke by-product by pure cultures of *Bifidobacterium bifidum*.

Fermentation time (h)		Concentration (μM)							Acetic:propionic:butyric
Sucrose	pH	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Caproic	Total	
0	7.5	661.47 ± 12.76 aA	26.88 ± 1.03 aB	21.15 ± 0.69 aA	83.76 ± 1.60 aA	15.28 ± 0.34 aA	23.92 ± 0.60 cdA	**832.5 ± 12.9 aA**	25:1:3
12	6.5	807.07 ± 2.53 bA	35.46 ± 0.34 bcdA	26.82 ± 0.33 bcA	106.39 ± 2.42 bB	14.58 ± 0.68 aA	22.84 ± 0.24 bcA	**1013.2 ± 3.6 bA**	23:1:3
24	6.5	1055.05 ± 18.74 cA	35.14 ± 1.75 bcA	27.78 ± 2.48 cdA	111.33 ± 0.75 bcB	14.74 ± 1.26 aA	23.02 ± 0.47 cA	**1267.1 ± 19.0 cA**	30:1:3
48	6.5	1895.11 ± 20.20 dA	33.94 ± 1.26 bA	25.08 ± 1.94 bA	116.80 ± 1.79 cA	14.33 ± 0.76 aA	21.84 ± 0.12 abA	**2107.1 ± 20.4 dA**	56:1:3
72	6.5	2702.61 ± 135.71 eB	37.97 ± 3.21 cdB	29.54 ± 0.25 deA	124.85 ± 6.16 dB	15.00 ± 1.70 aA	24.22 ± 1.31 dB	**2934.2 ± 135.9 eA**	71:1:3
96[a]	6	4113.85 ± 71.99 fB	38.23 ± 0.40 dA	31.64 ± 1.74 eA	126.99 ± 4.58 dA	15.37 ± 1.45 aA	21.25 ± 0.07 aA	**4352.4 ± 72.2 fA**	108:1:3
Artichoke									
0	7	1772.47 ± 35.41 bB	24.32 ± 0.79 aA	20.61 ± 0.76 aA	84.89 ± 2.02 aA	26.78 ± 1.97 bB	25.31 ± 1.18 bB	**1952.4 ± 35.6 bB**	73:1:3
12	6.5	1501.66 ± 24.47 aB	34.04 ± 1.36 bA	28.98 ± 0.49 bcA	101.19 ± 0.16 bA	18.74 ± 0.72 aB	22.70 ± 0.17 aA	**1707.3 ± 24.5 aB**	44:1:3
24	6	1987.46 ± 50.98 cB	34.83 ± 0.20 bA	27.63 ± 0.95 bA	103.97 ± 3.22 bA	17.52 ± 0.34 aB	22.68 ± 0.43 aA	**2195.2 ± 51.1 cB**	57:1:3
48	6	2527.25 ± 20.70 dB	34.57 ± 0.72 bA	28.44 ± 1.49 bB	112.17 ± 2.92 cA	18.78 ± 1.49 aB	23.20 ± 0.18 aB	**2744.4 ± 21.0 dB**	73:1:3
72	6	2564.93 ± 49.38 dA	34.34 ± 0.37 bA	31.17 ± 1.45 cdA	114.80 ± 1.22 cA	19.32 ± 0.41 aB	22.82 ± 0.49 aA	**2787.4 ± 49.4 dB**	75:1:3
96	6	2927.90 ± 15.50 eA	37.89 ± 1.64 cA	31.79 ± 2.02 dA	123.82 ± 2.03 dA	19.12 ± 1.06 aB	22.31 ± 0.37 aA	**3162.8 ± 15.9 eB**	77:1:3

Mean values ± SD (n = 3).
Lowercase letters mean significant differences in each SCFA along time in each type of sample.
Uppercase letters mean significant differences among samples for each SCFA at the same fermentation time.
SCFA determined by gas-liquid chromatography.
[a] Traces of valeric acid.

Table 4
Lactic acid content produced by *in vitro* fermentation of sucrose and globe artichoke by-product by pure cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum*.

	L. acidophilus		B. bifidum	
	Sucrose	Artichoke	Sucrose	Artichoke
Fermentation time (h)	Lactic acid (mM)			
0	1.74 ± 0.10 aA	1.62 ± 0.10 aA	1.74 ± 0.10 aA	1.69 ± 0.09 aA
12	2.57 ± 0.01 bA	2.36 ± 0.04 bA	2.21 ± 0.02 bA	2.36 ± 0.01 bB
24	2.57 ± 0.07 bA	6.90 ± 0.22 cB	2.35 ± 0.04 bA	2.30 ± 0.03 bA
48	46.78 ± 0.06 cB	10.21 ± 0.09 eA	2.76 ± 0.01 cA	2.86 ± 0.08 cA
72	60.98 ± 0.32 dB	9.22 ± 0.31 dA	2.41 ± 0.01 bA	2.76 ± 0.02 cB

Mean values ± SD (n = 3).
Lowercase letters mean significant differences in lactic acid content over time on each different substrate and for each individual bacterium.
Uppercase letters mean significant differences between samples at the same fermentation time and for each individual bacterium.
Lactic acid determined by ion chromatography.

fermentability should not only be evaluated by the pH reduction or SCFAs production, but also by the substrate consumed (Espinosa-Martos & Rupérez, 2009a, 2009b).

3.4. Lactic acid

Lactic acid produced was analyzed in *L. acidophilus* and *B. bifidum* over fermentation time (Table 4). Lactic acid can be substrate for other intestinal microbiota species and it can be degraded to short-chain fatty acids as propionic and butyric. Butyric acid is well known for its high anticarcinogenic potential (Commane, Hughes, Shortt, & Rowlan, 2005). The lactic acid content produced by both bacteria was very different. Regarding L. *acidophilus* culture, sucrose control showed a significantly higher content than artichoke sample at 24 h, and reached contents of 61 mM at 72 h. This increase in sucrose batch was 20-fold higher from 24 to 48 h, coinciding with the maximum growth of the colonies (Fig. 1A). However, the progression of lactate production on artichoke by-product was much slower than on sucrose control at 72 h and reached 9 mM vs 61 mM, respectively.

On the other hand, *B. bifidum* culture showed lower contents of lactic acid than *L. acidophilus* on both substrates. No significant differences were observed between substrates for the same incubation time, except for 24 h and 72 h. In addition, the maximum content of lactic acid was similar for both batches (2.76 vs 2.86 mM in sucrose and artichoke, respectively). Bifidobacteria can produce lactic acid to a lesser or greater extent depending on the specific gender and strain selected. Low or no lactic acid production is reported previously for other strains of *B. bifidum* different from the one used in this work (Scalabrini, Rossi, Spettoli, & Matteuzzi, 1998; Espinosa-Martos & Rupérez, 2009a).

3.5. Substrate consumption

The gravimetric determination of non-fermented insoluble residue in artichoke batch at 72 h was 56.2% and 41.8% for *L. acidophilus* and *B. bifidum*, respectively. Thus, artichoke by-product was consumed almost in half. The artichoke by-product consumption by both bacteria implies the bifidogenic capacity of artichoke-by product.

The present study is a first approach to the re-use of artichoke by-product as prebiotic. After further research, it could be considered as ingredient in foods, food supplements and nutraceuticals.

4. Conclusions

The artichoke by-product supported growth and was fermented *in vitro* by *Lactobacillus acidophillus* and *Bifidobacterium bifidum*, suggesting its potential prebiotic effect. This bifidogenic capacity has been shown by substrate consumption, bacterial growth, and production of short chain fatty acids and lactic acid. Valorisation of artichoke by-product could increase the benefit of its production process, minimize its environmental impact and favour circular economy.

CRediT authorship contribution statement

Francisca Holgado: Methodology, Formal analysis, Writing – original draft. **Gema Campos-Monfort:** Investigation. **Cristina de las Heras:** Methodology, Investigation. **Pilar Rupérez:** Conceptualization, Experimental design, Validation, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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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References

Apolinário, A. C., Goulart de Lima Damasceno, B. P., Macêdo Beltrão, N. E., Pessoa, A., Converti, A., & Da Silva, J. A. (2014). Inulin-type fructans: A review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers, 101*, 368–378. https://doi.org/10.1016/j.carbpol.2013.09.081

Campone, L., Celano, R., Piccinelli, A. L., Pagano, I., Carabetta, S., Di Sanz, R., et al. (2018). Response surface methodology to optimize supercritical carbon dioxide/cosolvent extraction of brown onion skin by-product as source of nutraceutical compounds. *Food Chemistry, 269*, 495–502. https://doi.org/10.1016/j.foodchem.2018.07.042

Castellino, M., Renna, M., Leoni, B., Calasso, M., Difonzo, G., Santamaria, P., et al. (2020). Conventional and unconventional recovery of inulin rich extracts for food use from the roots of globe artichoke. *Food Hydrocolloids, 107*, 105975. https://doi.org/10.1016/j.foodhyd.2020.105975

Cavini, S., Guzzetti, L., Givoia, F., Regonesi, M. E., Di Gennaro, P., Magoni, C., et al. (2020). Artichoke (*Cynara cardunculus* var. *scolymus* L.) by-products as a source of inulin: How to valorise an agricultural supply chain extracting an added-value compound. *Natural Product Research*, 2020. https://doi.org/10.1080/14786419.2020.1841188.

Claus, T., Maruyama, S. A., Palombini, S. V., Montanher, P. F., Bonaf, E. G., de Oliveira Santos Junior, O., et al. (2015). Chemical characterization and use of artichoke parts for protection from oxidative stress in canola oil. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 61*, 346–351. https://doi.org/10.1016/j.lwt.2014.12.050

Commane, D., Hughes, R., Shortt, C., & Rowlan, I. (2005). The potential mechanisms involved in the anti-carcinogenic action of probiotics. *Mutation Research, 591*, 276–289. https://doi.org/10.1016/j.mrfmmm.2005.02.027

Condezo-Hoyos, L., Pérez-López, E., & Rupérez, P. (2015). Improved evaporative light scattering detection for carbohydrate analysis. *Food Chemistry, 180*, 265–271. https://doi.org/10.1016/j.foodchem.2015.02.039

Costabile, A., Kolida, S., Klinder, A., Gietl, E., Baüerlein, M., Frohberg, C., et al. (2010). A double-blind, placebo-controlled, cross-over study to establish the bifidogenic effect of a very-long-chain inulin extracted from globe artichoke (*Cynara scolymus*) in healthy human subjects. *British Journal of Nutrition, 104*, 1007–1017. https://doi.org/10.1017/S0007114510001571

Cummings, J. H. (1981). Short chain fatty acids in the human colon. *Gut, 22*, 763–779. https://doi.org/10.1136/gut.22.9.763

El Sohaimy, S. A. (2014). Chemical composition, antioxidant and antimicrobial potential of artichoke. *The Open Nutraceuticals Journal, 7*, 15–20. https://doi.org/10.2174/1876396001407010015

Espinosa-Martos, I., & Rupérez, P. (2009a). Indigestible fraction of okara from soybean: Composition, physicochemical properties and *in vitro* fermentability by pure cultures of *Lactobacillus acidophilus* and. *Bifidobacterium bifidum. European Food Research and Technology, 228*, 685–693. https://doi.org/10.1007/s00217-008-0979-7

Espinosa-Martos, I., & Rupérez, P. (2009b). Indigestible fraction of okara from soybean: composition, physicochemical properties and in vitro fermentability by pure cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum. European Food Research and Technology, 228*, 847. https://doi.org/10.1007/s00217-009-1016-1. Erratum to: Eur Food Res Technol DOI 10.1007/s00217-008-0979-7.

Falleh, H., Ksouri, R., Chaieb, K., Karray-Bouraoui, N., Trabelsi, N., Boulaaba, M., et al. (2008). Phenolic composition of *Cynara cardunculus* L. organs, and their biological activities. *Comptes Rendus Biologies, 331*, 372–379. https://doi.org/10.1016/j.crvi.2008.02.008

FAO, Food and Agricultural Organization. The State of Food and Agriculture. (2019). *Moving forward on food loss and waste reduction. Rome*. Licence: CC BY-NC-SA 3.0IGO. Retrieved from: http://www.fao.org/3/ca6030en/ca6030en.pdf Accessed 20 September 2021.

Femenia, A., Robertson, J. A., Waldron, K. W., & Selvendran, R. R. (1998). Cauliflower (*Brassica oleracea* L), globe artichoke (*Cynara scolymus*) and chicory witloof (*Cichorium intybus*) processing by-products as sources of dietary fibre. *Journal of the Science of Food and Agriculture, 77*, 511–518. https://doi.org/10.1002/(SICI)1097-0010(199808)77:4<511::AID-JSFA74>3.0.CO;2-2

FEN 2015- Fundación Española de la Nutrición. (2015). *Verduras y hortalizas*. Alcachofa (*Cynara scolymus L.*). https://www.fen.org.es 〉 pdfs 〉 alcachofa.

Flint, H. J., Scott, K. P., Louis, P., & Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology & Hepatology, 9*, 577–589. https://doi.org/10.1038/nrgastro.2012.156

Gänzle, M. G., & Follador, R. (2012). Metabolism of oligosaccharides and starch in lactobacilli: A review. *Frontiers in Microbiology, 3*, 340. https://doi.org/10.3389/fmicb.2012.00340

García-Villalba, R., Giménez-Bastida, J. A., García-Conesa, M. T., Tomás-Barberán, F. A., Espín, J. C., & Larrosa, M. (2012). Alternative method for gas chromatography-mass spectrometry analysis of short-chain fatty acids in faecal samples. *Journal of Separation Science, 35*, 1906–1913. https://doi.org/10.1002/jssc.201101121

Gebhardt, R. (2001). Anticholestatic activity of flavonoids from artichoke (*Cynara scolymus L.*) and of their metabolites. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research, 7*, 316–320. PMID: 12211745.

Giambra, E. (2017). In A. Redondo-Cuenca, & P. Rupérez (Eds.), *Facoltà di Scienze Agrarie e Alimentari, Università degli Studi di Milano: 141. Enhancement of prebiotic functionality of artichoke by-products. Tesi di Laurea Magistrale*. Italy.

Gostin, A. I., & Waisundara, V. Y. (2019). Edible flowers as functional food: A review on artichoke (*Cynara cardunculus* L.). *Trends in Food Science & Technology, 86*, 381–391. https://doi.org/10.1016/j.tifs.2019.02.015

Gullón, P., Gullón, B., Astray, G., Carpena, M., Fraga-Corral, M., Prieto, M. A., et al. (2020). Valorization of by-products from olive oil industry and added-value applications for innovative functional foods. *Food Research International, 137*, 109683. https://doi.org/10.1016/j.foodres.2020.109683

Jiménez-Moreno, N., Cimminelli, M. J., Volpe, F., Ansó, R., Esparza, I., Mármol, I., et al. (2019). Phenolic composition of artichoke waste and its antioxidant capacity on differentiated Caco-2 Cells. *Nutrients, 11*, 1723–1738. https://doi.org/10.3390/nu11081723

Lattanzio, V., Kroon, P. A., Linsalata, V., & Cardinali, A. (2009). Globe artichoke: A functional food and source of nutraceutical ingredients. *Journal of Functional Foods, 1*, 131–144. https://doi.org/10.1016/j.jff.2009.01.002

Llorach, R., Espín, J. C., Tomás-Barberán, F. A., & Ferreres, F. (2002). Artichoke (*Cynara scolymus* L.) byproducts as a potential source of health-promoting antioxidant phenolics. *Journal of the Science of Food and Agriculture, 50*, 3458–3464. https://doi.org/10.1021/jf0200570

López-Molina, D., Navarro-Martín, M. D., Melgarejo, F. R., Hiner, A. N. P., Chazarra, S., & Rodríguez-López, J. N. (2005). Molecular properties and prebiotic effect of inulin obtained from artichoke (*Cynara scolymus* L.). *Phytochemistry, 66*, 1476–1484. https://doi.org/10.1016/j.phytochem.2005.04.003

Mena-García, A., Rodríguez-Sánchez, S., Ruiz-Matute, A. I., & Sanz, M. L. (2020). Exploitation of artichoke byproducts to obtain bioactive extracts enriched in inositols and caffeoylquinic acids by Microwave Assisted Extraction. *Journal of Chromatography A, 1613*, 460703. https://doi.org/10.1016/j.chroma.2019.460703

Meyer, D., & Stasse-Wolthuis, M. (2009). The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *European Journal of Clinical Nutrition, 63*, 1277–1289. https://doi.org/10.1038/ejcn.2009.64

Miadokova, E., Nadova, S., Vlckova, V., Duhova, V., Kopaskova, M., Cipak, L., et al. (2008). Antigenotoxic effect of extract from *Cynara carduncul*us L. *Phytotherapy Research, 22*, 77–81. https://doi.org/10.1002/ptr.2268

Moglia, A., Lanteri, S., Comino, C., Acquadro, A., De Vos, R., & Beekwilder, J. (2008). Stress-induced biosynthesis of dicaffeoylquinic acids in Globe Artichoke. *Journal of Agricultural and Food Chemistry, 56*, 8641–8649. https://doi.org/10.1021/jf801653w

Negro, D., Montesano, V., Grieco, S., Crupi, P., Sarli, G., De Lisi, A., et al. (2012). Polyphenol compounds in artichoke plant tissues and varieties. *Journal of Food Science, 77*, 244–252. https://doi.org/10.1111/j.1750-3841.2011.02531.x

Pandino, G., Lombardo, S., Mauromicale, G., & Williamson, G. (2011). Profile of polyphenols and phenolic acids in bracts and receptacles of globe artichoke (*Cynara cardunculus* var. *scolymus*) germplasm. *Journal of Food Composition and Analysis, 24*, 148–153. https://doi.org/10.1016/j.jfca.2010.04.010

Pham, M., Lemberg, D. A., & Day, S. (2008). Probiotics: Sorting the evidence from the myths. *Medical Journal of Australia, 188*, 304–308. https://doi.org/10.5694/j.1326-5377.2008.tb01627.x

Ruiz-Cano, D., Pérez-Llamas, F., Frutos, M. J., Arnao, M. B., Espinosa, C., López-Jiménez, J. A., et al. (2014). Chemical and functional properties of the different by-products of artichoke (*Cynara scolymus* L.) from industrial canning processing. *Food Chemistry, 160*, 134–140. https://doi.org/10.1016/j.foodchem.2014.03.091

Sabater, C., Sabater, V., Olano, A., Montilla, A., & Corzo, N. (2020). Ultrasound-assisted extraction of pectin from artichoke by-products. An artificial neural network approach to pectin characterization. *Food Hydrocolloids, 98*, 105–238. https://doi.org/10.1016/j.foodhyd.2019.105238

Scalabrini, P., Rossi, M., Spettoli, P., & Matteuzzi, D. (1998). Characterization of Bifidobacterium strains for use in soymilk fermentation. *International Journal of Food Microbiology, 39*, 213–219. https://doi.org/10.1016/s0168-1605(98)00005-1

Sleeth, M. L., Thompson, E. L., Ford, H. E., Zac-Varghese, S. E. K., & Frost, G. (2010). Free fatty acid receptor 2 and nutrient sensing: A proposed role for fibre, fermentable carbohydrates and short-chain fatty acids in appetite regulation. *Nutrition Research Reviews, 23*, 135–145. https://doi.org/10.1017/S0954422410000089

Tingirikari, J. M. R. (2018). Microbiota-accessible pectic poly- and oligosaccharides in gut health. *Food Function, 9*, 5059. https://doi.org/10.1039/C8FO01296B

United Nations. (2015). *Transforming our world: The 2030 agenda for sustainable development. A/RES/70/1*. Retrieved from: https://sdgs.un.org/sites/default/files/publications/21252030%20Agenda%20for%20Sustainable%20Development%20web.pdf Accessed: 20 September 2021.

Van den Abbeele, P., Ghyselinck, J., Marzorati, M., Villar, A., Zangara, A., Smidt, C. R., et al. (2020). *In vitro* evaluation of prebiotic properties of a commercial artichoke inflorescence extract revealed bifidogenic effects. *Nutrients, 12*, 1552. https://doi.org/10.3390/nu12061552

Villanueva-Suárez, M. J., Mateos-Aparicio, I., Pérez-Cózar, M. L., Yokoyama, W., & Redondo-Cuenca, A. (2019). Hypolipidemic effects of dietary fibre from an artichoke by-product in Syrian hamsters. *Journal of Functional Foods, 56*, 156–162. https://doi.org/10.1016/j.jff.2019.03.013

Zeaiter, Z., Regonesi, M. E., Cavini, S., Labra, M., Sello, G., & Di Gennaro, P. (2019). Extraction and characterization of inulin-type fructans from artichoke wastes and their effect on the growth of intestinal bacteria associated with health. *BioMed Research International, 2019*, 8 pages. https://doi.org/10.1155/2019/1083952. Article ID 1083952.

Zhu, X. F., Zhang, H. X., & Lo, R. (2005). Antifungal activity of *Cynara scolymus* L. extracts. *Fitoterapia, 76*, 108–111. https://doi.org/10.1016/j.fitote.2004.10.016

Zuorro, A., Maffei, G., & Lavecchia, R. (2016). Reuse potential of artichoke (*Cynara scolymus L.*) waste for the recovery of phenolic compounds and bioenergy. *Journal of Cleaner Production, 111*, 279–284. https://doi.org/10.1016/j.jclepro.2015.06.011