



Biorefinery of discarded carrot juice to produce carotenoids and fermentation products

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ARTICLE INFO

Handling editor: Cecilia Maria Villas Bôas de Almeida

Keywords:

Ethanol
Lactic acid
Fermentation
Pigments
Encapsulation

ABSTRACT

Discarded carrots are a major food waste that is produced from cultivation to sale. This waste has high humidity, which generates not only economic but also environmental problems, requiring a specific biorefinery for its valorization. The pulp and the juice were separated and received a different treatment. The juice was subjected to a process of separation and purification using several diafiltration cycles (30 kDa) giving rise to a fraction rich in carotenoids (4996.4 $\mu\text{g/g}$) and a fraction rich in sugars (84.83 \pm 3.26 g/L) and nutrients. The carotenoids have potential applications in the food and pharmaceutical industry; therefore, they were formulated through encapsulation with gum Arabic using spray drying and freeze drying. Encapsulation using spray drying was effective as it improved stability in water and reduced degradation by 51.9% compared to unencapsulated carotenoids. The sugar-rich fraction was valorized to obtain lactic acid and ethanol through three types of fermentation: with autochthonous microorganisms, with lactic acid bacteria, and with yeast, resulting in obtaining mostly lactic acid (17.64 \pm 1.54 g/L) or ethanol (49.46 \pm 0.28 g/L). The addition of 6% (w/v) of NaCl to the medium allowed the production of pure lactic acid with both autochthonous microorganisms and lactic bacteria. Sugar consumption was high (92.4–97.5%) except in cases with autochthonous microorganisms (23.3%) and lactic bacteria (43.8%) where a pH control seems to be necessary for total sugar consumption.

1. Introduction

In the European Union alone ca. 100 million tons of food waste are spoiled per year, which accounts for 35% of the total food production (Marić et al., 2018). Within the types of food waste, vegetable waste is at the top reaching a value of more than 24 million tons annually in Europe (Heredía-Guerrero et al., 2014). These residues are generated at various stages from cultivation to sale. Some examples are processing damage, lack of storage space, rejections by buyers, and supermarket waste due to reaching the expiry date (RajiniPriya et al., 2018). As an example, the percentages of waste generated in the processing industry for certain fruits reach figures of 30–50% for mango, 20% for banana, 40–50% for pomegranate, and 30–50% for citrus fruits (Banerjee et al., 2017).

The problem generated by this waste has both economic and environmental consequences. Vegetal waste has a high moisture content, so incineration is not an option due to its low efficiency (Banerjee et al., 2017). The management of this waste is therefore delicate, as there is no clear valorization. Most often a small part of the vegetal waste goes to animal feed while the majority goes to landfills. The disposal of this

waste in a landfill is associated with the emission of greenhouse gases. Waste from food processing has proven to be the third-largest source of greenhouse gas emissions after total emissions from China and the USA (Banerjee et al., 2017). For this reason, legal restrictions on the handling of these wastes have been tightened over the last decade (Nayak and Bhushan, 2019). Without forgetting that the best option is to prevent the generation of waste, the European Union guidelines have focused on its reuse and recycling so that energy or materials can be produced from these raw materials within a circular economy (Nayak and Bhushan, 2019). The companies that must deal with the problem of food vegetable waste usually have a relatively simple technology or are of medium size, so that currently the valorization of the waste is not applied in a majority way. Given the situation, it is to be expected that in the near future practices may change.

Carrot (*Daucus carota* L.) is a root vegetable widely consumed around the world, with a global annual production of close to 36 million tons (Encalada et al., 2019). It stands out as a source of valuable compounds such as vitamins (A, B, C, D, E, and K), carotenoids, minerals, and dietary fiber, all of which give its very healthy properties. Roughly 25–30% of

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<https://doi.org/10.1016/j.jclepro.2021.129139>

Received 30 March 2021; Received in revised form 22 July 2021; Accepted 20 September 2021

Available online 22 September 2021

0959-6526/© 2021 The Author(s).

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picked carrots are discarded by growers because they do not meet market quality standards such as color, size, diameter, length, or shape (Encalada et al., 2019). This results in the localized production of large tons of waste. Fruit and vegetable companies sometimes have inadequate space or equipment for handling carrot solid waste. Another type of carrot waste appears when carrots are discarded at the supermarket or at home. All these discards create an economic and environmental problem, as only 15–20% of discarded carrots are consumed by animals, the vast majority are being dumped, generating bad odors through decomposition, and the proliferation of insects or other pests (Clementz et al., 2019). The solution to this problem would be the recovery of the waste derived from the carrot for the valorization of its main components as well as the potential generation of high value-added products (Hiranvarachath and Devahastin, 2014). In a previous work we first juiced the discarded carrots and tackled the valorization of discarded carrot pulp (Ramos-Andrés et al., 2020). This work focuses on a valorization of the juice through its main components: carotenoids and sugars.

1.1. Carotenoids

Carotenoids are a family of natural pigments synthesized by certain plants, algae, and bacteria (Sun et al., 2010). They stand out for their antioxidant components, such as β -carotene with important health benefits including protection against cardiovascular disease and cataract disease (Yasuda et al., 2017). Most commercial carotenoids are produced through chemical synthesis (Kaur et al., 2019). In the extraction of carotenoids from agricultural residues, organic solvents such as hexane and acetone are normally used, resulting in a process that is harmful to the environment and risky for human consumption.

Once the carotenoids are extracted it is of great interest for their preservation to proceed to their formulation in solid state as a powdered product. This formulation improved storage conditions (Shishir and Chen, 2017). Likewise, the morphology in powder form makes it possible to meet the requirements for application in the food, pharmaceutical and cosmetic industries (Shishir and Chen, 2017). The most common drying methods in this sector are spray drying and freeze drying. Freeze drying is very suitable for drying heat-sensitive materials, as it takes place at room temperature and preserves the temperature-sensitive properties almost intact (Ballesteros et al., 2017). On the other hand, spray drying operates at high temperature but it is about 4–5 times more economical than freeze drying due to lower energy consumption combined with shorter drying times (Shishir and Chen, 2017). Within the drying process, microencapsulation stands out, where small particles of the material to be dried are covered by a thin film of a protective material. The coating protects the core from oxidative degradation and the evaporation of volatile substances (Mahfoudhi and Hamdi, 2015). The materials usually used as encapsulating agents are starches, gum Arabic, methylcellulose, gelatin, whey proteins, corn syrup, maltodextrins, disaccharides, pullulan, and sodium caseinate (Mahfoudhi and Hamdi, 2015). Gum Arabic stands out for its high water solubility, low viscosity, and emulsifying capacity (Mahfoudhi and Hamdi, 2015).

Several studies have focused on the drying of carrot extracts. Guldiken et al. (2019) used spray drying to obtain coated and uncoated liposomes with encapsulated black carrot extract, studying the degradation of anthocyanins. The liposomal encapsulated black carrot anthocyanins are physically and chemically more stable. Similar to the present work, Janiszewska-Turak et al. (2017) investigated the production of encapsulated carrot juice microcapsules using spray drying. Different encapsulating materials were used (maltodextrin, gum Arabic, mixtures of both, and whey protein isolate). The chemical and physicochemical properties of the microcapsules were studied, resulting in gum Arabic being the best encapsulating agent in terms of carotenoid retention and powder stability. A higher dry matter content, higher glass transition temperature, lower water activity, and better hygroscopic

properties were obtained with gum Arabic.

1.2. Free sugar monomers

Discarded carrots are rich in sucrose, glucose and fructose, and various minerals as well. The sugars and the other nutrients make carrots suitable for the growth of microorganisms (Aimaretti and Ybalo, 2012). The autochthonous microorganisms in carrots are lactic acid bacteria (LAB) with characteristics that vary depending on the quality of the vegetable, climate, and growing conditions (Gardner et al., 2001). Fermentation in carrots or their derivatives, as well as in other vegetables, is usually characterized by a succession of hetero- and homo-fermentative autochthonous lactic acid bacteria, with or without yeast, responsible for the co-production of ethanol and other compounds (Di Cagno et al., 2008).

Most of the work related to fermentation of carrots has been developed to improve some of their properties in the food industry, such as the production of probiotic products. Rakin et al. (2007) studied the nutritional improvement of vegetable juices through lactic fermentation using brewer's yeast autolysate as an additive. Gardner et al. (2001) performed a study of several LAB, most of them commercial, for the fermentation of cabbage, carrot, and beet-based vegetable products. In addition, they evaluated the development of the microorganisms during both the fermentation and storage period. The study showed that lactic acid fermentation of vegetables is a sequential process with a lactic fermentation followed by variations and secondary fermentation by yeast. This is not desirable in the industry so low ethanol production and rapid acidification are sought to improve stability.

Discarded carrots have also been used to produce bioethanol. It is worth mentioning the study of Aimaretti and Ybalo (2012), who used carrot and yeast discards for the production of second generation bioethanol, obtaining values of 0.41 g/g of ethanol yield and a productivity of 0.4 g/L/h without the need to make any modification to the broth (carrot must). The discarded carrots stood out for providing an appropriate C:N balance for ethanol fermentation. Clementz et al. (2019) also used discarded carrots for ethanol production. In their case, they used two different broths: the juice and a sugar-rich extract from the carrot obtained with hot water. The main difference between the two broths was the presence of carotenoids and fibres which were in the juice but not in the extract. Consequently, the broth used in the present work would be in some way equivalent to that of extracted sugars since neither carotenoids nor fibres were present. In their research, they showed that both broths were adequate without the need for enrichment.

The objective of the present research was to valorize discarded carrot juice through its two main components: carotenoids and free sugars. Fractionation was carried out through environmentally friendly physical processes of separation, purification and drying, using water and no chemical agents. Through several diafiltration cycles, the insoluble part of the juice (carotenoids) was separated from the soluble part (sugar and nutrients). To the best of our knowledge, there are no studies in which carotenoids are recovered from a vegetal residue through physical processes without the need for temperature treatment or chemical agents. Likewise, in the present work the fraction obtained was formulated through drying and microencapsulation using the two most common methods: spray drying and freeze drying. Previous research using carrots has not reported such an abundant fraction in carotenoids as the present fraction since it was purified thanks to the separation of the sugars. The microencapsulation using spray drying allowed to significantly reduce the degradation of carotenoids in the solid product and made it potentially suitable for use in food, pharmaceutical and/or cosmetic industries. The sugar-rich fraction was valorized for the production of ethanol and lactic acid through three types of fermentation including fermentation with autochthonous microorganisms. The effect of NaCl as an additive was analyzed for the three types of fermentation. Although certain studies have demonstrated the benefits of carrot

fermentation, the fermentation broth used in this work is notable for its abundant content of sugars and nutrients, and for its lack of components not required for fermentation such as fibres and carotenoids. The broth is also interesting because it has been obtained without the need to perform hot water extraction but only physical separation processes.

2. Materials and methods

2.1. Raw material preparation and characterization

The discarded carrots were provided by Muñozval S.L. (Valladolid, Spain). They were washed with water and stored in a cool, dry and dark place for very short periods while maintaining their properties. The juice was obtained from the pulp using a juice extractor (Moulinex ZU5008 Infinity Press Revolution) with 300 W of power. Every 1 kg of fresh carrots yielded approximately 0.455 kg of juice.

The juice of discarded carrots was characterized by determining the composition of soluble solids using HPLC according to the method in Section 2.6.1. The total solids of the juice were recovered using freeze drying after diafiltration process according to Section 2.4., and their structure was subsequently studied using FTIR (section 2.6.2). The non-soluble solids in the juice, containing most of the recovered carotenoids, are microparticles of carrot pulp, so it was assumed that their composition was the same as that determined in the pulp in our previous work (Ramos-Andrés et al., 2020).

2.2. Separation of sugars and pigments using diafiltration

The juice was subjected to a separation and purification process applying several cycles of diafiltration. In each cycle, a specific volume of water was added to the feed tank and subjected to ultrafiltration until the feed recovers its original volume. The added water was removed in the ultrafiltration through the permeate (diapermeate), dragging small substances considered impurities. Diafiltration cycles were applied until the concentration of impurities in the feed was reduced to the desired value. The feed after diafiltration (diafeed) maintained its volume and the components with the highest molecular weight, which are purified by reducing the presence of the remaining components. In the particular case of juice from discarded carrots, diafeed contained the non-soluble solids of the juice accompanied by a minimum sugar content while diapermeate contained the sugars, vitamins, minerals and other soluble substances of molecular weight lower than the molecular weight cut-off (MWCO) of the membrane.

The first step was microfiltration to remove the particles of size higher than 50 μm and prevent fouling problems. After microfiltration, the concentration of the feed was reduced by diluting the juice, decreasing the total soluble compounds in a 50% (v/v) (from 111.28 to 55.64 g/L). Subsequent purification of the retained pigments was performed by a multi-step diafiltration process. For this purpose, a Pellicon 2 Mini Biomax polymeric ultrafiltration membrane, flat sheet configuration, made of polyethersulfone was used, with MWCO of 30 kDa, and filtration area of 0.1 m^2 . During diafiltration, a known volume of water (50% of the feed volume) was added to the feed and it was collected in the permeate. By this process the concentration of small substances (mainly free sugars) is reduced as they are forced to cross the membrane. The determination of soluble solids during the treatment of the juice was carried out with a handheld refractometer measuring the concentration of soluble solids in degrees Brix ($^{\circ}\text{Bx}$). The feed underwent six diafiltration cycles until its $^{\circ}\text{Bx}$ and the $^{\circ}\text{Bx}$ of the feed were constant and reduced to almost zero.

Liquid samples were taken from feed liquor (feed), feed after diafiltration (diafeed), and permeates from all the diafiltration cycles (diaperm-1, diaperm-2, diaperm-3, diaperm-4, diaperm-5 and diaperm-6). The composition of these samples was analyzed using HPLC according to the method described in section 2.6.1. The concentration of total solids of the diafeed was determined gravimetrically, drying at

47 $^{\circ}\text{C}$ until constant weight in order to encapsulate the pigments with the proper ratio. Total carotenoid content (TCC) was also determined in diafeed according to section 2.6.3.

2.3. Spray drying of the encapsulated pigments

Pigments recovered in diafeed were encapsulated by mixing with gum Arabic in a ratio of 1:1 (w/w) considering the total concentration of solids of diafeed. The mixing was carried out using magnetic stirring for 12 h obtaining a homogeneous solution. The solution was dried using spray drying in a Mobile Minor spray dryer with a rotary atomizer, from Gea Niro. The liquid solution was pumped using a peristaltic pump (Watson-Marlow 520R) working with a flow rate of 1 L/h approx. Pressure in the dryer nozzle was fixed at 6 bar. The inlet air temperature was 150 $^{\circ}\text{C}$. The outlet air temperature was in the range 90–95 $^{\circ}\text{C}$, controlled by the feed liquid flow. After drying, a powder of encapsulated pigments was obtained (spray-1:1) and characterized structurally using FTIR. The TCC was also determined.

2.4. Freeze drying of the juice and pigments fractions

Freeze drying was used as an alternative method for drying pigments with and without the encapsulating agent. The total solids of diafeed were freeze-dried without encapsulation, and also a fraction was mixed for 12 h with different ratios of total-solids:encapsulating-agent: 1:0.5, 1:1, and 1:1.5 (freeze-1:0.5, freeze-1:1, and freeze-1:1.5, respectively). For this drying, the four liquid products were frozen overnight at -25°C and then freeze-dried under vacuum (0.180 mbar) and a condenser temperature of -50°C for 96 h using a Telstar Lyoquest -55°C (Terrassa, Spain). The TCC of diafeed, freeze-1:0.5, freeze-1:1, and freeze-1:1.5 was determined. Samples were characterized structurally using FTIR.

2.5. Fermentation of the sugar-rich fraction

Carrot juice from discarded carrots was ultrafiltered employing the membrane described in section 2.2 with a feed volume reduction of 80% (v/v). The permeate from this ultrafiltration was used as fermentation broth having a sugar concentration of 84.83 ± 3.26 g/L. Three kinds of fermentations were carried out: with native microorganisms, with bacteria to produce lactic acid (*Lactobacillus reuteri* RC-14® and *Lactobacillus rhamnosus* GR-1®), and with yeast to produce ethanol (*Saccharomyces cerevisiae*). Each of the three fermentations was performed with and without the addition of 6% (w/v) NaCl to the broth (30 kDa-permeate from carrot juice). These broths were used also as the incubation broths. The addition of 6% (w/v) NaCl was performed to study its effect on the prevention of external yeast invasion. Incubation was performed for 24 h under stirring, anaerobic conditions, and 35 $^{\circ}\text{C}$. The concentration of microorganisms in the inoculum was 2% (w/v) for *Saccharomyces cerevisiae* (Mondelēz International, EEUU) and 0.89% (w/v) for *Lactobacillus reuteri* and *Lactobacillus rhamnosus* (Chr. Hansen, Denmark). After that period the microorganisms were inoculated using the same fermentation broth in a ratio of 50% (v/v) forming a total volume of 20 mL in glass serum bottles. The experiments were carried out in duplicate. Liquid samples were taken periodically and analyzed to determine the microorganism concentration (g/L) and chemical composition using HPLC according to the method described in Section 2.6.1.

2.6. Analysis

2.6.1. Chemical characterization

Composition analysis of the liquid samples was done using high-performance liquid chromatography (HPLC) as in previous research (Ramos-Andrés et al., 2019). A SUGAR SH-1011 Shodex column was used for the identification and quantification of sugars, aldehydes,

organic acids, and ethanol. The column was at 50 °C and the mobile phase had a flow of 0.8 mL/min of 0.01 N sulfuric acid in Milli-Q water. The compounds were identified and quantified with a Waters IR detector 2414. All the samples were filtered (Pore size 0.22 µm, diameter 25 mm, Nylon; FILTER-LAB) before the HPLC analysis. The standards employed for the analysis were: sucrose (99%), galacturonic acid (97%), glucose (99%), fructose (99%), pyruvaldehyde (99%), formic acid (98%), acetic acid (98%), levulinic acid (98%), acrylic acid (99%), and ethanol (99.5%), all of them from Sigma-Aldrich.

2.6.2. Structural characterization

The encapsulation process was studied by analyzing the structural configuration of the dry pigments with and without the encapsulating agent, dried using spray drying and using freeze drying with different encapsulating ratios. The chemical structure was analyzed using attenuated total reflectance (ATR) – Fourier transform infrared spectroscopy (FT-IR) (Bruker, Alpha model, with a Platinum ATR single reflection diamond module). Absorbance spectra were determined in the wavenumber range from 4000 to 400 cm⁻¹, acquiring 64 scans per sample at a resolution of 4 cm⁻¹.

2.6.3. Determination of total carotenoid content (TCC)

The TCC was determined for the dry pigments with and without the encapsulating agent, dried using spray drying and using freeze drying. Carotenoids were extracted with a process similar to Knockaert et al. (2012). 0.05 g of sample was mixed with 2 mL of hexane and submitted to vigorous stirring in a vortex mixer for 5 min. After the extraction process, the sample was centrifuged for 3 min at 10000 rpm and the liquid was recovered. The liquid sample was diluted until the maximum absorbance was in the range 0.7–1.0 in a UV 2550 Shimadzu spectrophotometer (Kyoto, Japan). TCC in this liquid was calculated as follows:

$$TCC(\mu\text{g/g}) = \frac{A \times V \times 10^4}{E_{1\text{cm}}^{1\%} \times M}$$

where A was sample absorbance at 450 nm, V was the total volume of extract (mL), M was the sample weight (g), $E_{1\text{cm}}^{1\%}$ was the extinction coefficient (2560, for beta-carotene in hexane).

2.6.4. Measurement of the concentration of microorganisms

The biomass concentration of microorganism over time during fermentation was determined from samples of defined volume. Each sample was centrifuged for 3 min at 10000 rpm. The supernatant was separated and the solid residue was dried in an oven until constant weight. The dry mass was considered as the mass of the microorganism present in the given volume.

3. Results and discussion

3.1. Raw material characterization

The juice had a content of sugars of 111.28 g/L, valorized through the permeate. The composition was: sucrose (70.52 g/L), glucose (28.89 g/L) and fructose (11.87 g/L). The non-soluble solids were microparticles of the carrot pulp and contained the carotenoids. The TCC in the total solids of the juice was determined after diafiltration and it is discussed in section 3.3.

3.2. Separation of sugars and pigments from carrot juice using diafiltration

The feasibility of the process of separating sugars from pigments by applying several diafiltration cycles was evaluated. After microfiltration of carrot juice to remove particles bigger than 50 µm, and dilution by 50% (v/v), the diafiltration cycles were performed until the concentration of sugars in the feed was reduced. Throughout the process, the

diapermeates were sampled for composition analysis (Table 1). The concentration of the three sugars present in the feed was reduced with the cycles from initial values of 35.26 g/L of sucrose, 14.44 g/L of glucose, and 5.94 g/L of fructose to final values of 3.15 g/L of sucrose, 1.39 g/L of glucose, and 0.61 g/L of fructose. Consequently, the diafiltration process increased the purity of the carotenoids by reducing the total concentration of sugars by 90.7% (91.1% for sucrose, 90.4% for glucose, and 89.7% for fructose). Although the concentration of carotenoids did not increase because the volume of the feed remained constant, the ratio of carotenoids to total solids increased, resulting in a dry product highly concentrated in carotenoids, as discussed in section 3.3.

The amount of water required for the diafiltration process plays an important role and depends on the feed to be treated, the type of membrane, and the operating conditions. In this case, the removal of 90.7% of the sugars required a volume 7 times the original volume of the juice (considering the water used before diafiltration to dilute the juice to 50% (v/v)). The diapermeates were an excellent broth for fermentation, rich in both sugars and nutrients, as demonstrated in section 3.4. Alternatively, these diapermeates could be freeze-dried obtaining a solid sugary product enriched with vitamins and minerals with an aesthetically pleasing white colour (see Fig. 2).

Fig. 1 shows the transmembrane pressure (TMP) and permeate flow values during each diafiltration cycle. Permeate flow and TMP are typical variables that indicate membrane fouling (Nakajima, 2014). As the first diafiltration cycle took place, the evolution of the permeate flow was typical of an ultrafiltration operation: it starts a decline and then gradually decreases and finally reaches a certain steady state. This permeate flow decline was caused by membrane fouling which leads to an increase in hydraulic resistance, increasing also the TMP. The significant effect of fouling in this first diafiltration cycle increased TMP from 1.5 to 2.5 bar and the permeate flow was reduced from 500.0 to 299.3 mL/m²/min. The rapid initial drop in permeate flow was due to the blockage of the membrane pores, which were initially totally clean. This blocking process is fast compared to the cake formation as a single layer of particles is sufficient to block the pores (Nakajima, 2014). After the first diafiltration cycle, fouling occurred, but its effects stabilized with a smaller increase in the TMP, even stabilizing at 2.95 bar. The permeate flow also stabilized around 260 mL/m²/min. In the last diafiltration cycle, the permeate flow decreased again reaching a final value of 179.6 mL/m²/min. The fact that the flow decreased again could be due to the formation and growth of a cake layer on the membrane surface. The blocking of the pores increases the mass transfer resistance to permeate flow while the formation of a cake creates additional resistance (Nakajima, 2014). As the TMP increased from 1.5 to 2.95 bar, this pressure seemed a critical pressure, after which concentration polarization becomes enough for the retained solutes at the membrane surface to the formation of a cake. Once a cake layer has formed, further increase in TMP did not lead to further increase in the permeate flux, so they were not related anymore (Nakajima, 2014).

Once all the diafiltration cycles had been completed, the characterization of the purified feed (diafeed) was performed. The total solids content was determined to proceed with the microencapsulation of the pigments in the proper ratios. Soluble solids of diafeed were determined using HPLC (Table 1). Part of diafeed was freeze-dried and the TCC and their degradation were determined as explained in the following section.

3.3. Microencapsulation and drying of the pigments from carrot juice

3.3.1. Degradation during drying

The TCC was determined for each of the solid samples. These samples were diafeed freeze-dried (diafeed), diafeed encapsulated with the solid: encapsulating-agent ratio 1:1 and spray-dried (spray-1:1), and diafeed encapsulated with the ratios 1:0.5, 1:1 and 1:1.5 and freeze-dried (freeze-1:0.5, freeze-1:1, and freeze-1:1.5). According to the ratios of the encapsulating agent, each sample should have a carotenoid content according to the content of the control sample diafeed. However, the

Table 1
Composition of the diafiltration waters and the feed before and after the diafiltration cycles.

	Feed	Diaperm1	Diaperm2	Diaperm3	Diaperm4	Diaperm5	Diaperm6	Diafeed
Sucrose (g/L)	35.26	22.20	15.75	10.17	7.21	4.80	3.28	3.15
Glucose (g/L)	14.44	9.30	6.58	4.40	2.95	1.88	1.26	1.39
Fructose (g/L)	5.94	3.96	2.74	1.72	1.08	0.79	0.53	0.61

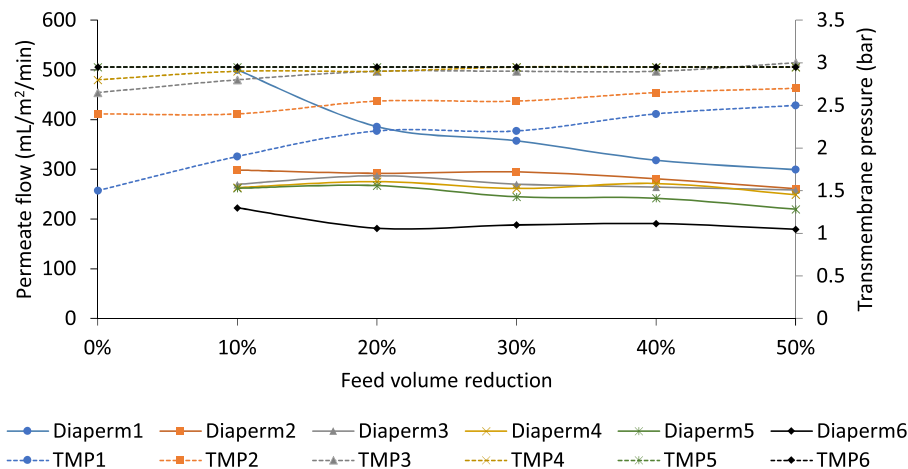


Fig. 1. Evolution of permeate flow (Diaperm, ml/m²/min) and transmembrane pressure (TMP, bar) during the six diafiltration cycles.

TCC was generally lower than expected, as can be seen in Table 2. This decrease in TCC after encapsulation has been attributed to a loss by degradation during the drying process, which was 54.7% (w/w) for spray drying, and 10.8% (w/w) and 19.8% (w/w) for freeze drying in ratios of 1:1 and 1:1.5, respectively. The freeze-1:0.5 sample did not seem to have suffered degradation since its content was even a 7% (w/w) higher than the theoretical content, which could be attributed to some experimental error. Consequently, spray drying was the method that degraded the carotenoids the most, even with encapsulant. During spray drying the carotenoids were in contact with high temperature air, which favours oxidation. On the other hand, during freeze drying, the drying process was performed at a condenser temperature of -50 °C and a pressure of 0.180 mbar. Part of the carotenoids was also degraded during freeze drying, since the sample was in contact with the surroundings during approximately 3 days. The degradation was greater when the proportion of encapsulant agent was higher. This may be because the encapsulating agent did not actually coat the pigments in the freeze drying encapsulation. Consequently, and as some studies have mentioned before (Mahfoudhi and Hamdi, 2015), the microporous structure resulting from freeze drying favours the diffusion of oxygen and light through the polymeric material, in this case gum Arabic, leaving the pigments more exposed to degradation. The greater the presence of the encapsulating agent and therefore the lesser the presence of pigments, the lighter weight the product and therefore the more microporous it is and the more prone to degradation due to the lack of encapsulation.

Table 2
Percentage of degradation of total carotenoids during drying and 15 days-storage depending on the drying method and the proportion of encapsulant.

	Total solids:Arabic gum	Drying method	TCC (µg/g sample)			Degradation (% w/w)	
			Theoretical before drying	After drying	After 15 days	Drying	Storage
Diafeed	1:0	Freeze drying		4996.4	4072.6		18.5%
Spray-1:1	1:1	Spray drying	2498.2	1131.7	1031.2	54.7%	8.9%
Freeze-1:0.5	1:0.5	Freeze drying	3330.9	3564.0	2046.6	n.d.	42.6%
Freeze-1:1	1:1	Freeze drying	2498.2	2228.4	1362.8	10.8%	38.8%
Freeze-1:1.5	1:1.5	Freeze drying	1998.5	1602.9	892.2	19.8%	44.3%

Janiszewska-Turak et al. (2017) carried out drying and formation of microcapsules of carrot juice using spray drying with an air inlet temperature of 160 °C. The encapsulations with gum Arabic proved to be more efficient. Their carotenoid concentration values were 1443 ± 86 µg/g dry matter for carrots and 860 ± 22 µg/g d.m. for carrot juice. Microcapsules encapsulated with whey protein isolate showed the highest concentration of carotene with a value of 573 µg/g d.m., while those encapsulated with maltodextrin had the lower concentration of carotenoids with a value of 423 µg/g d.m. Given their results, the spray drying encapsulation efficiency was between 48% for maltodextrin and 66% for whey protein isolate. In our case, the encapsulation efficiency with gum Arabic using spray drying was 45.3%. Due to the separation of the sugars from the juice using diafiltration, the concentration of carotenoids in the solids obtained in the present work was much higher. The non-encapsulate control solid (diafeed) had a concentration of carotenoids 4996.4 mg/kg, which would mean an increase in the carotenoid concentration close to 5.8-fold with respect to the juice solids because of the purification. The carotenoid concentration of the spray dried encapsulated product (spray-1:1) was 1131.7 mg/kg, which was close to the carotenoid concentration of carrots on a dry basis, but the carotenoids are much more stable and manageable in the spray-1:1 product.

3.3.2. Degradation during storage

The degradation of carotenoids over time was evaluated by comparing the content detected on the day of drying with the content

after 15-day storage in dark conditions at 4 °C. As can be seen in Table 2, the sample without encapsulation (diafeed) suffered degradation of 18.5% (w/w) of the TCC. The sample encapsulated using spray drying (spray-1:1) suffered less degradation, 8.9% (w/w). As it can be seen in Fig. 2, the solid product obtained after this drying was homogeneous, which seems to indicate effective encapsulation. In addition, the encapsulating agent was water-soluble, which increased the stability of the carotenoids in an aqueous medium, giving rise to a homogeneous dispersion in the liquid in which no visual suspended particles were present (Fig. 2). This increases the potential applications of the pigments in the food and pharmaceutical industry. On the other hand, degradation in the freeze-dried encapsulated pigments during storage was higher than in the spray-dried encapsulated sample and was even higher than in the freeze-dried non-encapsulated sample. This confirms that in this case, the use of Arabic gum in freeze drying did not lead to encapsulation of the carotenoids as it did not protect them from degradation. Besides not protecting them, it seems to favor degradation, as the values obtained were 42.6% (w/w) (1:0.5), 38.8% (w/w) (1:1), and 44.3% (w/w) (1:1.5) compared to 18.5% (w/w) without encapsulating agent. These values were relatively similar to each other which would indicate that the gum Arabic did not encapsulate effectively.

Nogueira et al. (2017) determined the efficiency of carotenoids encapsulation using freeze drying. The carotenoids were recovered in pure form, unlike in the present work where they were recovered as micro/nanoparticles of pulp. Encapsulation using freeze drying was efficient when working with carotenoids in their pure form. About 65%

of the total carotenoids were encapsulated in their work with soy protein. The remaining percentage stayed dispersed among the microparticles and therefore exposed to the environment and degradation. Finally, they explained that the encapsulant ratio does not significantly influence the encapsulation efficiency as long as there is enough encapsulant. In their work, Nogueira et al. (2017) explained that after freeze drying, the encapsulated product was in the form of microparticles. This was not the case in other studies that applied freeze drying (Sousdaleff et al., 2013), as well as in the present work.

We selected gum Arabic for our tests as it was proven to be the best encapsulant in a previous work on encapsulation of carrot juice by spray drying (Janiszewska-Turak et al., 2017). Similarly, gum Arabic encapsulation of the carotenoids in the juice using spray drying was adequate in the present work. However, the same encapsulation using freeze-drying was not good. Fig. 2 shows how in freeze drying encapsulation there was a phase separation between the encapsulant and the pigments, probably during the freezing process. Before discarding the encapsulation using freeze drying it would be convenient to evaluate the feasibility of other types of encapsulants but it is out of the scope of this work.

3.4. Fermentation of the sugar-rich fraction from carrot juice

Both the permeate of the ultrafiltration and the diapermeates of the diafiltration of the juice are fractions of great interest. They contain a high concentration of sugars and no fermentation inhibiting compounds,

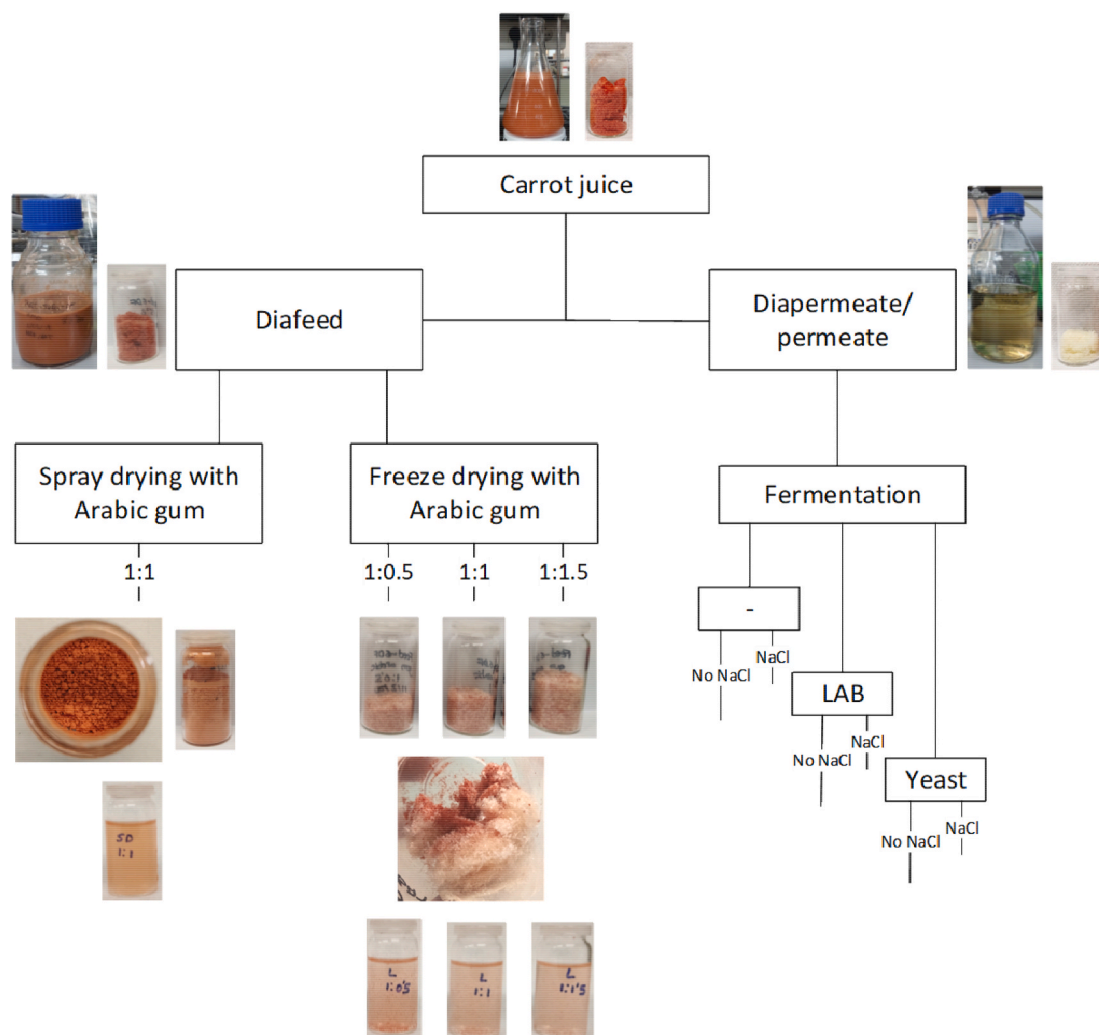


Fig. 2. Valorization of the juice of discarded carrots by drying and encapsulating the pigments and fermenting the sugars.

unlike other broths from hydrolysis. In addition, the juice is a fraction that naturally contains nutrients such as vitamins and minerals and is therefore a potential fermentation broth without the need for additives. The viability of the 30 kDa permeate from the juice of discarded carrots was studied for different fermentation options. The aim of this work was to study the viability of the 30 kDa permeate as a fermentation broth to obtain two typical compounds: lactic acid and ethanol. To obtain them, fermentation was performed with LAB and yeast, but with

autochthonous microorganisms was also evaluated so that it could be considered as a fermentation method without the need for external microorganisms.

3.4.1. Substrate

Two different broths were used: the 30 kDa permeate from the juice of discarded carrots with and without 6% (w/v) of NaCl, with the idea that NaCl acts as an inhibiting agent against invasive yeast. The broth

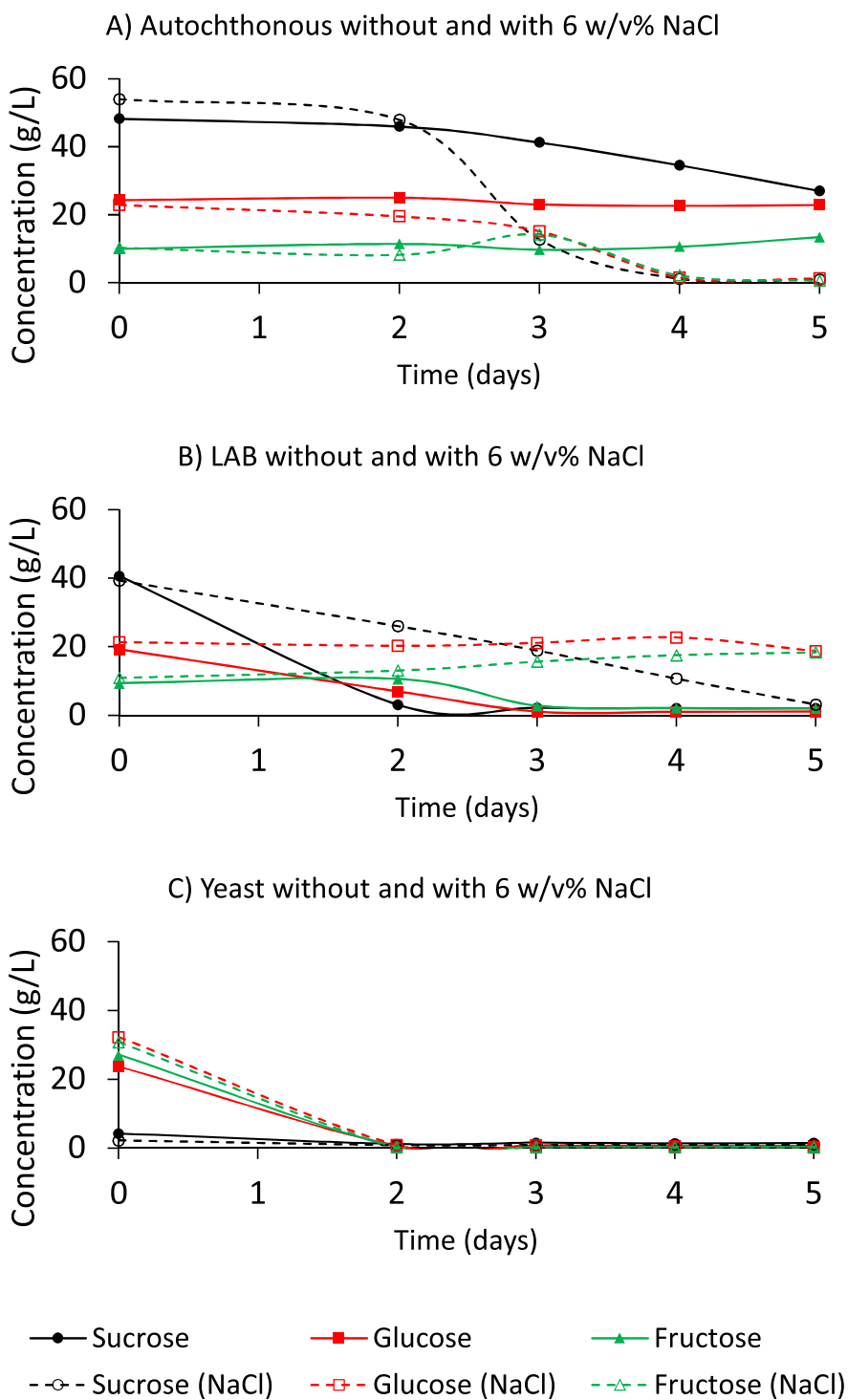


Fig. 3. Evolution in the concentration of sucrose, glucose and fructose during fermentation with A) autochthonous microorganisms, B) with LAB, and C) with yeast, without and with the addition of 6% (w/v) NaCl.

used in each experiment was the same in the inoculum and in the fermentation. The sugar composition of the 30 kDa permeate was 51.13 ± 1.30 g/L of sucrose, 23.59 ± 0.34 g/L of glucose, and 10.11 ± 0.36 g/L of fructose. As already mentioned, since this is a fraction of the discarded carrot juice it is known to contain important nutrients such as vitamins, minerals, and amino acids. In the three cases studied – with autochthonous microorganisms, with LAB, and with yeast – the permeate was a suitable fermentation broth that allowed the obtaining of bioproducts. As can be seen in Fig. 3, the consumption of the three sugars was almost total, with values between 92.4 and 97.5% (w/w) except in two cases: 1) autochthonous microorganisms and NaCl in the broth, and 2) LAB and NaCl in the broth. In these two cases, 23.3% (w/w) and 43.8% (w/w) of the initial sugar were consumed, respectively. This could be due to the fact that LAB were the microorganisms present in these experiments (autochthonous and allochthonous), and they are more sensitive to variations in the pH of the medium and did not resist the conditions (Gardner et al., 2001). In the cases where NaCl was not present, the high consumption of substrate was entirely or mostly due to invasive yeast. The pH of the two broths was measured at the beginning of fermentation and was 5.42 and 5.83 with and without NaCl, respectively.

3.4.2. Microorganisms and fermentation products

Fig. 4 shows the evolution in the production of fermentation products. Two types of products can be distinguished: those which are the main ones and for which the microorganisms were focused (ethanol and lactic acid) and those which were present in smaller proportions and which were produced as co-products in the fermentation (pyruvaldehyde, formic acid, acetic acid, levulinic acid, and acrylic acid). Regarding microorganisms, LAB and yeast produced the expected compounds: lactic acid and ethanol, respectively. The lactic acid

fermentation was contaminated by yeast, which caused a co-production of ethanol (Fig. 4c). However, the addition of NaCl at a concentration of 6% (w/v) to the medium prevented contamination resulting in the exclusive production of lactic acid (Fig. 4d). In the fermentation with autochthonous microorganisms, only lactic acid was obtained in the NaCl assay (Fig. 4b), which leads to the conclusion that the autochthonous microorganisms present in the carrot are LAB, coinciding with previous research (Di Cagno et al., 2008). It would be expected that the essay with the addition of external LAB and NaCl would result in much more lactic acid than the essay with native microorganisms and NaCl, as more LAB would be present. However, the microorganism concentrations showed similar values (Table 3). This may be because growing these different strains together does not result in increased growth of each group of strains. Furthermore, as explained, LAB are very sensitive to pH, which should limit their growth and lactic acid production regardless of the concentration of microorganism present. According to the productivity of microorganisms in the case of allochthonous microorganisms, both LAB and yeast had zero productivity during the stage of fermentation, experiencing growth only in the inoculum (Table 3). Growth during fermentation was just in the case of contamination of LAB by yeast (Table 3). The contamination that took place from yeast was similar for native LAB and for allochthonous LAB (Fig. 4a and c).

3.4.3. Effect of 6% (w/v) NaCl

The effect of adding NaCl to the culture medium was previously studied by Di Cagno et al. (2008), who mentioned that a concentration in the range 5–7% (w/v) acts to inhibit yeast growth. Considering the results, it is confirmed that this is also true for the culture medium used in this work. As can be seen in Table 3, the addition of NaCl in fermentation with autochthonous microorganisms reduced the final

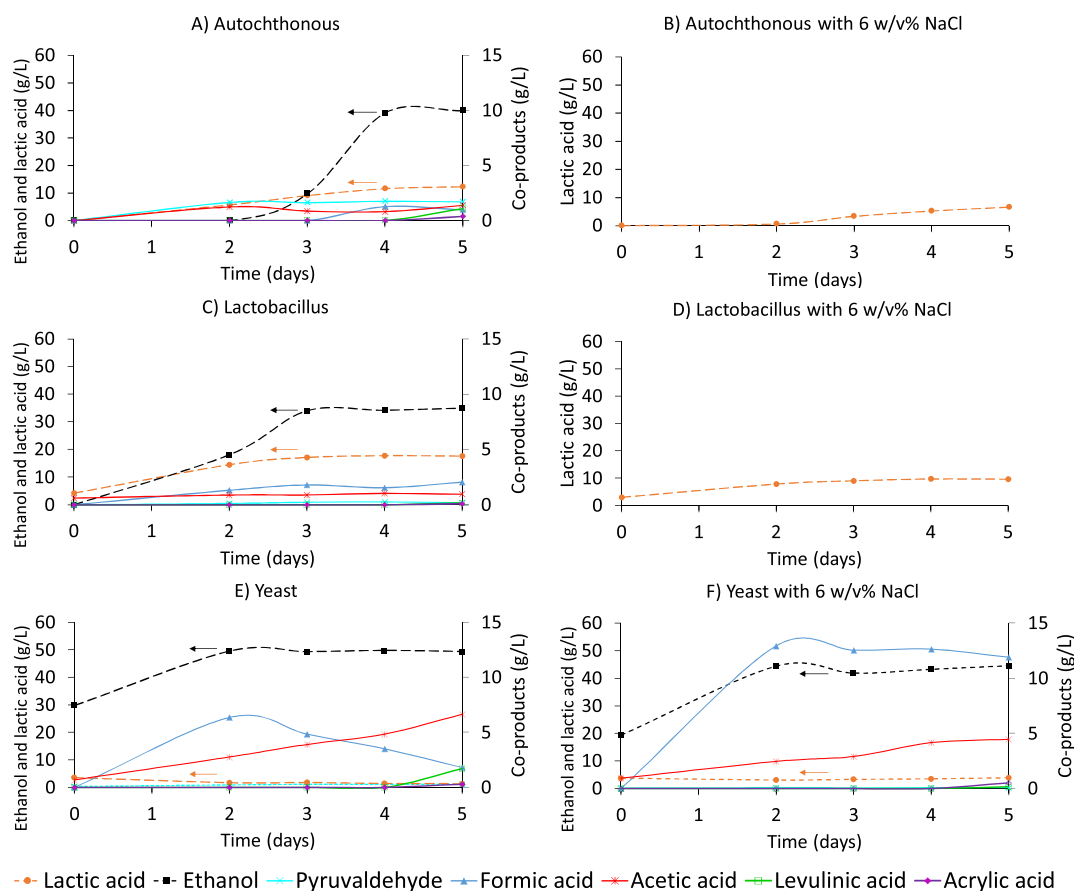


Fig. 4. Evolution in the concentration of fermentation products during fermentation with a) autochthonous microorganisms, b) with LAB, and c) with yeast.

Table 3

Final values of production (g/L) and yield (g product/g substrate consumed) for fermentations with and without NaCl of autochthonous microorganism, LAB and yeast.

	Autochthonous		LAB		Yeast	
	No NaCl	6% (w/v) NaCl	No NaCl	6% (w/v) NaCl	No NaCl	6% (w/v) NaCl
Pyruvaldehyde (g/L)	1.70 ± 0.31	n.d.	0.20 ± 0.04	n.d.	1.22 ± 0.12	0.28 ± 0.01
Lactic acid (g/L)	12.30 ± 1.06	6.62 ± 0.79	17.64 ± 1.54	9.66 ± 0.51	1.39 ± 0.15	3.94 ± 0.07
Formic acid (g/L)	0.97 ± 0.16	n.d.	2.05 ± 0.47	n.d.	1.81 ± 0.08	11.91 ± 0.40
Acetic acid (g/L)	1.38 ± 0.24	n.d.	0.96 ± 0.01	n.d.	6.64 ± 0.28	4.47 ± 0.23
Levulinic acid (g/L)	1.09 ± 0.30	n.d.	0.17 ± 0.01	n.d.	1.71 ± 0.19	0.15 ± 0.03
Acrylic acid (g/L)	0.37 ± 0.08	n.d.	0.11 ± 0.02	n.d.	0.29 ± 0.02	0.53 ± 0.03
Ethanol (g/L)	39.89 ± 0.91	n.d.	35.01 ± 1.75	n.d.	49.46 ± 0.28	44.60 ± 0.13
Total (g/L)	57.71 ± 0.88	7.27 ± 0.79	56.13 ± 1.59	9.74 ± 0.51	62.52 ± 0.26	65.88 ± 0.18
X initial (g/L)	n.d.	n.d.	2.60 ± 0.60	5.20 ± 0.01	20.60 ± 1.00	16.00 ± 0.80
X final (g/L)	2.33 ± 0.01	1.70 ± 0.10	2.10 ± 0.17	1.50 ± 0.43	9.63 ± 1.57	5.80 ± 0.07
Pyruvaldehyde (g/g)	0.020	n.d.	0.002	n.d.	0.011	0.003
Lactic acid (g/g)	0.146	0.344	0.185	0.167	0.012	0.038
Formic acid (g/g)	0.012	n.d.	0.022	n.d.	0.016	0.116
Acetic acid (g/g)	0.016	n.d.	0.010	n.d.	0.059	0.044
Levulinic acid (g/g)	0.013	n.d.	0.002	n.d.	0.015	0.001
Acrylic acid (g/g)	0.004	n.d.	0.001	n.d.	0.003	0.005
Ethanol (g/g)	0.473	n.d.	0.368	n.d.	0.441	0.435
Total (g/g)	0.684	0.344	0.590	0.167	0.558	0.642
X inoculum (g/g)	n.d.	n.d.	0.083	0.196	0.345	0.411
X ferment. (g/g)	0.028	0.088	0.012	n.d.	n.d.	n.d.

production of ethanol, formic acid, acetic acid, and levulinic acid from 39.89 g/L, 0.97 g/L, 1.38 g/L, 1.09 g/L, and 0.37 g/L, respectively, to 0 g/L (or below the detection limit). The production of lactic acid had a certain decrease (from 12.30 g/L to 6.62 g/L). The presence of NaCl in external LAB fermentation also reduced the production of ethanol (35.01 g/L), formic acid (2.05 g/L), acetic acid (0.96 g/L), levulinic acid (0.17 g/L), and acrylic acid (0.11 g/L) to values below the detection limit. Lactic acid became the only product although it experienced a decrease in concentration from 17.64 g/L to 9.66 g/L. In the case of the addition of NaCl in yeast fermentation, the effect was different. A higher formic acid production took place to the detriment of ethanol, increasing the concentration from 1.81 g/L to 11.91 g/L (Table 3). This may be due to an effect on the metabolic pathway of the yeast, as already mentioned in previous works (Hohl and Joslyn, 1941). Formic acid is an intermediate in the production of ethanol and is formed because yeasts are in an unfavourable environment to their growth. The addition of NaCl in yeast fermentation did not act by eliminating the yeast but by creating this unfavourable environment. This effect was shown in a lower production of ethanol when compared to without NaCl and with NaCl: 49.46 g/L vs 44.60 g/L, as well as a slight reduction in ethanol yield: from 0.441 g/g to 0.435 g/g. It is worth mentioning the production of acetic acid in yeast fermentations, a compound that is also considered an intermediate in ethanol production (Paraggio and Fiore, 2004). It should be noted that when NaCl was added to the medium, in addition to avoiding contamination by yeast, the LAB were partially affected, as the production of lactic acid decreases slightly: 46.2% with indigenous LAB and 45.2% with non-indigenous LAB. This may be due to the pH changes that affect LAB.

In addition to the main products already discussed (ethanol, lactic acid, formic acid and acetic acid), pyruvaldehyde, acrylic acid, and levulinic acid were also present. Pyruvaldehyde or methylglyoxal is a by-product derived from the glycolysis of triose phosphate through the triose-phosphate isomerase, therefore it is an intermediate and has been present in the essays with alcoholic fermentation. Previous research has shown that glycolaldehyde maximum production corresponds to the maximum production of ethanol and the maximum sugar consumption (Martins et al., 2001).

3.4.4. Time evolution of the fermentations

Regarding the temporal evolution in the products and co-products, in the case with autochthonous microorganisms (Fig. 4a) the production of lactic acid was from the beginning of fermentation while that of ethanol

(due to invasive yeasts) began on the second day. Both products peaked around day four. Regarding the co-products present, pyruvaldehyde and acetic acid were present from day one, while formic acid was present from the time ethanol production increased, i.e. from day three. Levulinic acid and acrylic acid did not appear until the last day of fermentation. With the addition of NaCl to the medium only lactic acid was produced (Fig. 4b), with a concentration increasing moderately from day 2 until the end of fermentation.

When allochthonous LAB were used (Fig. 4c), at the beginning of the fermentation there was a low concentration of lactic acid due to an initial production in the inoculum. From the initial moment, ethanol production also took place, and both ethanol and lactic acid products reached their maximum concentration on the third day of fermentation. Regarding the co-products, in the case of autochthonous microorganisms, the most abundant co-product was pyruvaldehyde while with the addition of LAB it was formic acid. The co-products were formed from the first day except for levulinic acid and acrylic acid which again did not appear until the last day of fermentation. With the addition of NaCl (Fig. 4d), lactic acid was present from the beginning of fermentation and increased moderately until stabilizing around day three. With the addition of NaCl the production of lactic acid started earlier in the case of allochthonous LAB than in the case of autochthonous LAB (two days difference). This could be due to the lower concentration of autochthonous LAB. Ethanol production by invading yeast was also faster for allochthonous LAB than for autochthonous LAB.

In the yeast fermentation (Fig. 4e), the process was much faster already from the inoculum, where a large amount of ethanol was formed, as the initial concentration in the fermentation was 29.83 g/L. The production of ethanol during fermentation continued until it stabilized on the second day at around 49.61 g/L. Interestingly, the production of lactic acid decreased over time. This presence of lactic acid would be associated with autochthonous bacteria. Regarding co-products, formic acid associated with ethanol production presented a maximum when the ethanol maximum was reached (on the second day), then decreasing perhaps due to its transformation into other components. Acetic acid increased its concentration from the beginning of fermentation to the end, as did pyruvaldehyde but more slightly. Levulinic acid and acrylic acid did not appear until the last day of fermentation. With the addition of NaCl (Fig. 4f), a similar production of ethanol took place, stabilizing on the second day, and a much higher production of formic acid (11.91 g/L versus 1.81 g/L), which peaked on the second day and then remained approximately constant. The other

co-products increased slightly during fermentation with values like those without NaCl. This shows that the addition of 6% (w/v) NaCl acts against invading yeast but its effect is minimal against non-invading yeast, it only creates an unfavourable environment causing the increased production of formic acid. It should be noted that lactic acid production by autochthonous bacteria was slower with NaCl than without NaCl (its initial concentration was lower) but remained approximately constant at a higher value than without NaCl (3.94 g/L versus about 1.39 g/L).

Gardner et al. (2001) performed solid-state lactic acid fermentation of carrot/cabbage vegetable mix. They showed that inoculation of allochthonous LAB produces ethanol by yeast invasion, which was also seen in the present work: 39.89 ± 0.91 g/L of ethanol with native LAB versus 35.01 ± 1.75 g/L of ethanol with allochthonous LAB. As in our study, during lactic fermentation, certain sugars were not totally consumed. This was associated with an excessive drop in pH that could slow down fermentation. Gardner et al. (2001) also added NaCl during fermentation, but ethanol was present in all essays. This ethanol came from an invasion of yeast as the graphs show an ethanol production not coupled to that of lactic acid, so it is not associated with LAB. They conclude that LAB fermentation was generally a sequential process that undergoes secondary fermentation by yeast. However, it is important to note that by adding NaCl in the right proportion, this invasion was avoided in the present work. As for the type of microorganism that is present in a native way in carrots, previous investigations have detected the presence of *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Weissella soli/Weissella koreensis*, *Enterococcus faecalis*, *Pediococcus pentosaceus*, and *Lactobacillus fermentum* (Di Cagno et al., 2008). Therefore, the LAB strains used in the present essays did not coincide with any of the native strains of carrot, and in fact, it seems possible that they did not act in a synergic way, since the production when both were present increased but not by a considerable factor compared to when only the native ones were present. This moderate increase may also be because of the effect of pH on fermentation.

With regard to spontaneous fermentation, Di Cagno et al. (2008) carried it out on solid carrots instead of in juice, but a comparison of results is possible. Their production was 13.96% (w/w) lactic acid, 6.08% (w/w) acetic acid, and 79.95% (w/w) ethanol. In our case, spontaneous fermentation without NaCl resulted in 21.32% (w/w) lactic acid, 2.40% (w/w) acetic, and 69.13% (w/w) ethanol, among other minority co-products (7.15% (w/w)). In the case of using external LAB strains, their production was as follows: 61.37% (w/w) lactic acid, 9.28% (w/w) acetic acid, and 29.36% (w/w) ethanol. The essay of the present study in which LAB was added without NaCl addition was: 31.42% (w/w) lactic acid, 1.71% (w/w) acetic acid, 62.38% (w/w) ethanol, and other minority co-products (4.49% (w/w)). It can be seen how in the essay of the present study the contamination by yeast was higher, which could be due to the fact that Di Cagno et al. (2008) added NaCl to the medium. Considering the essays of the present study in which NaCl was added to the LAB, a production of 100% (w/w) of lactic acid was obtained. This total inhibition of yeast invasion could be explained by the concentration of NaCl used, 6% (w/v), which is in the line with the values recommended in the literature for total inhibition (Di Cagno et al., 2008). Another factor that contributes to mitigating contamination is the rapid growth of LAB. This was consistent with slightly lower contamination in the LAB fermentation that is spontaneous, as the concentration of LAB during fermentation was higher in the latter than in the allochthonous LAB fermentation.

3.5. Structural characterization

The ATR-FTIR spectra were determined for the fraction rich in sugars without carotenoids (freeze-sugar) and for the carotenoids-rich fraction (diafeed), both obtained using diafiltration and freeze-dried before analysis (Figure A1A). The sample diafeed also contained sugars, so its spectrum was similar to freeze-sugar. The most important difference was

the peaks that the diafeed curve had but they were not present in the sugar-freeze curve. These peaks could be associated with compounds that were present in the juice with a molecular weight higher than 30 kDa or as microparticles, standing out the carotenoids. According to the curves, the sample diafeed differed from freeze-sugar in the following peaks. The peak 2850 cm^{-1} associated to symmetrical C-H stretch of CH_3 group, which was not present in sugars but in carotenoids (Mohamed et al., 2011). 1740 cm^{-1} associated with stretching C=O ester that could be due to the presence of methyl esters and O-acetyl esters of hemicelluloses and pectins, present in the microparticles of diafeed (Liu et al., 2019). 1656 cm^{-1} corresponding to vibrations of the polyene chain related to H-C=C-H stretching of beta-carotene (Mohamed et al., 2011). 1529 cm^{-1} assigned to C=C stretching vibration of the polyene chain (Schlücker et al., 2003). 1503 cm^{-1} due to the aromatic ring in lignin, since it is known that lignin cannot be present in freeze-sugar as it contained only water-soluble compounds, but it can be in the microparticles of pulp found in diafeed (Célino et al., 2013). Finally, 1383 cm^{-1} because of vibration in the carotenoids due to the symmetry deformation CH_3 attributed to the beta-ionone ring of the beta-carotene (Espericueta et al., 2018).

ATR-FTIR spectra were also determined to show the effect of encapsulation and the effect of the drying method. The spectra from the samples diafeed, spray-1:1, and freeze-1:1 were compared (Fig. A1B). In both types of drying, the encapsulation generated a significant change in the level of absorbance in the $1185\text{--}891\text{ cm}^{-1}$ band. In this band, the presence of encapsulant increased the absorbance and the peaks in the band were no longer present and were replaced by a single big peak. This could be explained by a higher absorbance in that band by the encapsulant, exceeding that of the starting material (diafeed). The peaks found in that band in the sample diafeed were the same as those present in the sugar-freeze sample, so they could be associated with sugars as mentioned in previous studies. These peaks were 1132 , 1109 , 1044 , 997 , and 930 cm^{-1} . All of them are characteristic of sucrose (Cadet et al., 1997), which was the main sugar, and represent vibrations of C-O, CC, and CCO bonds. It should be noted that the polysaccharides present in the sample could also contribute to this type of vibrations. As far as the rest of the regions are concerned, all of them also increased in absorbance after the action of the encapsulant except the $3700\text{--}3190\text{ cm}^{-1}$ band. This band is associated with the -OH group, which remained the same. Comparing spray drying with freeze drying, in the spray drying some of the peaks associated with carotenoids had a slightly higher absorbance than in the case of freeze drying (2850 cm^{-1} , 1740 cm^{-1}). This could be associated to degradation from one type of carotenoid isomer to another during drying (Sun et al., 2010). The difference in any case was small.

In Fig. A1B, the amount of encapsulant was the same in spray drying as in freeze drying, and it was corroborated by the same absorbance in the $1185\text{--}891\text{ cm}^{-1}$ band. The ATR-FTIR spectra of freeze-1:0.5, freeze-1:1, freeze-1:1.5, and diafeed were also compared to study the effect of encapsulate ratio (Fig. A1C). The difference was shown in the encapsulant's band, $1185\text{--}891\text{ cm}^{-1}$, which had a higher absorbance as the encapsulant:material ratio was higher. The rest of the graph was practically identical regardless of the amount of encapsulant, which implies that the type of bonds present was the same. From this, it would be concluded that a higher presence of encapsulant in the freeze drying process did not change the interaction between it and the carotenoids. Both in Fig. A1B and A1C, no clear differences in carotenoid-associated peaks can be seen before and after encapsulation. This may be due to the way the carotenoids are present, embedded in the cells found in the carrot pulp microparticles. Nevertheless, encapsulation by spray drying could be confirmed according to the results in Table 2.

4. Conclusions

Separation and purification of the juice using diafiltration cycles at room temperature resulted in a carotenoid-free fraction rich in sugars

(84.83 ± 3.26 g/L) and nutrients, and a fraction with high carotenoid concentration (4996.4 µg/g) and minimized sugar content. Recovery of the carotenoid fraction allowed obtaining a solid pigment encapsulated in gum Arabic using spray drying with a carotenoids content of 1131.7 µg/g and whose degradation did not exceed 10% over 15 days. On the contrary, encapsulation using freeze drying was not effective in this case. This encapsulated solid pigment has potential applications in the food and pharmaceutical industry due to its stability both in water and against oxidation. The sugar-rich fraction was subjected to different types of fermentation to study its suitability as broth for the production of important chemicals such as lactic acid and ethanol. The fermentations were conducted with autochthonous microorganisms as well as LAB and yeast. The addition of 6% (w/v) of NaCl to the medium prevented the invasion by yeasts and allowed pure production of lactic acid with both autochthonous microorganisms (6.62 ± 0.79 g/L) and allochthonous LAB (9.66 ± 0.51 g/L). This production could be increased by controlling the pH. Yeast fermentation resulted in high ethanol production (49.46 ± 0.28 g/L). The sugar-rich fraction was a suitable broth for the two types of microorganisms studied (LAB and yeast).

CRedit authorship contribution statement

Marta Ramos-Andrés: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Beatriz Aguilera-Torre:** Validation, Investigation. **Juan García-Serna:** 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.

Acknowledgments

The authors thank the Spanish Ministry of Economy and Competitiveness, The Spanish Ministry of Science, Innovation and Universities and FEDER Funds EU for funding with the projects reference: CLU-2019-04 (Consejería de Educación, Junta de Castilla y León, FEDER, EU) and PID2019-105975GB-I00 (MICINN/FEDER, EU). Marta Ramos-Andrés would like to thank the Spanish Ministry of Education, Culture and Sports for the research-university professor training contract (reference FPU15/06366). The authors wish also to thank the company Muñozval S.L. for the raw material and the advice.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2021.129139>.

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