Peer-reviewed version available at Nanomaterials 2018, 8, 868; doi:10.3390/nano8110868

Article

On the Use of Electrosprayed Agave Fructans as Nanoencapsulating Hydrocolloids for Bioactives

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Abstract: High degree of polymerisation agave fructans (HDPAF) are presented as a novel encapsulating material. Electrospraying coating (EC) was selected as the encapsulation technique and β-carotene as the model bioactive compound. In case of direct electrospraying, two encapsulation methodologies (solution and emulsion) were proposed to find the formulation which provided a suitable particle morphology and an adequate concentration of β-carotene encapsulated in the particles. SEM images showed spherical particles with sizes ranging from 440 to 880 nm depending on the concentration of HDPAF and processing parameters. FTIR analysis confirmed interaction and encapsulation of β-carotene with HDPAF. Thermal stability of β-carotene encapsulated in HDPAF was evidenced by thermogravimetric analysis (TGA). The study showed that β-carotene encapsulated in HDPAF by the EC method remained stable for up to 50 h of exposure to UV light. Therefore, HDPAF is a viable option to formulate nanocapsules as a new encapsulating material. In addition, EC allowed increasing the ratio β-carotene:polymer as well as its photostability.

Keywords: HDPAF 1; β-carotene 2; electrospraying 3; encapsulation 4; photoprotection 5

1. Introduction

Fructans from Agave tequilana consist of a complex mixture of fructooligosaccharides (fructose polymer obtained by enzimatic hydrolisis of high polymerization degree Agave fructans (HDPAF) by FEH and 1-FFT enzymes) containing principally β-(2→1) fructosyl- fructose linkages, but also β-(2→6) and branch moieties [1,2]. The physico-chemical and functional properties of fructans are linked to the degree of polymerization (DP) as well as the presence of branches. The short-chain fraction, oligofructose, is much more soluble and sweeter than native and long-chain fructans, and can contribute to improve mouthfeel because its properties are closely related to those of other sugars. The high DP (> 40 fructose units) fraction can be used as a fat substitute in low-fat or reduced-fat products since it is less soluble, more viscous and more thermostable than native fructans, which allows modifying the rheological and sensorial properties of dairy products. In this case, fructans act as a filler or as breaker of structure in the same way as fat globules do [1,3,4].

Up to our knowledge, little work was done on the exploration of the technological applications for fructans. In this way, Furlán et al. [5], evaluated high, medium and low polymerization degree agave fructans from Agave tequilana Weber as lyoprotectant agents on bovine plasma proteins during spray drying and storage. They concluded that the agave fructans were able to cryoprotect food proteins. Thus, agave fructans are a valuable alternative as a functional ingredient for food formulation. Ortiz-Basurto et al. [6], studied the characteristics and applications of medium and high

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polymerisation degree agave fructans from Agave tequilana Weber, as microencapsulating material of the pitanga or Surinam cherry (Eugenia uniflora L.) juice by spray drying. The powders from both fractions were stable and able to protect the bioactive compound during and after the spray-drying process. These good results together with its characteristics as biopolymer, classified as biodegradable and GRAS [7]; make fructans a really interesting encapsulating material for food, pharma and cosmetic applications.

Up to now, several techniques have been used to encapsulate bioactive components for the food industry, such as, extrusion methods [8], fluidized bed coating [9], spray cooling [10] or spray drying [11]. Nowadays, spray drying is the most common and cheapest technology in the food industry to produce microencapsulated additives for food applications [12]. The electrohydrodynamic processing, including both electrospinning and electrospraying techniques, has recently arisen as an alternative technology that can also be used for encapsulation [13,14]. The basic setup for electrospraying consists of four main components: (1) a high-voltage source (1–30 kV) usually operated in direct current mode, though alternating current mode is also possible, (2) a blunt-ended stainless steel needle or capillary, (3) a syringe pump, and (4) a grounded collector in the form of a flat plate. The electrospraying process involves the application of a strong electrostatic field between two electrodes and imposed on a polymer solution. Increasing the electrostatic field up to a critical value, charges on the surface of a pendant drop destabilize the shape of the solution from partially spherical into conical, i.e., the so-called Taylor's cone effect. As the charged jet accelerates toward regions of lower potential, the solvent is evaporated [15]. Besides being a very simple technique, the solvent is evaporated at room temperature, thus, it constitutes an ideal method for protecting sensitive encapsulated ingredients.

The aim of this work was to study the ability of fructans to form capsules by electrospraying and to asses, as an example, the viability of this polysaccharide as encapsulating material. For that purpose, β-carotene was selected as a model substance. The produced particles were characterized in terms of morphology and photoprotective effect.

2. Materials and Methods

2.1. Materials

High polymerization degree Agave fructans (HDPAF) were purchased from Campos Azules Co, (Ciudad de Mexico, Mexico). TEGO SML (sorbitan fatty acid ester) from Evonik Inc., (Essen, Germany). HPLC grade methanol, absolute ethanol and β-carotene from Sigma-Aldrich (Steinheim, Germany). Deionized water was used throughout the study.

2.2. Preparation of formulation

In order to demonstrate the ability of HDPAF to form nanocapsules, different solutions and emulsions were prepared. Solutions contained different concentrations of HDPAF (5, 10, 20, 30, 40 and 50% w/w), TEGO SML (1%) as a surfactant and a hydroalcoholic solution (water-ethanol, 9:1) as solvent. They were prepared under magnetic stirring at 350 RPM for 5 min (Agimatic-S model 7000242). Oil in water emulsions (O/W) were formulated at a ratio of 10:90. The continuous phase consisted in different HDPAF concentrations (4, 9, 19, 29, 39 and 49%) dissolved in the hydroalcoholic solution (water-ethanol, 9:1). The disperse phase consisted in olive oil. TEGO SML (5% of total emulsion volume) was used as a surfactant to aid emulsion stability and decrease surface tension. The two phases were first mixed in a high-shear mixer at 16,800 RPM for 2 min (Ultra Turrax T25, IKA, Staufen, Germany) in order to prepare the pre-emulsion. The emulsion process was carried out with an ultrasonic homogenizer model Sonopuls 2200 (Bandelin Electronic Gmbh& Co, Berlin, Germany) at 20 kHz for 1 min. The temperature was maintained at 25 ± 1ºC using an ice bath [16].

The ability of electrospraying solutions and emulsions were evaluated and then, particle morphology and size were analysed to select the most adequate solution and emulsion for the photoprotection study.

For solutions containing β-carotene, β-carotene (0.1%) was incorporated in ethanol and then mixed with the solution containing water, TEGO SML and HDPAF. The mixtures were homogenized

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under continuous stirring, at 350 RPM for 30 min. For the emulsions, β-carotene (1%) was previously incorporated in dichloromethane (1 mL) and was gradually added to the olive oil. When the oily phase was saturated with β-carotene, dichloromethane was separated for 24 h by natural evaporation in the extraction chamber and then, the mix was incorporated to the ethanol. The oily phase was added to the solution containing water, TEGO SML and HDPAF, following the same emulsion preparation procedure previously stated.

2.3. Characterization of different solutions and emulsions

The apparent viscosity (ga) was determined using a rotational viscosity meter Visco Basic Plus L from Fungilab S.A. (San Feliu de Llobregat, Spain) with a Low Viscosity Adapter (LCP). The surface tension was measured using the Wilhemy plate method in an EasyDyne K20 tensiometer (Krüss GmbH, Hamburg, Germany). The conductivity was measured using a conductivity meter XS Con6 (Labbox, Barcelona, Spain). All measurements were made at 25°C.

2.4. Preparation of capsules by electrospraying

The electrospraying apparatus, equipped with a variable high-voltage 0–30 kV power supply, was a Fluidnatek® LE-10 from BioInicia S.L. (Valencia, Spain, Solutions and emulsions with and without β-carotene were introduced in a 12 mL plastic syringe and were electrospun under a steady flow rate using a stainless-steel needle of 700 μm of diameter. The needle was connected through a PTFE tube to the syringe. The syringe was lying on a digitally controlled syringe pump while the needle was in horizontal towards the collector. The electrospraying conditions for obtaining the capsules were optimized and fixed at 0.1 mL/h of flow-rate, 17 kV of voltage and a tip-to-collector distance of 22 cm. The samples were stored in darkness, until analysis.

Additionally, a different encapsulation strategy named electrospraying coating (EC) patented by Lagaron et al. [17] and reported by Librán et al. [18] was used. The coating was a three-step process carried out at room temperature. In the first step, an initial layer of fructans were electrosprayed over the collector. Secondly, 2% of β-carotene with respect to the solution electrosprayed was spread out over the initial electrosprayed material layer. Finally, a top coating layer of fructans was electrosprayed directly over the material to achieve full encapsulation. The capsules were then collected and mechanically mixed and homogenized. The basic setup of Fluidnatek™ LE10 (Bioinicia S.L., Spain) was used to conduct the electrospraying process.

2.5. Scanning electron microscopy (SEM)

The morphology and size of the encapsulation structures was examined using SEM on a Hitachi microscope (Hitachi S-4100) after having been sputtered with a gold–palladium mixture under vacuum for 3 min (SC7640, Polaron, Kent, United Kingdom). All SEM experiments were carried out at 10 kV. Capsule diameters were measured by means of the Adobe Photoshop CS3 software from the SEM micrographs in their original magnification.

2.6. Fourier transform infrared spectroscopy

Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) was used to evaluate the interaction between β-carotene and nanocapsules (Thermo Scientific Nicolet, iS5 iD5). Spectra were recorded from 600 to 4000 cm−1 with a resolution of 8 cm−1.

2.7. Thermogravimetric analysis (TGA)

Thermogravimetric analyses of the nanocapsules were done using TGA 550 equipment (TA Instruments, USA) and TRIOS 4.3.0.38388 was the analysis software. The analyses were conducted under the following conditions: 3-6 mg of sample, heating from 25 to 500ºC, at a heating rate of 5ºC/min under nitrogen flow.

2.8. Ultraviolet (UV) photostability

With the aim of accelerating the oxidation of β-carotene and simulating the radiation of natural sunlight, an Osram Ultra-Vitalux (300 W) lamp was used. This blend of radiation is generated by a quartz discharge tube and a tungsten filament [19]. Nanocapsules with β-carotene and free β-carotene were exposed to the UV radiation (13.6 W) at 37ºC. After irradiation at different times (0, 6, 12, 24 y 48 h), extraction of β-carotene from 2.5 mg of nanocapsules was carried out. The polymeric capsule wall was opened with water (1 mL) under magnetic stirring (200 RPM, 1 min). β-carotene was extracted from the mixture by adding 0.75 mL of chloroform and separated by centrifugation (10000 RPM, 1 min). An aliquot of the organic phase was taken and the absorbance was measured at 466 nm in a spectrophotometer (Spectrum SP- 2000 UV), chloroform was used as blank. Oxidation was reported as a function of the relative β-carotene content (% absorbance). Analysis were made in triplicate.

3. Results and Discussion

3.1 Solution properties

The successful development of encapsulation structures using electrospraying technology strongly depends on the solution properties and, hence, an initial characterization of solution viscosity and viscoelasticity was carried out. From a screening study, it was seen that HDPAF solutions led to very low apparent viscosity values at 5 % (see Table 1), which resulted in unstable jetting and no structures were formed from these solutions. In order to increase the viscosity of solution, HDPAF concentration was increased. Viscosity values at 10 to 30% HDPAF concentration provided viscosity values previously reported as adequate for electrospraying [17].

Table 1. Physical properties (conductivity, surface tension, and viscosity) of solutions and emulsions at different HDPAF concentration.

Concentration (% w/v)	Viscosity (cP)	Surface Tension (mN/m)	Conductivity (μS/cm)
Solutions			
5	1.61 ± 0.05 [a]	24.35 ± 0.05 [a]	69.39 ± 0.03 [a]
10	2.37 ± 0.07 [b]	24.37 ± 0.02 [a]	82.14 ± 0.03 [b]
20	3.42 ± 0.01 [c]	24.85 ± 0.05 [b]	101.20 ± 0.05 [c]
30	6.82 ± 0.02 [d]	23.65 ± 0.05 [c]	93.30 ± 0.06 [d]
40	46.05 ± 0.05 [e]	23.51 ± 0.05 [d]	76.73 ± 0.05 [e]
50	162.22 ± 0.60 [f]	23.46 ± 0.05 [d]	52.86 ± 0.01 f
Emulsions			
5	2.65 ± 0.03 [a]	22.42 ± 0.04 [a]	41.81 ± 0.04 [a]
10	3.40 ± 0.03 [b]	22.91 ± 0.02 [b]	45.82 ± 0.04 [b]
20	8.83 ± 0.08 [c]	24.05 ± 0.03 [c]	52.70 ± 0.02 [c]
30	12.26 ± 0.09 [d]	23.20 ± 0.02 [d]	50.89 ± 0.02 [d]
40	45.70 ± 0.12 [e]	22.73 ± 0.03 [e]	39.11 ± 0.04 [e]
50	93.54 ± 0.24 [f]	21.13 ± 0.03 [f]	30.26 ± 0.01 [f]

a–f: Different superscripts within the same column indicate significant differences among the samples ($p < 0.05$).

Formulations with 40% of HDPAF, produced a significant increase in viscosity. This could be attributed to the high amount of HDPAF added, but also to the high molecular weight of HDPAF, since they consist of a mixture of long polymers and fructooligosaccharides [6], which have been reported to contribute to increase viscosity [18].

Similar values of surface tension in all formulations were obtained (Table 1). It was also observed that, even though high HDPAF concentrations (30-50%) were used, the profile of surface tension values of the aqueous solutions decreased but the range were still adequate. This behaviour allows solutions and emulsions to be processed by electrospraying to obtain capsules. It has been previously reported that solutions with low surface tension, favour the electrospraying process [20] and, thus, capsules formation [17], because the intensity of the electrical field must overcome the solution surface tension, expelling an electrified jet from the Taylor's cone formed on the needle tip [21]. Therefore, during drying by electrospraying, the Taylor's cone was held stable for formulations with low surface tension, which is in agreement with the work reported by Jaworek [22], who affirmed that solutions with surface tension above 50 mN/m cannot be electrosprayed. This decrease in surface tension could be attributed to the ethanol addition to solubilize β-carotene in the formulations, because ethanol surface tension is lower than water surface tension [23]. The conductivity values were increased from 5 to 20% of HDPAF added, then conductivity decreased (Table 1), however, conductivity values were always low and in the range reported as adequate to be processed by electrospraying [18]. Finally, electrical conductivity should not be too high to avoid the destabilization of the electrospraying jet [23]. Emulsions and solutions properties showed the same behaviour with respect to the physical properties evaluated (Table 1).

3.2 Capsules Morphology

SEM images demonstrated the ability of HDPAF to form capsules when HDPAF concentrations between 10 and 50% (w/v) were used as shown in Figure 1. Nanocapsules with spherical morphology, sizes between 440 to 880 nm; without cracks, dents or deformations and without being agglomerated (Figure 1) were obtained. The absence of pores or cracks on the capsule surface is important to ensure a low oxygen permeability that could lead to the degradation of the encapsulated antioxidant compounds [24]. However, Ortiz-Basurto et al. [6] observed an irregular surface and several indentations on the microparticles of HDPAF encapsulating pitanga juice obtained by spray drying.

It is important to note that some elongated capsules were observed. This behaviour can be attributed to the properties of the solutions or emulsions and to the electrospraying process conditions, such as voltage and the needle to collector distance [15,19].

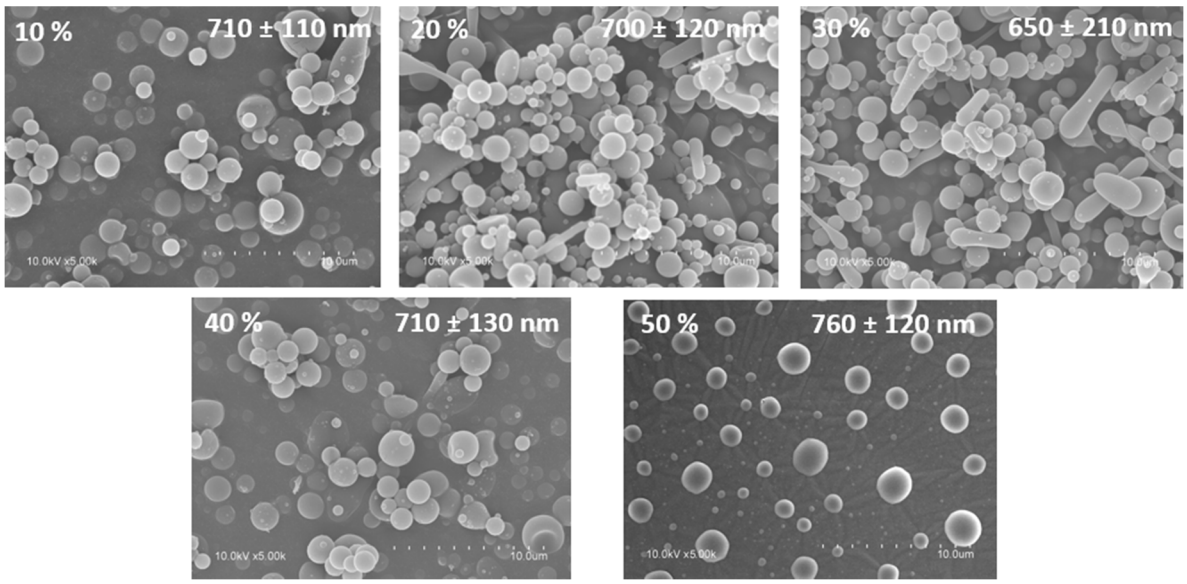

Figure 1. Micrographs obtained by SEM of HDPAF nanocapsules at different HDPAF concentrations (% w/v 10, 20, 30, 40 and 50) obtained by electrospraying.

3.3 β-carotene encapsulation

The encapsulation of the β-carotene supposes a technical challenge due to its high instability to light, its hydrophobicity and its low solubility in common organic solvents. The great interest of the food industry in this compound has motivated researchers to try the encapsulation of β-carotene by several methods. Tan and Nakajima [25] suggested the nanodispersion of β-carotene by the solvent evaporation method. Ribeiro et al. [26] proposed the encapsulation in PLA and PLGA by the solvent displacement method and Astete et al. [27] the encapsulation in calcium alginate. However, all these proposals presented the disadvantage of using organic solvents such as acetone [26], hexane [25] or chloroform [27]. Traces of these solvents would make the encapsulates unsuitable for food applications, and, therefore, it is of great interest to find a methodology to obtain nanocapsules based on the use of eco-friendly ingredients and with a high encapsulation efficiency, which could reach the character "generally recognised as safe (GRAS)" granted by the FDA.

Our first proposal was to use a solution to encapsulate the β-carotene. Nevertheless, the low solubility of β-carotene in conventional solvents prevented obtaining capsules with β-carotene concentrations over 0.1%. The second attempt was to use an emulsion, but for that option the use of dichloromethane was required. The residual organic solvent concentration in the capsules was evaluated by headspace-solid-phase microextraction–gas chromatography (HS-SPME-GC) according to Camelo-Méndez et al., [28] with some modifications, and no traces of dichloromethane were detected in samples. Despite this good result, the concentration of the β-carotene in the particles was less than 1%. On the other hand, β-carotene encapsulated by the EC method allowed higher concentration of β-carotene and consequently, the whole study was focused on this option.

3.4 FTIR analysis of the encapsulation structures

Interaction between β-carotene and HDPAF nanocapsules was evaluated by Infrared spectroscopy (FTIR) according to Peinado et al. [29]. The FTIR spectra of β-carotene shown a broad peak at 3411 cm−1 that represents the presence of O-H stretching of the hydroxyl group, which is likely due to the interaction of β-carotene with oxygen in the air [30]. The peaks at 2929 and 2869 cm−1 indicate the CH2 asymmetry and symmetry stretching, respectively (Figure 2a). The presence of carbonyl groups and stretching symmetry of C-H bond group, was evidenced in peaks at 1717 and 1366 cm−1, respectively. The sharp peak at 965 cm−1 marks the deformation mode of trans-conjugate alkenes as the specific areas of trans=CH used for identification of β-carotene [30,31].

The FTIR spectra of HDPAF nanocapsules (Figure 2b) shown the most intensive broad band with maximum at 1050 cm−1 with two shoulders at 940 and 1130 cm−1. Moreover, two overlapped bands are at 2930 and 2870 cm−1 characteristic of carbohydrates [32]. The band from 2800 to 3000 cm−1 is similar to inulin spectra reported by Grube et al. [32] and Apolinário et al. [33]; this band is attributed to C-H stretching. The broad stretching peak around 3492 cm−1 indicated the presence of hydroxyl groups (-OH) of carbohydrates [33].

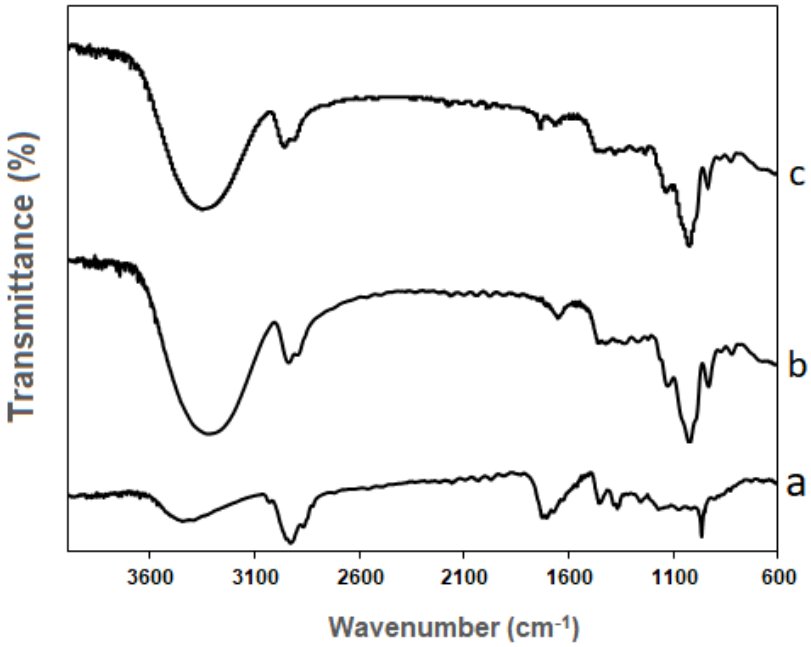

Figure 2. FTIR spectra. β-carotene (a), HDPAF nanocapsules (b) and HDPAF/β-carotene nanocapsules produced by the EC process(c).

The comparison of nanocapsules of HDPAF and HDPAF/β-carotene obtained by the EC process (Figure 2b-c) proved HDPAF as the dominating component. The main differences in nanocapsules of HDPAF and HDPAF/β-carotene spectra appeared in 1700-1800 cm−1, region that indicates the interaction C = O of fructose molecules with β-carotene presenting a stretching of the peak. The low intensity of β-carotene suggests that only a slight amount is located on the surface of the HDPAF nanocapsules [29]. Nanocapsules with a low surface intensity observed by FTIR suggests a centripetal distribution of β-carotene, where the highest concentration is in the core of the nanocapsule. Such confinement, likely due to the hydrophobicity of β-carotene, is desired, as it would create a barrier against oxygen and a protection against thermal decomposition processes [29].

3.5 Thermal stability of β-carotene and HDPAF nanocapsules

The purpose of the thermogravimetric analysis was to evaluate the thermal resistance to degradation of β-carotene encapsulated in HDPAF nanocapsules. Thermograms shown, according with the derivate of thermogram, a thermal decomposition of pure β-carotene between 150.58 and 354.16°C (Figure 3a), similar decomposition temperature range (150-450°C) was reported by Busolo & Lagaron, [34]. HDPAF nanocapsules degraded between 205.48 and 257.70°C (Figure 3b). These differences in stability can be associated to the structure of the molecules since the HDPAF is a complex mixture of fructooligosaccharides [1,2] and may have functional properties linked to the degree of polymerization.

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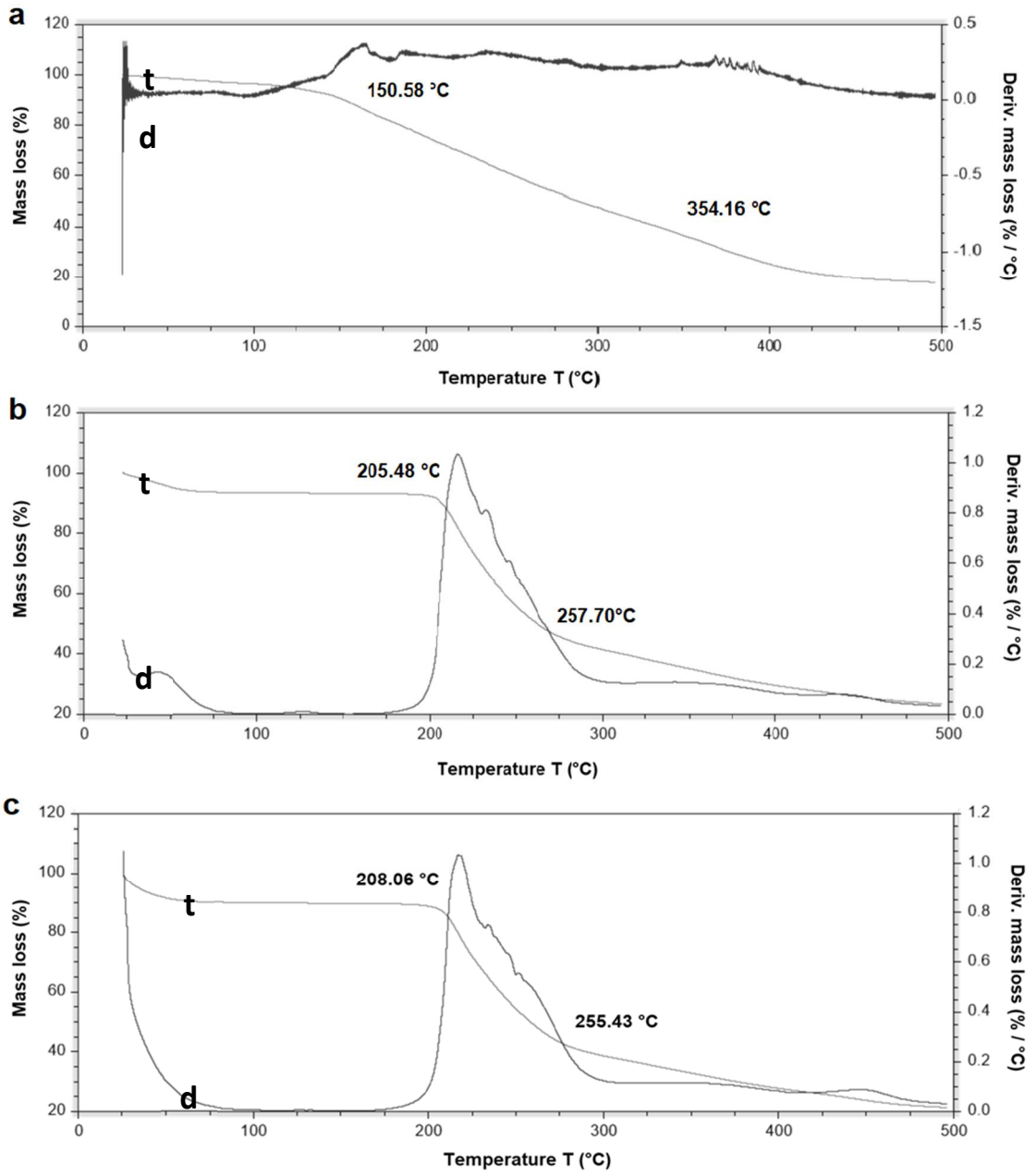

Figure 3. Thermogravimetric profile. β-carotene (a), HDPAF nanocapsules (b) and HDPAF/β-carotene nanocapsules obtained by EC (c). Curve t represents the thermogram and d the thermogram derivate.

β-carotene encapsulated in HDPAF nanocapsules was decomposed between 208.60 and 255.43°C (Figure 3c). This result supports the thermal protective effect of the HDPAF nanocapsule on β-carotene, similar to that reported by Peinado et al. [29] for the encapsulation of β-carotene in electrospun nanofibers of poly (ethylene oxide). The thermal stability of antioxidants as β-carotene depends on whether the molecules are totally encapsulated in the nanocapsules or on the surface [34]. Thermal stability of HDPAF/nanocapsules with and without β-carotene did not show a significant difference (Figure 3). Therefore, HDPAF exerts a protective role against the thermal degradation of β-carotene.

290 *3.6 Ultraviolet (UV) photostability of encapsulated β-carotene*

291 β-carotene is highly susceptible to photoxidation (oxidation or isomerization) due to the
292 presence of conjugated double bounds in the molecule [19]. The exposure of β-carotene to UV light
293 led to the damage in the molecule producing a decrease in the absorbance (measured at 466 nm)
294 (Figure 4). Degradation of unprotected β-carotene has been also reported by Fernandez et al. [19] and
295 de Freitas Zômpero et al. [21]. However, the β-carotene encapsulated in HDPAF showed a higher
296 stability to UV light even after 48 h of exposure (Figure 4).

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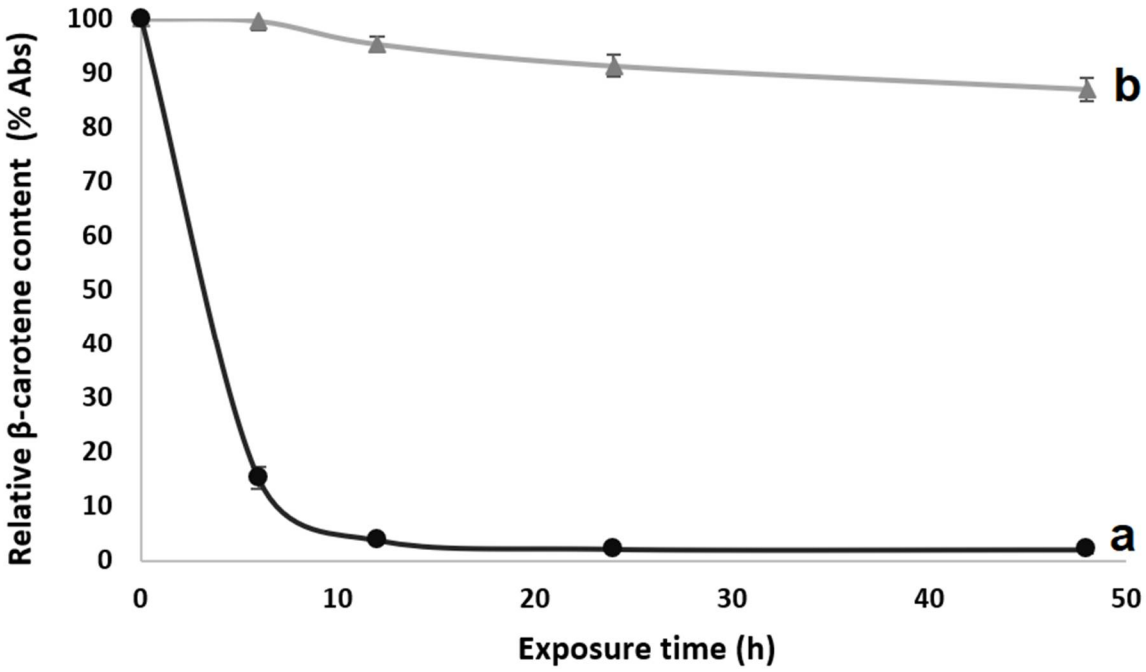

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299 Figure 4. Relative decay in absorbance percentage (% Abs), as a function of exposure time UV (0, 6,
300 12, 24 and 48 h) in β-carotene (UV-vis a 466 nm). β-carotene (a), HDPAF/β-carotene (EC) (b).

301

302 Photoisomerization under UV-light exposure is thought to be able to take place in free bioactive
303 compounds, but not very readily in dried particles [19]. López-Rubio and Lagaron [35] produced
304 hydrocolloid films (whey protein concentrate, zein, soy protein and gelatin) containing β-carotene
305 which were able to maintain the β-carotene stability even after 50 h of UV light exposure. De Freitas
306 Zômpero et al. [21] reported that a double encapsulation (nanoliposome + polymeric fiber) by
307 electrospinning was useful to guarantee the β-carotene stability during 6 h of UV light exposure.
308 Therefore, the utilization of the HDPAF as encapsulating material could be a novel option, to be used
309 in the nanocapsules manufacture as a new material to protect active compounds. In this case, the β-
310 carotene loaded in HDPAF presented a similar behaviour as compared with other
311 polymers/hydrocolloids used before. However, this behaviour was obtained with a low HDPAF
312 concentration in the particle.

4. Conclusions

In this paper, high degree of polymerisation agave fructans (HDPAF) are presented as a novel encapsulating material. First, their ability to form capsules by electrospraying was tested. The best results, in terms of morphology and capsule size, were obtained when concentrations of 30 and 40% of fructans were used. β-carotene was encapsulated in HDPAF by direct electrospraying and by EC. However, the EC method presented advantages in comparison with emulsion or solution direct electrospraying, since it was possible to obtain particles with higher bioactive-polymer ratios. Moreover, the particles obtained by the EC method showed a good photoprotection. Results shown in this work, evidence that the HDPAF have the capacity to improve the stability of β-carotene. Additionally, HDPAF are appropriate for human consumption, therefore they could be a really interesting encapsulation polymer for the food industry.

Author Contributions: Conceptualization was devised by J.AR.-S. and J.M.L.; methodology, validation, and formal analysis was carried out by J.A.R.-H., J.A.R.-S., M.C.-S., R.I.O.-B., C.P., and J.M.L.; investigation, resources, data curation, writing—original draft preparation and writing—review & editing was performed by J.A.R.-H., J.A.R.-S., M.C.-S., R.I.O.-B., C.P., and J.M.L; visualization, supervision, project administration, funding acquisition was carried out by J.A.R.-S and J.M.L.

Funding:

Acknowledgments: The authors would like to thank the "Plan de Movilidad AGARED 2017" ("AGARED- Red Temática Mexicana Aprovechamiento Integral Sustentable y Biotecnología de los Agaves"; the MINECO project AGL2015-63855-C2-1-R for funding and the CONACYT (Mexico) for the scholarship granted to Jorge Alberto Ramos-Hernández.

Conflicts of Interest: The authors declare no conflict of interest.

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