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Inulin as a carrier of β-carotene: Particle preparation and characterisation

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ABSTRACT

Inulin, a linear polysaccharide consisting of fructose units linked by β-(2 → 1) glycosylic bonds, has applications in the food, biotechnology and pharmaceutical industries. The study aims to encapsulate β-carotene using inulin as a food grade material. β-Carotene is a well-known antioxidant and has health-promoting properties. However, it is sensitive to visible/UV light, degrades in the presence of oxygen and is insoluble in water. β-Carotene-inulin particles were fabricated by the complexation method using different β-carotene/inulin ratios. Raman and ^{1}H NMR spectroscopy and the differential scanning calorimetry method were used to confirm the formation of the complex. Based on complexation yield, encapsulation efficiency and loading capacity, the β-carotene/inulin ratios of 1:10 and 1:20 (w/w) were found to be suitable for particle preparation. The water solubility of the complexes was 45–50 mg/mL. The applicability of newly prepared β-carotene-inulin particles was demonstrated in a real food system, that is, apple juice and birch sap.

1. Introduction

Inulin is a linear polysaccharide that consists of fructose units with a degree of polymerisation (DP) ranging from 2 to 60 units. Its molecules are usually terminated by a glucopyranose residue linked by a sucrose-type linkage. Inulin is therefore a non-reducing polysaccharide. What is interesting about inulin is that fructose units are connected by β-(2 → 1) glycosylic bonds that do not incorporate any sugar ring in the biopolymer backbone. This gives more flexibility to the molecule. If the DP is not higher than 10, inulin is termed an oligofructose (Apolinário et al., 2014; Mensink et al., 2015).

Native inulin has a sweet taste and is found in several plants, such as globe artichokes, asparagus, garlic, leeks, bananas, and others. However, β-(2 → 1) glycosylic bonds are indigestible to humans (Roberfroid, 2007). On the contrary, gut bacteria, including *Bifidobacterium* or *Lactobacilli* can metabolise inulin, improving the gut microbiota (Sheng et al., 2023) and lowering the risk of developing colon or other types of cancer. Therefore, inulin is a valuable dietary fibre that improves the health of the human body. Native inulin powder, which is a mixture of moieties with different DP, is also a healthy and natural sweetener that does not contribute to diabetes. Inulin with a higher DP is less sweet compared to short-chain inulin (Mensink et al., 2015). In addition, it has many other therapeutic benefits. As a fibre, inulin reduces constipation in people of all ages and increases calcium ion absorption and bone mineralisation. The ability of inulin to reduce intestinal inflammation has also been demonstrated (Gupta et al., 2019; Tawfick et al., 2022).

Industrially, inulin is produced primarily from chicory root and Jerusalem artichoke (Shoaib et al., 2016). It has a health claim from the European Food Safety Authority (EFSA) and is generally recognised as safe (GRAS) in the United States (Mensink et al., 2015; Scientific Opinion, 2015 on the Substantiation of a Health Claim Related to 'Native Chicory Inulin' and Maintenance of Normal Defecation by Increasing Stool Frequency Pursuant to Article 13.5 of Regulation (EC) No 1924/2006," 2015). First, inulin has applications in the food industry as a fat replacer in dairy products and baked cakes and biscuits, and as a sugar replacer in products such as beverages, chocolate, and cakes. Inulin is also used as a texture modifier in dairy desserts (Jackson et al., 2023). Inulin, especially hydrophobically modified inulin, is used as an emulsifier in cosmetic formulations (Yang et al., 2022). In the cosmetic industry, inulin derivatives, that is, carboxymethyl and quaternized inulin, can replace hyaluronic acid and act as humectants (Bhanja, Sutar, & Mishra, 2022). Inulin and inulin-rich feedstocks are renewable and inexpensive materials for the biotechnology industry. High fructose syrup, bioethanol, citric and lactic acids, sugar alcohols, and other chemicals can be produced in bioprocesses using inulin or inulin-rich materials as a carbohydrate source (Chi et al., 2011; Singh et al., 2022).

The pharmaceutical industry is another very important and large field for inulin applications. It is widely used for the development of

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drug delivery systems (Afinjuomo et al., 2021), because natural polysaccharides have optimal drug delivery properties, including targeting (Giri et al., 2021) and drug solubility (Z. Liu et al., 2008; Srinarong et al., 2009). Another application of inulin is as a vaccine adjuvant (Ferrell et al., 2021; Petrovsky & Cooper, 2015; Stewart et al., 2022). For example, it has improved the efficacy of vaccines against SARS (Stewart et al., 2022), influenza (Petrovsky & Cooper, 2015), and hepatitis B (Saade et al., 2013). Inulin can be used as a protein stabiliser in spray and freeze drying (Mensink et al., 2015) and as an encapsulant for probiotic microorganisms mainly in spray drying (Gruskiene, Lavelli, & Sereikaite, 2024).

β-carotene (CAR) is a member of the carotenoid family and is also known as provitamin A. It is a lipophilic molecule composed of a polyene system with eleven conjugated double bonds. At each end of the chain there are β-rings. (Martín Ortega & Segura Campos, 2021; Rodriguez-Concepcion et al., 2018). When consumed, it is converted to vitamin A, also known as retinol. CAR is important for a healthy immune system and vision, reduces oxidative stress, and can prevent chronic diseases such as cancer or cardiovascular disease (Kim et al., 2021; Palace et al., 1999). It has been reported that the use of a mixture of different carotenoids or carotenoids with other antioxidants such as tocopherol can increase the protective effect (Stahl et al., 1998, 2000).

CAR is generally found in yellow, orange, or dark green leafy vegetables such as carrots, tomatoes, spinach, sweet potatoes, and fruits such as apricots, pumpkins, mango, and others (Meléndez-Martínez et al., 2022). However, people do not consume enough fruits and vegetables, and therefore do not intake enough CAR to maintain their health. Often, CAR can be consumed as supplements, used as a pigment, or as a food additive to improve the quality and appearance of food. The European Food Safety Authority has proposed that the use of supplemental CAR should be limited to 3.9 mg/day for women and 4.5 mg/day for men (Turck et al., 2024).

In the market, most CAR is chemically synthesised. Only a few percent of the market is CAR derived from bioresources such as yellow/orange vegetables or micro/macro algae (Hamed et al., 2023; Meléndez-Martínez et al., 2022).

CAR is very sensitive to visible/UV light and degrades rapidly in the presence of atmospheric oxygen (Scita, 1992). Furthermore, CAR is soluble in oil, making it difficult to incorporate into water-based foods. To improve their stability and solubility, carotenoids are usually formulated with other materials and incorporated into colloidal particles. Carotenoids encapsulation deserves attention because the carotenoids formulation determines their stability and solubility not only during food processing and storage, but also in gastrointestinal fluids (Hamed et al., 2023; Meléndez-Martínez et al., 2021). Emulsions are the most explored system for CAR delivery (Lavelli & Sereikaitė, 2022; Molet-Rodríguez et al., 2025). However, flocculation of traditional emulsions and nanoemulsions can occur to varying degrees during the production of functional beverages. The disadvantages of microemulsions are the risk of precipitation and the high level of surfactant required (Sereti et al., 2025). Gum Arabic, a natural emulsifier, is often used for this purpose. It is a costly material with limited supply (Prasad et al., 2022). As a carrier of CAR, cyclodextrins can also be used. Because of weak interactions, an additional coating is needed to stabilise the cyclodextrin-CAR complex (Wang et al., 2022). This study hypothesises that inulin, as a food grade material, could be used for CAR formulation. Previously, carrot waste extract was encapsulated by freeze and spray drying method using a mixture of whey protein and inulin as wall material (Šeregelj et al., 2021). This study aims to encapsulate CAR using a bottom-up approach (Joye & McClements, 2014), that is, to prepare β-carotene-inulin (CAR-IN) particles using the complexation method and to investigate their physicochemical properties.

2. Materials and methods

2.1. Materials

Inulin (IN) from dahlia tubers with a relative molecular mass (M_r) of 5000 and a solubility in water of 100 mg/mL was purchased from Sigma-Aldrich. β-Carotene (CAR), 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were also purchased from Sigma-Aldrich. Hydrochloric acid and NaOH were purchased from Roth. $FeCl_3$ and potassium persulfate were obtained from Fluka. Sodium acetate, acetone, hexane, and ethanol were purchased from Merck, Chempur, Reachem Slovakia s.r.o., and Vilniaus degtinė (Vilnius, Lithuania), respectively.

2.2. Preparation of CAR-IN particles

The CAR-IN complexes were prepared at the different ratios of CAR/IN (w/w) (1:5, 1:10, 1:20, 1:40, 1:60). First, 40–400 mg of inulin were dissolved in 10 mL of deionised water and heated to 67 °C by stirring on a magnetic stirrer. For higher concentrations of inulin, the water was heated to 85–90 °C until the inulin was dissolved, then the temperature was lowered to 67 °C. Second, 20 mL of 0.2 mg/mL CAR solution in acetone was prepared in a brown glass bottle stirring for at least 30 min. The CAR solution was then added dropwise to the inulin solution in the flask wrapped in aluminium foil to prevent the CAR from being damaged by sunlight. The CAR-IN solution was then kept at 67 °C by stirring until the acetone evaporated. The solution was then cooled to room temperature, filtered through a 40 μm glass filter to remove any unreacted CAR and frozen at −20 °C in the freezer. Finally, the samples were lyophilised for at least 48 h using a Telstar LyoQuest freeze dryer: first frozen at −75 °C and then dried at 0.1 mbar pressure. The dry complexes prepared were stored at −20 °C and protected from light.

2.3. Characterisation of CAR-IN particles

To determine the complexation yield, the lyophilised samples were weighted and the yield was calculated using the following equation:

$$\text{Yield}\ (\%) = (m_{CAR-IN} / (m_{CAR} + m_{IN})) \times 100\% \quad (1)$$

where $m_{CAR\text{-}IN}$, m_{CAR} and m_{IN} are the mass of the complex, CAR and inulin, respectively.

The encapsulation efficiency (EE) was determined by CAR extraction from freshly prepared samples. The extraction was carried out as follows: the sample was dissolved in water at a concentration of 3 mg/mL by shaking at 850 rpm for 30 min. The sample was then mixed with ethanol and hexane at a ratio of 1:2:3 (v/v/v), respectively, and gently shaken. The extraction was repeated until the aqueous solution became colourless. The concentration of CAR in the hexane fractions was determined by UV/Vis spectroscopy and calculated according to the following equation (Celitan et al., 2021; Corte-Real et al., 2016):

$$c_{CAR} = \text{Abs} \times \text{DF} \times 10 / (A^{1\%} \times d) \quad (2)$$

where c_{CAR} is the concentration of CAR in the sample (mg/mL); Abs is the absorbance at 450 nm; DF is a dilution factor of the sample; $A^{1\%}$ is the CAR mass extinction coefficient in hexane equal to 2590 L/(g × cm); d is the length of the cuvette equal to 1 cm.

The mass of the complexed CAR was calculated on the basis of c_{CAR}. The EE (%) was then calculated using the following equation:

$$\text{EE}\ (\%) = (m_{CAR-complexed} / m_{CAR-initial}) \times 100 \quad (3)$$

where $m_{CAR\text{-}complexed}$ is the mass of complexed CAR, and $m_{CAR\text{-}initial}$ is the initial mass of CAR taken for complexation.

The loading capacity (LC, %) was calculated using the following equation:

$$LC\ (\%) = \left(m_{CAR-complexed} \,/\, m_{CAR-IN}\right) \times 100 \quad (4)$$

where $m_{CAR\text{-}complexed}$ is the mass of complexed CAR and $m_{CAR\text{-}IN}$ is the mass of the prepared complex.

The solubility of the complex was determined by first weighing dry Eppendorf tubes followed by dissolving 30 mg of the CAR-IN complex in 0.5 mL of deionised water by shaking at 850 rpm for 30 min. The samples were then centrifuged at 5450×*g* for 10 min. The remaining insoluble complex was dried using an Eppendorf Concentrator Plus centrifuge, then weighted, and the concentration of the dissolved sample was calculated.

2.4. Physicochemical properties of CAR-IN particles

The hydrodynamic diameter, polydispersity index (PDI) and zeta potential (Z_p) were determined by dynamic light scattering (DLS) method using a Malvern Zetasizer Nano ZS instrument equipped with a 4 mW HeNe laser at a wavelength of 633 nm. Measurements of the intensity of the scattered light were performed at 25 °C and an angle of 173°. Data were analysed using Malvern Zetasizer software 7.03.

The thermal properties of the CAR-IN complexes were determined by the differential scanning calorimetry (DSC) method using a PerkinElmer STA 6000 Simultaneous Thermal Analyzer. An amount of 5 mg of the freeze dried sample was heated in non-hermetically sealed pans from 25 °C to 400 °C at a rate of 10 °C/min. A nitrogen atmosphere was used at a flow rate of 40 mL/min.

The colour characteristics were measured using the Konica Minolta CM-700d colour spectrophotometer and presented in CIELAB colour coordinates. The spectrophotometer was calibrated against a white background and the samples were measured twice. Chroma (C^*) and hue angle (h^*) parameters were calculated using equations (5) and (6) (Pathare et al., 2012):

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (5)$$

$$h^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (6)$$

For thermal stability experiments, aqueous solutions of CAR-IN complexes at a concentration of 3 mg/mL were prepared by shaking for 30 min at 850 rpm. The 0.6 mL samples were then incubated in Eppendorf safe-lock microtubes at 40, 60, 80 and 100 °C for 30 min in the dark and immediately transferred to an ice bath. The samples were then centrifuged at 1000×*g* for 10 min. The absorbance at 450 nm was measured and the relative stability was calculated using the following equation:

$$\text{Relative stability}\ (\%) = (A_T \,/\, A_0) \times 100$$

where A_T is the absorbance of the CAR-IN sample kept at an appropriate temperature for 30 min, and A_0 is the initial absorbance of the sample.

2.5. Antioxidant activity of CAR-IN particles

The antioxidant activity was determined using three different methods: DPPH, ABTS and ferric reducing/antioxidant power (FRAP) (Xiao et al., 2020).

The solution of 0.1 mM DPPH in ethanol was freshly prepared by stirring on a magnetic stirrer for 1 h. Then the 3 mg/mL solutions of the CAR-IN samples were prepared. The DPPH and CAR-IN solutions were mixed at a 1:1 (v/v) ratio and incubated for 2 h at 18 °C in the dark. The absorbance was measured in a 96-well plate at 517 nm and the radical scavenging activity (RSA) was calculated using the following equation:

$$RSA\ (\%) = \left(1 - \left(A_{s-dpph} - A_s\right) \,/\, A_{blank}\right) \times 100 \quad (7)$$

where RSA (%) is the radical scavenging activity, $A_{s\text{-}dpph}$ – is the absorbance of the sample mixed with DPPH solution in ethanol, A_s – is the absorbance of the sample mixed with ethanol, A_{blank} – is the absorbance of blank control, that is, DPPH solution in ethanol mixed with only deionised water.

Next, the sample concentration required to reduce the initial DPPH concentration by 50 % (IC_{50}) was determined by plotting the dependence of RSA (%) on the sample concentration. The equivalent antioxidant capacity of Trolox (TEAC, mg/g) was determined by plotting a calibration curve at a Trolox concentration from 0.00025 up to 0.05 mg/mL and then calculated using the following equation:

$$TEAC = IC_{50}(\text{Trolox, mg / mL}) / IC_{50}(\text{sample, g / mL}) \quad (8)$$

For the determination of antioxidant activity using the ABTS method, stock solutions of 7 mM ABTS and 2.45 mM $K_2S_2O_8$ were prepared in 20 mM acetic acid buffer, pH 4.5, and kept in the dark. For measurement, they were mixed at a 1:1 (v/v) ratio and kept overnight in the dark at room temperature. The next day, 2.8 mL of the mixed solution was diluted to 65 mL with 20 mM acetic acid buffer, pH 4.5, and kept at room temperature for 30 min. Then 200 μL of the prepared solution was mixed with 10 μL of each sample solution in a 96-well plate and kept in the dark for 30 min. Absorbance was measured at 734 nm and TEAC was determined by constructing a Trolox calibration curve at concentrations from 0.00025 up to 0.05 mg/mL and using the following equation:

$$TEAC = c \times V \,/\, m \quad (9)$$

where TEAC is expressed as mg of Trolox equivalent (TE) per g of dry sample, c is the concentration of Trolox (mg/mL) in the calibration curve corresponding to the absorbance of the sample, V is the volume of the sample (mL) and m is the mass of the dry sample (g).

For the FRAP method, the solutions of 10 mM tripyridyltriazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$ in deionised water were prepared. The FRAP solution was obtained by mixing 0.3 M acetic acid buffer solution pH 3.6, 10 mM TPTZ and 20 mM $FeCl_3$ solutions at a ratio of 10:1:1 (v/v/v), respectively, and then preheated at 37 °C. Then 180 μL of freshly prepared FRAP solution was mixed with 20 μL of each sample in a 96-well plate and incubated at 37 °C for 30 min. Absorbance was measured at 593 nm and TEAC was determined as described above using equation (9).

2.6. Spectroscopy measurements of CAR-IN particles

The ^{1}H NMR spectra of pure inulin, the CAR-IN complex and the physical mixture of β-carotene and inulin were recorded in D_2O (Aldrich) using a Bruker 400 Ascend™ nuclear magnetic resonance spectrometer (400 MHz) at 22 °C. Chemical shifts for ^{1}H NMR spectra were reported in parts per million (ppm, δ). Acetone resonance signal (δ 2.22 ppm) was used as an external reference (Fontana & Widmalm, 2023).

A PerkinElmer Raman Station 400 F spectrometer with a 785 nm laser was used to record the Raman spectra. All samples were analysed as lyophilised powder. Spectra were recorded in the range of 3500–800 cm^{-1}. The measurement conditions were as follows: laser power and exposure time were 120 mW and 5 s for complexes, 120 mW and 0.5 s for physical mixtures, 25 mW and 1 s for β-carotene and 250 mW and 2 s for inulin.

2.7. Statistical analysis

All data are presented in this paper as the mean ± standard deviation of at least three parallel experiments. A one-way analysis of variance (ANOVA, $P < 0.05$) was used to compare the parameters of the CAR−IN complexes and define statistically significant results.

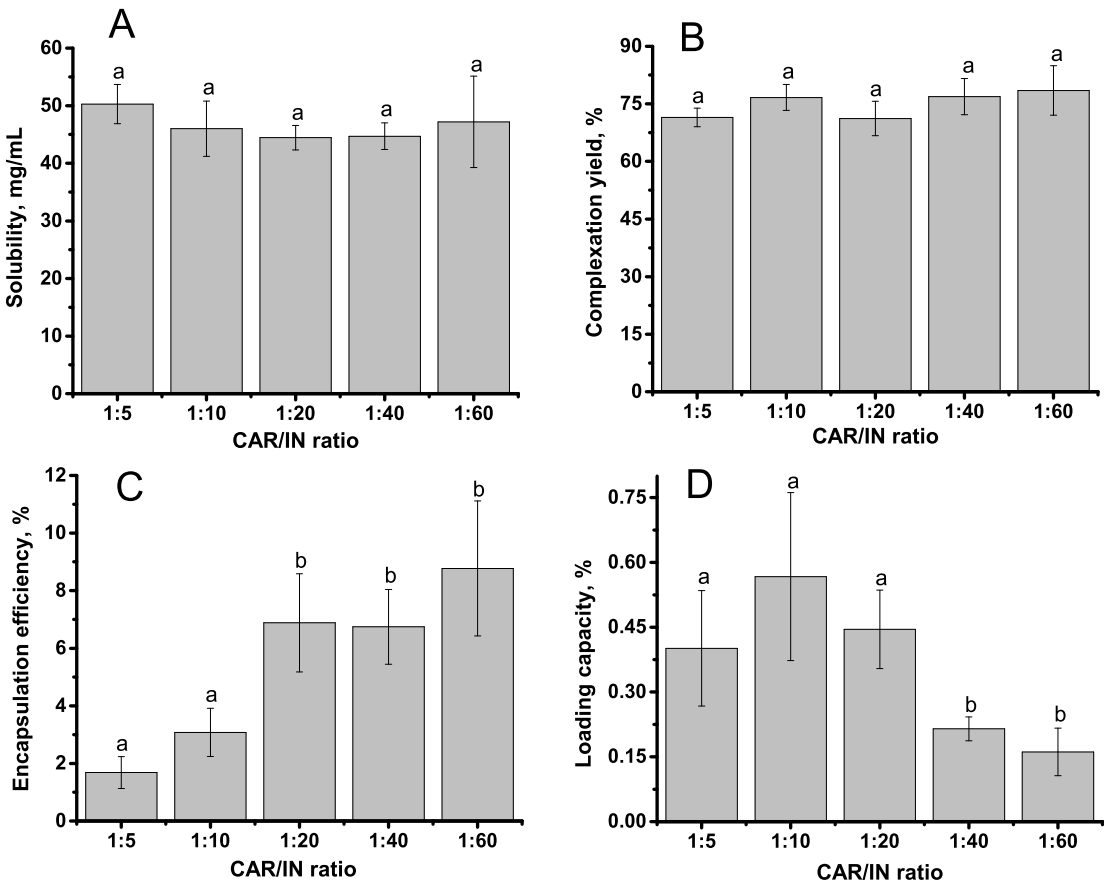

Fig. 1. Preparation of β-carotene-inulin particles (CAR-IN) at different CAR/IN ratios; solubility (A), complexation yield (B), encapsulation efficiency (C) and loading capacity (D) of obtained CAR-IN complexes. Data are presented as mean ± standard deviation of three particle preparation experiments. Different letters represent significant differences in the mean of each parameter depending on the CAR/IN ratio ($P < 0.05$).

Table 1
Characterisation of CAR-IN particles using the DLS method [a].

CAR/IN ratio	Size, nm	PDI	Z_p, mV
1:5	296.9 ± 30.3	0.569 ± 0.062	−23.7 ± 3.3
1:10	271.6 ± 67.8	0.428 ± 0.120	−28.7 ± 6.5
1:20	196.2 ± 71.9	0.451 ± 0.138	−32.1 ± 6.2
1:40	283.0 ± 157.7	0.426 ± 0.113	−27.8 ± 2.5
1:60	311.4 ± 170.4	0.629 ± 0.291	−21.8 ± 6.5

[a] There was no significant difference in the mean of each parameter depending on the CAR/IN ratio as determined by statistical analysis (ANOVA, $P < 0.05$).

3. Results and discussion

3.1. Synthesis and characterisation of CAR-IN particles

To prepare β-carotene-inulin particles, the previously optimised complexation method for the synthesis of β-carotene-cyclodextrin complexes was used (Celitan et al., 2021). The complexes were obtained by slow addition of CAR in the organic solvent acetone to an aqueous inulin solution, followed by evaporation of the organic solvent. CAR was complexed with inulin at different weight ratios. As can be seen, the complexation yield varied from ~71 to ~78 % (Fig. 1B), which is comparable to previously reported β-carotene - xylan complexation (Straksys et al., 2022). The encapsulation efficiency showed a tendency to increase with increasing amount of inulin used for complexation (Fig. 1C). However, the encapsulation efficiency and the loading capacity (Fig. 1D) were lower compared to the encapsulation with xylan (Straksys et al., 2022). On the one hand, different methods were used to prepare the complex, and on the other hand, a different mode of

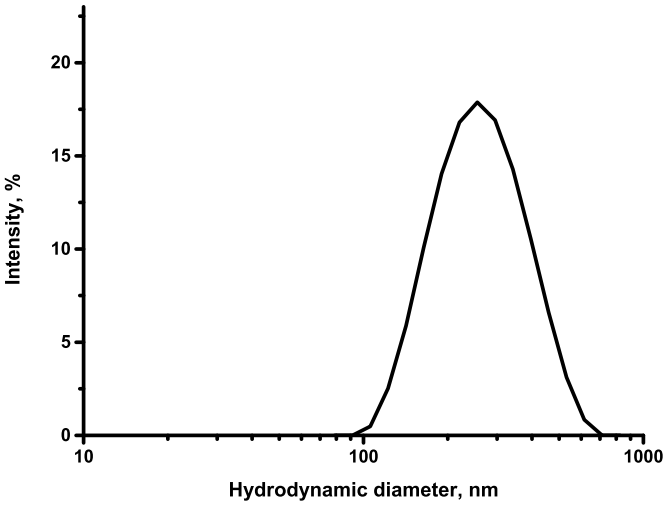

Fig. 2. Size distribution of the CAR-IN complex prepared at a CAR/IN ratio (w/w) of 1:10.

complexation is possible as discussed in Section 3.2. Nevertheless, CAR-IN particles were soluble in water (Fig. 1A). Solubility was similar to that of β-carotene – xylan (Straksys et al., 2022) and about 1.5 times higher than that of β-carotene – chitooligosaccharides complexes (Bockuviene & Sereikaite, 2019). Table 1 shows the CAR-IN parameters determined by the DLS method. There was no significant difference in CAR-IN particle size, PDI and Zp as a function of the CAR/IN ratio as

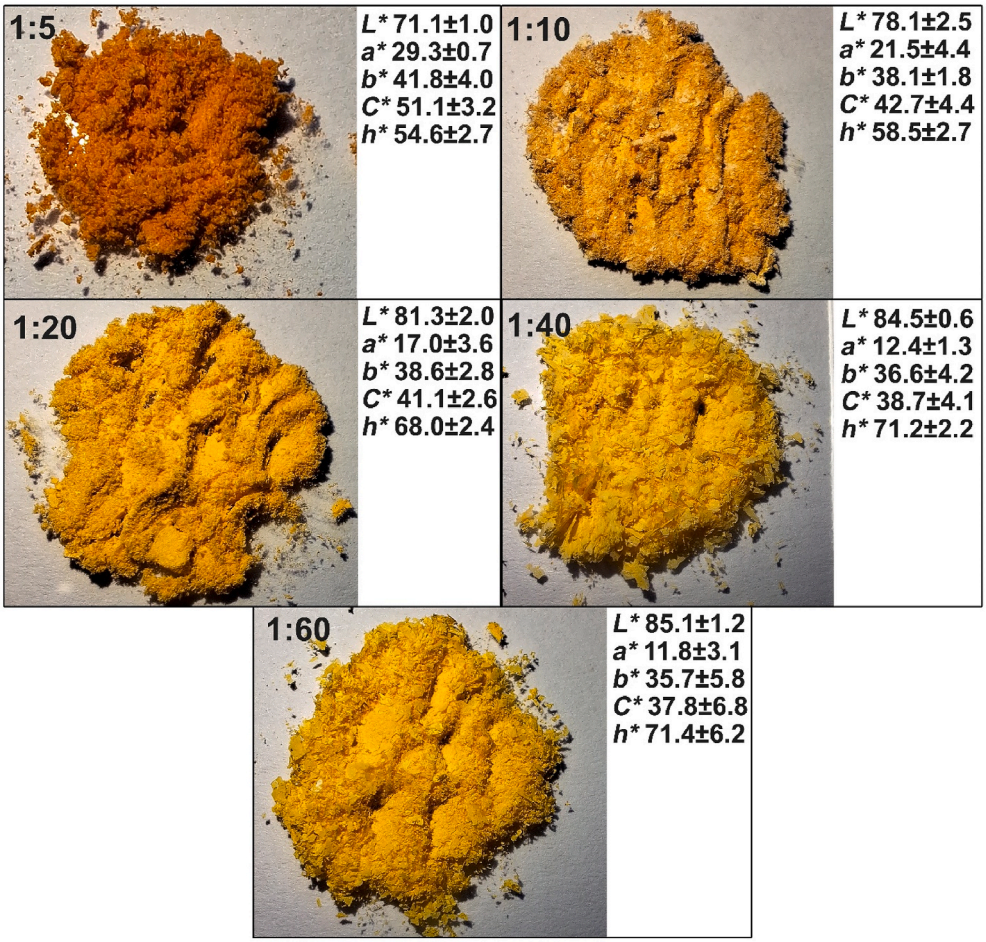

Fig. 3. Photographs and CIELAB colour parameters of the prepared CAR-IN complex powder at different CAR/IN ratios (w/w).

determined by statistical analysis (ANOVA, $P < 0.05$). The particles had a negative surface charge due to the negative zeta potential of inulin (Krivorotova et al., 2016; Mandracchia et al., 2014) that ranged from −21.8 to −32.1 mV. Usually, a surface charge of ±20 mV ensures the stability of the system (Tamjidi et al., 2013). Since the PDI obtained by the DLS method was greater than 0.4, the particles were characterised as broadly polydisperse (Bhattacharjee, 2016). As an example, the size distribution of the CAR-IN complex prepared at a CAR/IN ratio (w/w) of 1:10 is presented in Fig. 2. Taking into account the data in Fig. 1 and Table 1 and it can be concluded that the most appropriate CAR/IN ratios for complex preparation are 1:10 and 1:20.

Photographs of all CAR-IN complexes and their colour parameters are presented in Fig. 3. The colour parameters of the dry samples were determined in CIELAB colour coordinates, as commonly used in the food industry. In the CIELAB colour system, the L^* parameter describes the darkness/lightness of the sample and varies from 0 (for black) to 100 (for white). The C^* (Chroma) parameter describes the intensity or saturation of the colour. The higher the C^* value, the more intense the colour is. Parameters a^* and b^* describe the colour of the sample, i.e., a^* indicates the greenness/redness and varies from negative values (for green) to positive ones (for red) and b^* indicates the blueness/yellowness and varies from negative values (for blue) to positive ones (for yellow). The h^* (hue angle) is calculated from the values of b^* and a^*. An angle of 0^o or 360^o represents red hue, 90^o – yellow hue, 180^o – green hue, and 270^o – blue hue (Pathare et al., 2012). Any angle between these values represents a mixture of hues. As can be seen, the colour parameters depended on the CAR/IN ratio used for the complex preparation. The lightness L* of the samples increased, and the colour intensity (C^*) decreased with increasing amount of inulin. The hue angle showed the change in colour from dark orange (h^* 54.6, 1:5) to yellowish (h^* 71.4, 1:60) (Fig. 3).

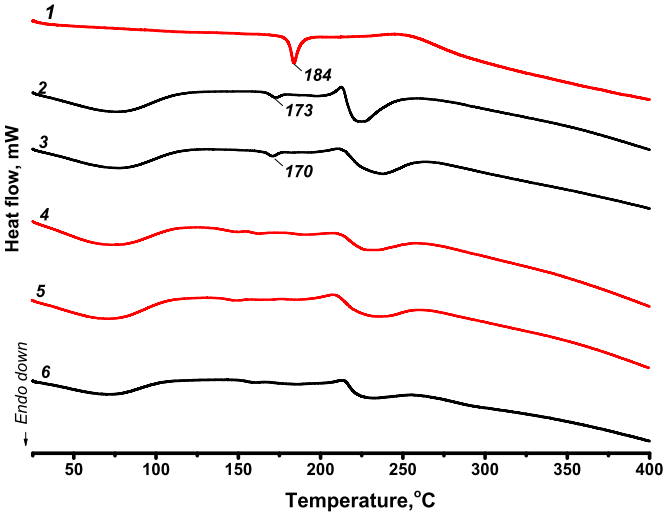

Fig. 4. Differential scanning calorimetry thermograms of β-carotene (1), physical mixtures of β-carotene and inulin (2, 3) and CAR-IN complexes (4, 5) prepared at the CAR/IN ratios (w/w) of 1:10 (2, 4) and 1:20 (3, 5), and inulin (6).

The thermal properties of CAR, inulin, their physical mixtures, and the prepared CAR-IN complexes were analysed using the DSC method

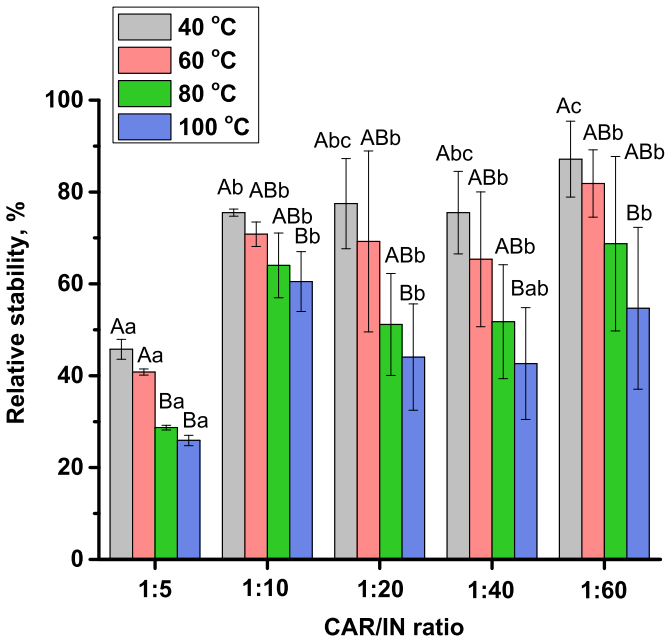

Fig. 5. Thermal stability of CAR in the CAR-IN complexes prepared at different CAR/IN ratios. Different lowercase letters represent significant differences in the mean of relative stability within the same temperature, but at different CAR/IN ratio, and different uppercase letters represent significant differences in the mean of relative stability within the same CAR/IN ratio, but at different temperature ($P < 0.05$).

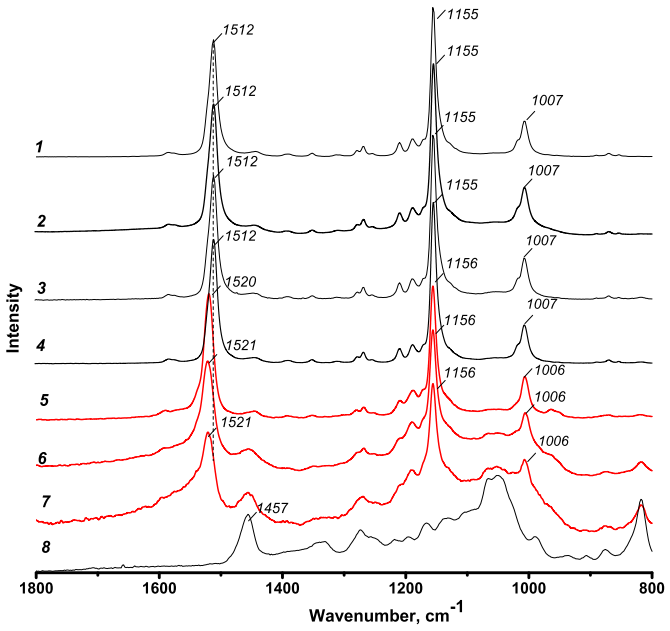

Fig. 6. Raman spectra of pure β-carotene (1), physical mixtures of β-carotene (2, 3, 4) and the CAR-IN complexes (5, 6, 7) prepared at the CAR/IN ratios (w/w) of 1:10 (2, 5), 1:20 (3, 6) and 1:40 (4, 7), and inulin (8).

(Fig. 4). As can be seen, all thermograms of the inulin-containing samples showed two broad endothermic peaks i.e., one of them up to ~100 °C was attributed to the loss of adsorbed water, and another one between 210 and 260 °C corresponded to the onset of thermal degradation of inulin (Ronkart et al., 2007, 2010). The DSC thermogram of CAR showed an endothermic peak corresponding to the melting point at 184 °C (Martini et al., 2010). However, the CAR peak disappeared in the thermograms of the complexes (Fig. 4, curves 4–5) (Bockuviene & Sereikaite, 2019; Straksys et al., 2022). This shows that complexation and/or amorphization has occurred and is in good agreement with previous studies (W. Y. Liu et al., 2023). The melting temperature of CAR in the physical mixtures prepared at the CAR/IN ratios of 1:10 and 1:20 were determined to be at ~173 and ~170 °C, respectively (Fig. 4, curves 2–3). A significant shift of the melting point of CAR in physical mixtures compared to pure CAR and broadening of the peaks were observed. The shift could be due to the different environments of CAR in the physical mixture and in the pure form. Furthermore, the interaction of CAR with a solid inulin cannot be excluded. The shift of the melting point of CAR was also previously observed analysing the physical mixture of CAR and xylan (Straksys et al., 2022).

Complexation with inulin increased the thermal stability of CAR at elevated temperatures (Fig. 5). The effect is noticeable at CAR/IN ratios of 1:10 and higher compared to previously published data for pure CAR. Incubation of 0.1 % (w/v) β-carotene dissolved in ethyl acetate at 60 and 80 °C for 30 min resulted in approximately 50 and 20 % CAR retained, respectively (Chen & Zhong, 2015). Complexation of CAR with chitooligosaccharides has a similar thermal stabilisation effect as inulin (Bockuviene & Sereikaite, 2020).

3.2. Spectroscopy measurements of CAR-IN particles

Raman spectra were recorded to confirm the formation of the complex (Fig. 6). β-Carotene exhibits characteristic Raman bands corresponding to three primary vibration modes: C=C– stretching (ν_1) at 1512 cm^{-1}, asymmetric deformation of –C=C–H (ν_2) in-plane at 1155 cm^{-1}, and –C–CH_3 deformation (ν_3) at 1007 cm^{-1} (De Oliveira et al., 2011). The band assigned to the ν_1 mode is highly sensitive to the chemical environment and provides a good probe for structure monitoring (De Oliveira et al., 2011). The ν_1 mode observed at 1512 cm^{-1} in the Raman spectrum of pure CAR (Fig. 6, curve 1) remained unchanged in the spectra of the physical mixtures (Fig. 6, curves 2–4). In the spectra of the CAR-IN complex prepared at the CAR/IN ratios of 1:10, 1:20, and 1:40, the peak at 1512 cm^{-1} became broader and shifted to the higher wavenumbers of 1520, 1521 and 1521 cm^{-1}, respectively (Fig. 6, curves 5–7). This happens due to the structural changes induced by complex formation and the loss or significant decrease in CAR multiple double bond conjugation (De Oliveira et al., 2011; Letona et al., 2017; Macernis et al., 2021; Straksys et al., 2022).

Since the signals of the Raman spectrum of polysaccharides are significantly weaker than those of the conjugated C=C bond π system of carotenoids, Raman signals attributed to inulin were not observed in the spectrum of the complex prepared at a CAR/IN ratio of 1/10 (Marinopoulou et al., 2021). As can be seen, a decrease in the ratio of CAR to IN led to an increase in the intensity of the Raman signals associated with inulin. In the spectra of the complex prepared at the CAR/IN ratios of 1:20 and 1:40, a band at 1457 cm^{-1} was observed, which is attributed to the bending vibration of the methylene group of inulin. Furthermore, bands corresponding to the C–O stretching vibrations of the C–OH and C–O–C inulin groups appeared in the region between 1100 and 950 cm^{-1} (Silva et al., 2024).

The ^{1}H NMR spectra of the inulin physical mixture and the CAR-IN complex are presented in Fig. 7. In the inulin spectrum (Fig. 7A), an intense doublet at 4.25 ppm ($J = 8.5$ Hz) and a triplet at 4.10 ppm ($J = 8.5$ Hz) were related to H-3 and H-4 of fructose, respectively. The appearance of a triplet of C4 proton at 4.10 ppm is typical for (2→1) linked residues in inulin chains. The protons of the AB-spin system are assigned to H-1 and H-6 of the fructose unit, showing chemical shifts at 3.71 and 3.93 ppm for H-1, and 3.77 and 3.85 ppm for H-6, respectively (Oka et al., 1992). The H-5 signal (δ 3.86 ppm) overlapped strongly with the H-6_A signals. The chemical shifts of the signals agree well with the data from the literature (Frasch et al., 2017; Meng et al., 2020; Sun et al., 2020).

Normally, the NMR technique can be used to assess the formation of the inclusion complex. However, in this study, the discrepancies in the

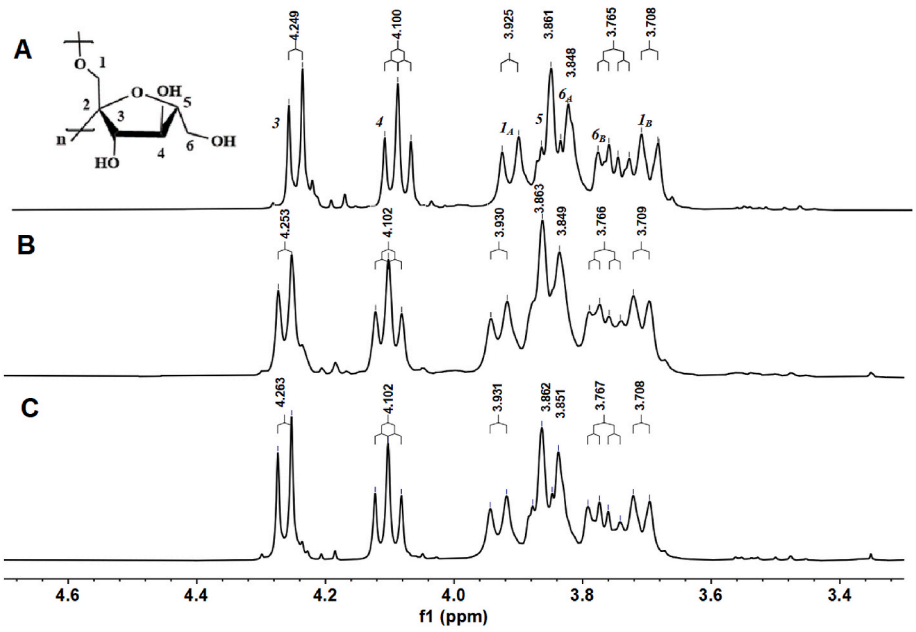

Fig. 7. ^{1}H NMR spectra of pure inulin (A), the CAR-IN complex (B) and the physical mixture of β-carotene and inulin (C) prepared at a CAR/IN ratio (w/w) of 1:10.

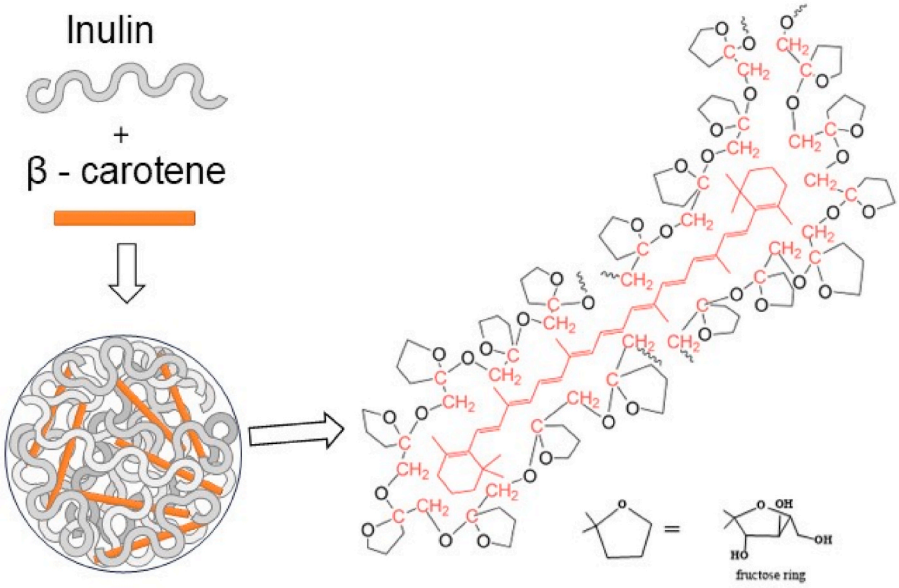

Fig. 8. Scheme of the formation of the β-carotene-inulin complex.

chemical shifts between the proton signals of inulin in the complex and of pure inulin are less than 0.01 ppm (Fig. 7A and B). Therefore, the formation of the inclusion complex between inulin and CAR cannot be confirmed. This phenomenon has already been pointed out in previous works that analyse the complexation of red bell pepper pigments with β-cyclodextrin (Gomes et al., 2014) or 2-hydroxypropyl-β-cyclodextrin (De Lima Petito et al., 2016) and can be expected in cases where an inclusion host-guest complex has a low value of the association constant. On the other hand, the inulin macromolecule does not form a stable helical structure stabilised by hydrogen bonding with hydrophobic cavities, as seen in levan-type polysaccharides or polysaccharides composed of glucose-based units (Vereyken et al., 2003). The backbone of β-(2 → 1) linked inulin is similar to poly(ethylene oxide) chains and does not incorporate any sugar ring. This contributes to increased chain mobility (Afinjuomo et al., 2021; Mensink et al., 2015). Therefore, the discrepancies in the chemical shifts between the internal and external hydrogens of inulin in the spectra of pure inulin and inulin in the complex were small.

The ^{1}H NMR spectra of pure inulin (Fig. 7A) and the physical mixture of inulin and β-carotene (Fig. 7C) show well-resolved proton signals of inulin. The spectrum of the physical mixture is similar to that of pure inulin, indicating no interaction between the hydrophobic molecule and inulin. Meanwhile, the spectrum of the complex shows a decrease in the mobility of the inulin chain due to the interaction with hydrophobic β-carotene (Fig. 7B). The motional freedom of polysaccharide protons is reduced in the presence of hydrophobic β-carotene. This restriction in motion increases the correlation time (τc), which is the characteristic time for molecular reorientation in the NMR analysis, and leads to a broadening of the NMR resonances (Van Hees et al., 2019; Wei et al., 2018).

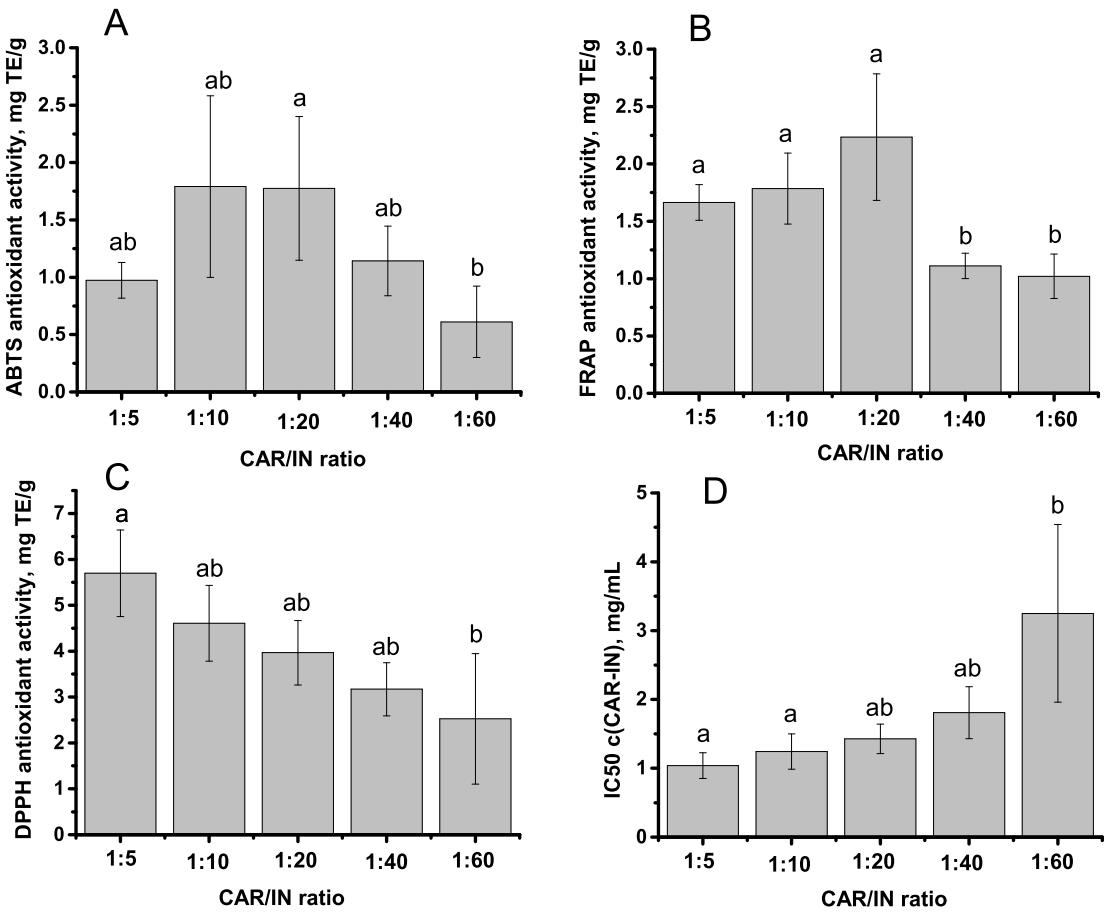

Fig. 9. Antioxidant activity of CAR-IN particles determined by ABTS (A), FRAP (B) and DPPH (C) assays in Trolox equivalents, and CAR-IN concentration required to reduce the initial concentration of DPPH by 50 % (D). The data are presented as the mean ± standard deviation of three experiments on the preparation of the particles. Different letters near the columns represent significant differences ($P < 0.05$).

On the basis of spectroscopic measurements and DSC analysis, it is evident that the complexation of CAR with inulin occurred. However, the inclusion complexes were not formed as in the case of amylose or xylan (Kong et al., 2018; Straksys et al., 2022). The main reason for this is the high flexibility of the inulin chain and the inability to form a stable helical structure. As mentioned above, due to the absence of a fructose ring in the backbone of inulin and its similarity to poly(ethylene oxide), the most plausible interaction mechanism is the entrapment of CAR between inulin molecules and the hydrophobic interaction of CAR with the backbones of inulin (Fig. 8). Previous experiments have demonstrated the hydrophobic properties of inulin in its interaction with lipid systems. The CH_2 groups in the inulin backbone constitute the hydrophobic part of the molecule. Furthermore, longer inulin chains (DP > 20) are more hydrophobic than shorter ones. (Stopar et al., 2023; Vereyken et al., 2001, 2003).

3.3. Antioxidant activity of CAR-IN particles

Considering the application of a new β-carotene formulation as a food additive, it is important to find out whether the newly prepared complexes would still be useable as antioxidants. In this study, three methods, DPPH, ABTS and FRAP, were used to investigate the antioxidant activity of the prepared CAR-IN particles. These methods are widely used to assess the antioxidant activity of various compounds (Dudonné et al., 2009; Rumpf et al., 2023; Thaipong et al., 2006). Data are shown in Fig. 9. As seen, in the case of ABTS and FRAP, the tendency of the changes of ABTS radical scavenging activity and ferric reducing power practically correlates with loading capacity. At lower CAR/IN ratios, that is, higher amount of inulin used for particle preparation, ABTS antioxidant activity and ferric reducing ability decrease. Inulin itself has a weak antioxidant activity and ferric reducing power compared to strong antioxidants as determined in this and previously published study (Shang et al., 2018). The DPPH radical scavenging activity showed a tendency to decrease with increasing amount of inulin used for particle preparation, and the CAR-IN concentration required to reduce the initial concentration of DPPH by 50 % increased (Fig. 9C and D). There is some discrepancy in this tendency compared to the ABTS and FRAP methods. Previously published studies have also found a good correlation between ABTS and FRAP methods. However, the correlation between ABTS and DPPH, and FRAP and DPPH was found to be weak. This depends not only on the reaction mechanism but also on the origin and structure of the samples (Rumpf et al., 2023; Wootton-Beard et al., 2011).

To sum up, β-carotene encapsulated in inulin retains its antioxidant activity. Therefore, CAR-IN particles could serve as an additive for fortifying foods with beneficial compounds. This is in good agreement with previously published studies. Encapsulation does not usually mask the antioxidant properties of β-carotene (Nazir et al., 2024; Straksys et al., 2024; Zarif et al., 2023).

To test the feasibility of using CAR-IN complexes to fortify beverages with CAR, freshly pressed apple juice and store-bought birch sap, a popular beverage in Lithuania and northern Europe, were used. A CAR-IN complex prepared at a CAR/IN ratio of 1:20 was dissolved in the beverages to achieve a final concentration of 1 mg/mL of the complex. This corresponded to the maximum level of 0.0045 mg/mL of CAR recommended by the German Federal Institute for Risk Assessment

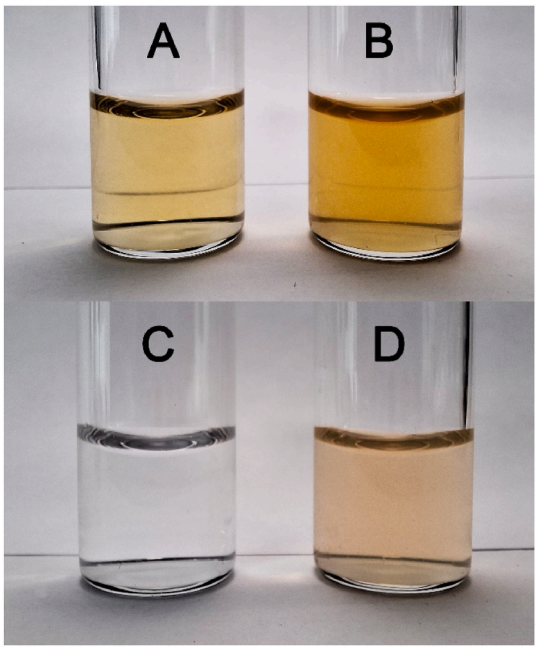

Fig. 10. Photographs of apple juice (A) and birch sap (C), and apple juice (B) and birch sap (D) enriched with the CAR-IN complex prepared at a CAR/IN ratio (w/w) of 1:20. The final concentration of the CAR-IN complex was 1 mg/mL corresponding to 0.0045 mg/mL of CAR.

("Updated recommended, 2021 Maximum Levels for the Addition of Vitamins and Minerals to Food Supplements and Conventional Foods: BfR Opinion No 009/2021 of March 15, 2021," 2021) (Fig. 10). The antioxidant activity of birch sap, as measured by the DPPH method, increased more than twofold, increasing from 0.005 ± 0.001 to 0.012 ± 0.002 mg TE/mL. As apple juice is rich in antioxidants (Vallée Marcotte et al., 2022), the addition of the CAR-IN complex had an insignificant effect on antioxidant activity of the juice, which remained at 1.9 ± 0.2 mg TE/mL. Nevertheless, the apple juice was enriched with CAR as a vitamin A precursor. The addition of the CAR-IN complex changed the colour of both beverages. From a^* and b^*parameters calculated, C^* of apple juice increased from 13.6 ± 1.1 to 17.1 ± 1.5 and h^* decreased from 87.3 ± 2.2 to 73.8 ± 2.8. The colour became more intense and reddish. Birch sap is a clear colourless liquid. After the addition of the CAR-IN complex, the a^* and b^* parameters reached values of 3.9 ± 0.01 and 5.3 ± 0.01, respectively. The C^* and h^* parameters were 6.6 ± 0.7 and 54.2 ± 1.9, respectively. This shows the appearance of red-yellow tones in the fortified birch sap sample.

Therefore, the use of CAR-IN complexes for food fortification was demonstrated. However, more detailed investigations into the physicochemical stability of these complexes are needed. The long-term stability of CAR-IN complex powder should be investigated during storage at different temperatures. It is also important to investigate the stability of the encapsulated β-carotene in soluble form within a real food product. Finally, the bioaccessibility of the CAR complexed with inulin should be analysed and compared with that of other β-carotene-polysaccharide complexes previously prepared (Bockuviene & Sereikaite, 2019; Straksys et al., 2022).

4. Conclusions

The study demonstrated the possibility of using inulin for the development of a β-carotene delivery system. On the basis of the spectroscopic analysis, the most plausible mode of particle formation is the entrapment of CAR between inulin molecules and the hydrophobic interaction of CAR with the backbones of inulin. The encapsulated β-carotene showed its antioxidant activity and the complexes were soluble in water. These properties of newly prepared complexes are essential when considering the use of particles for water-based food fortification. This is the first time that β-carotene has been complexed with inulin. This expands the potential applications of β-carotene as an additive.

CRediT authorship contribution statement

Inga Gabriunaite: Writing – original draft, Investigation, Conceptualization. **Tatjana Kavleiskaja:** Visualization, Investigation. **Rūta Gruškienė:** Validation, Conceptualization. **Jolanta Sereikaitė:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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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Data availability

Data will be made available on request.

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