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Synthesis and Characterisation of Water-Soluble β-Carotene–Xylan Complexes

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ABSTRACT

β-Carotene is a useful compound for food fortification because it has beneficial effects on human health. However, it is insoluble in water and sensitive to light, oxygen and temperature. To increase the solubility and stability of *β*-carotene, its complexation with polysaccharides is used. The study aimed to develop and optimise a new method for *β*-carotene–xylan (CAR–XYL) complex fabrication. The physical and chemical properties of the resulting complexes were investigated. The *β*-carotene:xylan ratios of 1:3 (w/w) and 1:5 (w/w) were concluded to be the most applicable to *β*-carotene formulation. The solubility of the complexes was 36.8 and 38.3 mg/mL, respectively. The particles were small in size. Their hydrodynamic diameters were 300.4 ± 10.4 nm and 242.2 ± 2.6 nm, respectively. The thermal stability of the complexes at different temperatures for 30 min was not significantly affected by the *β*-carotene:xylan ratio. However, the shelf life of *β*-carotene under UV irradiation and long-term storage under different conditions showed a tendency to decrease in *β*-carotene:xylan ratios of 1:10 (w/w) and 1:20 (w/w). *β*-Carotene complexed with xylan did not lose its antioxidant activity. Furthermore, for the use of the *β*-carotene–xylan complexes in functional foods, the bioaccessibility of *β*-carotene complexed with xylan was studied using the INFOGEST protocol. For CAR–XYL complexes fabricated at CAR:XYL ratios of 1:3 and 1:5, bioaccessibility was 54.3 ± 5.6% and 59.03 ± 3.3%, respectively.

1 | Introduction

β-Carotene (CAR) is a desirable component for the fortification of functional foods and beverages. It positively affects human health because of its anti-inflammatory and high antioxidant activity (AA). Consumption of CAR reduces the risk of some diseases such as cardiovascular disease, cancer and type 2 diabetes. It is also important for human vision, reducing the number of cases of blindness. In humans, CAR is also a precursor of vitamin A [1–3]. CAR is usually obtained from a diet that includes fruits and vegetables. If people's diets do not contain enough fruits and vegetables, they could obtain CAR from fortified foods and beverages. However, CAR is a lipophilic compound that has a polyene system with 11 conjugated double bonds and two *β*-rings at each end of the chain in its structure. It is insoluble in water and undergoes chemical degradation under the action of oxygen, temperature and light [3, 4].

In order to deliver CAR into various foods, particularly those based on water, and to increase its chemical stability, various encapsulation techniques are being investigated. Over the last decade, many studies have been carried out on the use of conventional and Pickering emulsions as CAR delivery systems [5–8]. Although emulsions are the most widely used colloidal system, their instability and stratification cause many problems. To extend their shelf life and facilitate commercial applications and handling, conversion of them from a liquid to a powder was proposed using spray drying or freeze drying [8, 9].

Another way to increase the solubility and stability of CAR is to complex it with cyclodextrins or polysaccharides. For that, β-cyclodextrin [10–12] or modified forms such as 2-hydroxypropyl- and H_2N- β-cyclodextrins [13, 14] or cyclodextrin-based nanosponges [15] have been used. To provide additional protection, CAR complexes formed with cyclodextrin can be coated with polysaccharides, such as chitosan or pectin [16, 17]. Polyakov et al. [18] fabricated the first water-soluble carotenoid complex with polysaccharides. Canthaxanthin was complexed with arabinogalactan by a mechanochemical method. CAR complexes were later obtained with chitooligosaccharides [19], inulin [20] and xylan [21].

Xylan is extracted from plants and algae. It is found in the cell wall and is composed of a linear chain of β-(1 ⟶ 4)-linked xylopyranose residues. The backbone can be substituted with neutral sugars, such as α-D-galactose and α-D-arabinose, as well as α-D-glucuronic acid and 4-O-methyl-α-D-glucuronic acid. In addition, acetyl groups as well as ferulic, coumaric and phenolic acids are found as side groups. The structure of xylan depends on the extraction process and the source of the biopolymer [22, 23]. Xylan is used in industrial biotechnology as a carbohydrate source for biofuel and value-added chemical production [24, 25]. In addition, due to its biodegradability and high biocompatibility, xylan is being studied for biomedical applications such as drug and gene delivery or tissue engineering [26, 27]. Xylan is biologically active and exhibits antioxidant and prebiotic properties [28, 29]. Xylooligosaccharides, which are produced from xylan by hydrolysis, also find their application in the food industry as valuable components of functional foods [30].

In previous work [21, 31], the β-carotene–xylan (CAR–XYL) complex was obtained using the mechanochemical method, which involved shaking followed by freeze-drying. This process is time-consuming, with the shaking step taking at least 24 h. In this study, a new method based on immediate solvent evaporation was proposed and optimised for the formulation of CAR with xylan.

2 | Materials and Methods

2.1 | Materials

Beechwood xylan (≥ 95%; monosaccharides (%)—xylose:glucuronic acid:other sugars = 84:10.3:5.7) was purchased from Megazyme. CAR (≥ 93%) and (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (97%, Trolox) were obtained from Sigma-Aldrich. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Alfa Aesar. Methanol (HPLC grade) and acetone (puriss. p.a.) were purchased from Honeywell. Tetrahydrofuran for HPLC and ethanol (96%) were purchased from Roth and the local distributor 'Vilniaus Degtine', respectively. The digestive enzymes and bile used for in vitro digestion were obtained from Sigma-Aldrich: pepsin from porcine gastric mucosa (P7012; ≥ 2500 units/mg), pancreatin from porcine pancreas (P7545; 8xUSP specification) and bovine bile (B3883).

2.2 | Synthesis of Water-Soluble CAR–XYL Complexes

To synthesise the CAR–XYL complex, 4.2 mg of CAR was dissolved in 20 mL of acetone and slowly added dropwise with rapid stirring to 12.6 mg of xylan dissolved in 10 mL of water heated to 65°C. The final CAR:XYL ratio was 1:3 (w/w). The reaction was performed in the flask, which was wrapped in aluminium foil to prevent ambient light damage to CAR. The reaction was carried out using a heating plate with a temperature sensor until the acetone had completely evaporated. Finally, the CAR–XYL solution was filtered using a disposable syringe and a polyester sulfone (PES) filter (pore size 5 µm) to remove unreacted CAR. The aqueous CAR–XYL solution was lyophilised for 48 h in a Telstar LyoQuest freeze-dryer. The same procedure was used to prepare CAR–XYL complexes in CAR:XYL ratios of 1:1, 1:5, 1:10 and 1:20 (w/w).

To determine the amount of CAR encapsulated, the aqueous solution of the CAR–XYL complex was mixed with n-hexane and ethanol in a ratio of 1:3:2 (v/v/v), respectively. The extraction was repeated three times, and the hexane phase was collected. To determine the CAR concentration, the absorbance of the hexane phase was recorded at 450 nm. The CAR concentration was found using the formula [32]: $c\ (\mathrm{CAR}) = (\mathrm{Abs}/(A^{1\%} \times d)) \times \mathrm{DF} \times 10$, where c (CAR) is the CAR concentration in the sample (mg/mL); Abs is the absorbance at 450 nm; $A^{1\%}$ is the CAR mass extinction coefficient in hexane equal to 2590 L/(g × cm); d is the cuvette length equal to 1 cm; and DF is a sample dilution factor.

Finally, the loading capacity (LC, µg/mg) was determined according to the formula: LC = mass of the complexed CAR/mass of the prepared CAR–XYL complex.

The encapsulation yield (YE, %) and the encapsulation efficiency (EE, %) were found using the formulas as follows: YE (%) = (final mass of complex/initial mass of CAR and XYL) × 100 and EE (%) = (encapsulated mass of CAR/initial mass of CAR) × 100.

2.3 | Characterisation of CAR–XYL Complexes Obtained in Different CAR:XYL Ratios

A Zetasizer NanoZS instrument (Malvern Instruments) equipped with a 4 mV HeNe laser at 633 nm was used to determine the zeta potential and hydrodynamic diameter of the complexes. The intensity of the scattered light was registered at an angle of 173°. Experiments were carried out at 20°C. To analyse the data, Malvern Zetasizer software 7.03 was used.

FT-IR spectra of the samples were recorded using a Perkin Elmer Frontier 65 spectrometer with a Universal ATR Sampling Accessory under the following conditions: a scan range of 800–1800 cm^{-1} and 20 scans at a resolution of 2 cm^{-1}.

The HPLC analysis of CAR was carried out using a C30 YMC column (250 × 4.6 mm I.D., particle size of 5 µm, YMC Co., Ltd., Japan). A mixture of ethanol–methanol–tetrahydrofuran (75:20:5, v/v/v/) was used as a mobile phase. The isocratic elution was carried out at 1 mL/min, and the absorbance was recorded at 450 nm. The initial CAR and CAR extracted from the water-soluble CAR–XYL complex were dissolved in the mobile phase and analysed.

The ^{1}H NMR spectrum of the extracted CAR was recorded using a Bruker 400 Ascend™ 400 MHz (UltraShield™ Plus 9.4 T magnet) spectrometer at 29°C. The sample was dissolved in C_6D_6 (^{1}H NMR δ = 7.16 ppm), and the ^{1}H NMR spectrum was recorded with an acquisition time of 6.8 s and with 96 scans.

To determine the solubility of the CAR–XYL complexes, 20 mg of the lyophilised complex was dissolved in 500 μL of water under magnetic stirring for 30 min. The solution was then centrifuged at 3800 × *g* for 10 min. The remaining insoluble complex was dried and weighed. The solubility was calculated and expressed in mg/mL and was corrected for the moisture content of the lyophilised complex samples, which did not exceed 3.5%.

To evaluate the relative stability of the encapsulated CAR, 0.7 mL of aqueous solutions of the CAR–XYL complex in screw-capped microtubes were kept in the light at room temperature (22°C) and in the dark at 4°C and room temperature (22°C) for four weeks. At certain time points, the changes in absorbance were measured at 450 nm.

To investigate the effect of UV on the stability of encapsulated CAR, 250 μL of aqueous solution of complexes with absorbance at 450 nm close to 1 was poured into a 96-well plate and kept for 2 h at 15 cm away from the UV source. The samples were irradiated using a UV-C lamp (254 nm, 30 W, Philips, The Netherlands). The absorbance of the samples was recorded at certain time points.

For thermal stability experiments, 0.5 mL samples in Eppendorf safe-lock microtubes were incubated at 20, 40, 60, 80 and 100°C for 30 min in the dark. To stop the reaction, the samples were cooled down in an ice bath, and the absorbance of the samples was registered at 450 nm.

In all cases, the relative stability of the encapsulated CAR was calculated as follows: Relative stability $(\%) = (A_t/A_0) \times 100$.

To measure the AA of the CAR–XYL complexes, the DPPH method was used. First, a 0.1 mM ethanolic DPPH solution was prepared and then mixed with the test samples in a 1:1 ratio (v/v). The resulting solution was incubated at room temperature for 2 hours. Then, 250 μL of each sample was poured into a 96-well plate, and the absorbance was measured at 517 nm. To calculate the radical scavenging activity (RSA, %), the formula was used as follows: RSA $(\%) = (1 - (A_S - A_E)/A_C) \times 100$, where A_C is the absorbance of the ethanolic DPPH solution when water is added instead of the sample; A_S is the absorbance of a mixture of DPPH and sample solutions; A_E is the absorbance of a mixture of sample solution and ethanol when ethanol is added instead of the ethanolic DPPH solution.

The AA was presented as mg of Trolox equivalent (TE) per g of complex according to the formula: AA (mg TE/g) $= (C_T \times 1000)/C_S$, where C_T is the Trolox concentration (mg/mL) corresponding to RSA (%) of the sample and determined from the calibration curve obtained by plotting the RSA (%) against the Trolox concentration; and C_S is the sample concentration used for analysis (mg/mL).

2.4 | Evaluation of In Vitro Digestibility of CAR–XYL Complexes

In vitro digestion of CAR–XYL complexes was performed using the standardised INFOGEST method [33, 34]. To simulate the oral, gastric and intestinal phases of digestion, the simulated salivary (SSF), gastric (SGF) and intestinal (SIF) fluids containing electrolytes were prepared as described by Brodkorb et al. [33]. Aqueous CAR–XYL complexes fabricated in CAR–XYL ratios of 1:3 and 1:5 were used in the study. To simulate the oral phase, 1 mL of the sample was mixed with 1 mL of SSF. The pH was adjusted to 7.0, and the resulting 2 mL mixture was incubated for 2 min at 37°C. Then, 2.0 mL of the mixture obtained after the oral phase was mixed with SGF and 0.32 mL of pepsin was added to achieve a final pepsin concentration of 2000 U/mL. The pH was then adjusted to 3.0, and the sample in the final volume of 4 mL was simulated in the gastric phase for 2 h at 37°C with rotation at 30 rpm. To simulate the intestinal environment, 4.0 mL of the sample obtained after the SGF phase was mixed with SIF, 1.0 mL of pancreatin at a concentration of 133.3 mg/mL and 0.6 mL of 9% bile dissolved in SIF buffer. The pH was adjusted to 7.0, and the resulting 8 mL sample was incubated for 2 h at 37°C with rotation at 30 rpm. To obtain the micellar fraction containing bioaccessible CAR, an aliquot of the digesta was centrifuged for 10 min at 5000 × *g* and 4°C.

To determine the bioaccessibility of CAR, the initial CAR–XYL sample, the digested sample and the micellar fraction were used for CAR extraction. A volume of 0.5 mL of sample was well mixed with 1.2 mL of an organic solvent consisting of 2:3 (v/v) hexane/isopropanol. The mixture was then centrifuged at 2000 × *g* for 1 min, and the well-separated upper hexane layer was collected.

The absorbance of the hexane phase containing dissolved CAR was then measured at a wavelength of 450 nm. The CAR content was calculated as described in Section 2.2.

CAR bioaccessibility was calculated as follows: Bioaccessibility $(\%) = C_M/C_0 \times 100$, where C_0 and C_M are the amounts of CAR present in the mixture before digestion and in the micellar fraction, respectively.

To calculate the recovery of CAR, which indicates its stability during in vitro digestion, the following formula was used: Recovery $(\%) = C_D/C_0 \times 100$, where C_D is an amount of CAR in the digesta.

2.5 | Statistical Analysis

Data are shown as the mean ± standard deviation of three independent experiments unless otherwise stated. One-way analysis of variance (ANOVA, $p < 0.05$) with post hoc Tukey's HSD test was used to define statistically significant data.

3 | Results and Discussion

3.1 | Fabrication of CAR–XYL Complexes in Different Component Ratios

A water-soluble CAR system was prepared according to the scheme shown in Figure 1. The method is based on the slow addition of CAR dissolved in acetone to an aqueous xylan solution, followed by immediate evaporation of the organic solvent. In order to ensure the water solubility of CAR and its high stability during storage and protection against various environmental factors, different ratios of CAR:XYL (w/w) were tested.

As can be seen in Table 1, the yield of the process was quite high, ranging from 83% to 89%, and did not depend on the ratio of the components. The complexes were negatively charged. The zeta potential ranged from −21.8 to −28.9 mV. According to Megazyme data, the xylan used for complexation consists of units of xylose, glucuronic acid and other sugars in the 84:10.3:5.7 (%) ratio. Since the pK_a of glucuronic acid is approximately 3.2 [35],

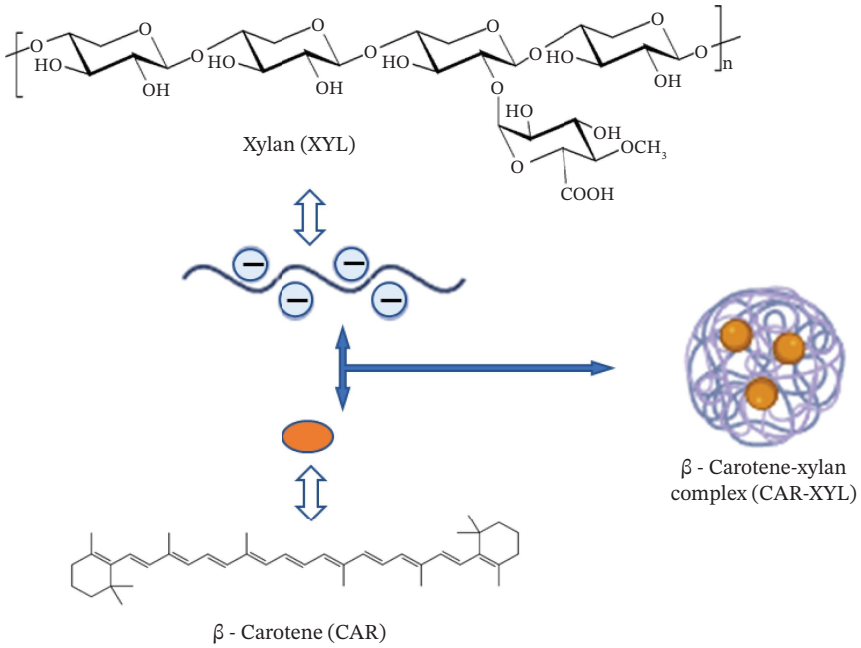

FIGURE 1 | Scheme of *β*-carotene–xylan complex preparation.

the zeta potential of xylan is −25.0 ± 0.98. Consequently, the zeta potential of the resulting complexes is also negative. Zeta-potential values show that a system is expected to be stable. Typically, a minimum value of ± 20 mV is required to ensure the stability of a colloidal solution [36].

The hydrodynamic diameter of the complexes ranged from 250 to 300 nm. The system was moderately polydisperse, as the PDI ranged from 0.11 to 0.21 [37]. As an example, the size distribution of the CAR–XYL complex prepared in a 1:5 CAR:XYL ratio is presented in Figure 2.

The lowest solubility of the complexes obtained was in a 1:1 CAR:XYL ratio, increased to 36.8 mg/mL at 1:3 and varied slightly with increasing xylan portion. Based on the solubility of the complexes, as well as the EE and LC, the CAR:XYL ratios of 1:3 and 1:5 were found to be applicable for the synthesis of soluble CAR–XYL complexes.

Lyophilised examples of synthesised complexes and their aqueous solutions are presented in Figure 3. The solutions of complexes prepared in CAR:XYL ratios of 1:10 and 1:20 are lighter in colour due to lower LC.

To demonstrate the formation of CAR–XYL complexes, FT-IR spectra were recorded (Figure 4).

In the spectrum of pure CAR, the bands at 1450 and 1370 cm^{-1} are assigned to $=CH_2$ scissoring and $-CH_3$ umbrella vibrations, respectively. The band at 964 cm^{-1} corresponds to the conjugated alkene −CH=CH− out-of-plane deformation mode [38]. The spectra of xylan, CAR–XYL complex and physical mixture show the characteristic asymmetric and symmetric stretching vibrations of carboxylate at ~1600 and 1415 cm^{-1}, respectively [39]. The intense band at ~1033–1040 cm^{-1} is attributed to the COH and C−C vibrations of the xylopyranose ring [40]. However, the characteristic CAR bands at 1370 and 964 cm^{-1} disappear in the CAR–XYL complex spectrum, suggesting intermolecular interactions between CAR and xylan. On the contrary, these bands are observed in the spectrum of the physical mixture of CAR and xylan.

To verify that CAR did not undergo isomerisation and degradation during complex synthesis, HPLC was performed and the purity of CAR used for synthesis was compared with that extracted from the complex (Figure 5). The results showed that 94.0% of the initial CAR was all-*trans*-*β*-carotene as obtained from the supplier. After complexation, approximately 70%–75% of the extracted CAR remained all-*trans*-*β*-carotene, and the percentage was similar for all CAR:XYL ratios used in the experiments.

TABLE 1 | Fabrication of *β*-carotene–xylan complexes in different CAR:XYL ratios.

CAR:XYL ratio (w/w)	YE (%)	EE (%)	LC (μg/mg)	Solubility (mg/mL*)	Size (d·nm)	PDI	Zp (mV)
1:1	88.8 ± 4.5^{a}	6.1 ± 1.2^{a}	31.6 ± 4.9^{a}	30.4	302.4 ± 3.2^{a}	0.161 ± 0.002^{ab}	-23.2 ± 0.9^{ac}
1:3	85.2 ± 9.5^{a}	10.3 ± 4.8^{bc}	25.8 ± 11.1^{a}	36.8	300.4 ± 10.4^{a}	0.160 ± 0.031^{ab}	-21.8 ± 0.9^{a}
1:5	85.9 ± 10.3^{a}	13.8 ± 4.4^{c}	23.1 ± 7.3^{a}	38.3	242.2 ± 2.6^{b}	0.112 ± 0.008^{a}	-24.7 ± 1.0^{c}
1:10	83.1 ± 6.6^{a}	7.7 ± 2.4^{ab}	7.0 ± 2.2^{b}	38.6	258.6 ± 8.0^{b}	0.212 ± 0.066^{b}	-28.4 ± 0.7^{b}
1:20	87.8 ± 6.1^{a}	6.1 ± 0.4^{ab}	2.9 ± 0.2^{c}	40.0	297.0 ± 3.4^{a}	0.211 ± 0.002^{ab}	-28.9 ± 0.4^{b}

Note: Data are shown as mean ± SD ($n = 3$). Different letters show significant differences ($p < 0.05$) in the mean within each column.
*Data are shown as the mean of two independent experiments.

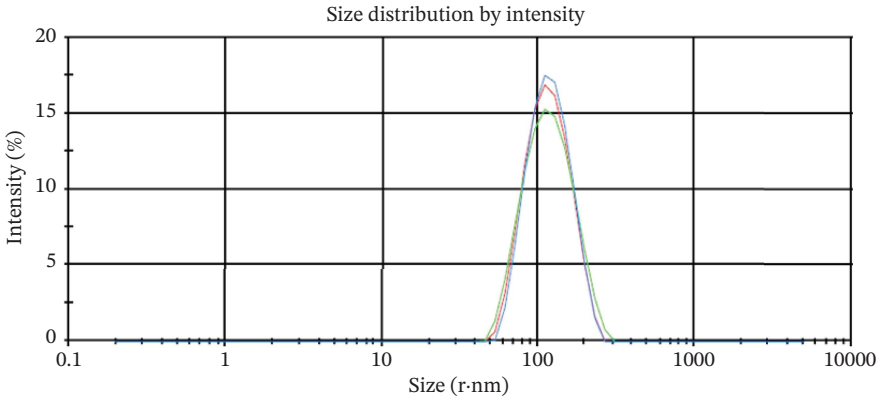

FIGURE 2 | Size distribution of β-carotene–xylan complex prepared at a 1:5 CAR:XYL ratio.

In addition, the ^{1}H NMR spectrum of the extracted CAR was recorded (Figure 6). In the olefinic region of the ^{1}H NMR spectrum, doublets or doublets of doublets at 6.32, 6.36, 6.39, 6.49, 6.68, 6.78 and 6.81 ppm correspond to protons 8/8′, 10/10′, 7/7′, 12/12′, 14/14′, 12/12′, 15/15′ and 11/11′, respectively. Unfortunately, the signals of the aliphatic proton region overlapped with the signals of impurities from the extraction mixture. Nevertheless, the spectrum pattern in the aliphatic proton region was comparable with that of CAR [41]. In the spectrum of the extracted CAR, the methyl groups appeared as a singlet at 1.16 (16/16′ and 17/17′), 1.78 (18/18′) and 1.92 (19/19′ and 20/20′) ppm. The methylene groups 3/3′ and 4/4′ of the rings were observed as multiplets at 1.61 and 1.99 ppm, respectively.

The physical properties of the CAR–XYL complexes obtained are comparable to those of other previously published CAR–polysaccharide complexes [20, 21]. As an example, the size of the CAR–inulin (CAR–IN) complexes is similar to that of the CAR–XYL complexes, but the polydispersity index is about three times higher. The complexation yield and EE of the CAR–IN complex are slightly lower than those of the CAR–XYL complex [20]. CAR–XYL complexes fabricated by Straksys et al. [21] exhibited a similar PDI to that obtained in this study but had a particle size that was approximately 1.5 times lower. Due to the different methodologies used to determine the LC, it is difficult to compare the parameters obtained in this study with those previously published for CAR–XYL complexes [21]. The LC of the CAR–XYL complex prepared in this study at a CAR:XYL ratio of 1:5 is approximately six times higher than that of the CAR–IN complex at the same component ratio [20]. At a ratio of 1:10, this parameter is similar for both the CAR–XYL and CAR–IN complexes. The solubility of the CAR–XYL complex is similar to that of complexes fabricated by Straksys et al. [21], but slightly lower than that of CAR–IN complexes. CAR–chitooligosaccharide complexes prepared at a 1:1 component ratio [19] had the same solubility as CAR–XYL complexes prepared at the same ratio.

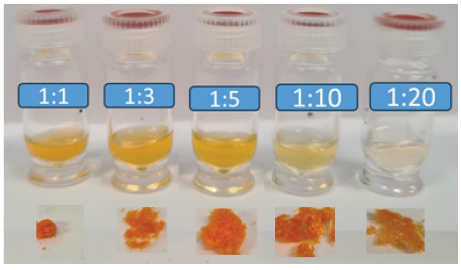

FIGURE 3 | Lyophilised complexes and their aqueous solutions at 0.7 mg/mL.

3.2 | Stability and AA of Encapsulated CAR in Complexes Obtained in Different CAR:XYL Ratios

Taking into account the effect of the CAR:XYL ratio on the stability of encapsulated CAR, thermal stability was studied by keeping the samples at different temperatures for 30 min (Figure 7). At the same temperature, the variation in thermal stability did not depend significantly (ANOVA, $p > 0.05$) on the CAR:XYL ratio used for the synthesis of the complexes. At 60°C, the relative stability of CAR was quite high, with about 70% of the complexed CAR remaining. At 100°C, it decreased to 22%–35%.

In Tables 2 and 3, the kinetic parameters of the complexed CAR stability are presented under storage at different conditions. As can be seen, the half-lives of the complexed CAR show a tendency to decrease in the CAR:XYL ratios of 1:10 and 1:20. This is especially evident when the samples are kept under UV irradiation. Since the xylan chain forms a helical structure in the solution, CAR may insert into the hydrophobic cavity of the helix, as previously supported by experiments with iodine–xylan and CAR–xylan complexes [21, 22, 42]. Furthermore, in solution, xylan molecules show a tendency for self-association [43]. Therefore, at a higher concentration of xylan as a result of the self-association of molecules, the formation of CAR–xylan complex is possibly reduced and more CAR adsorbs on the surface of the xylan associates. This results in a higher sensitivity to light and UV irradiation.

As shown in Figure 8, the CAR complexed with xylan did not lose its antioxidant properties. Furthermore, the complexes retained their antioxidant properties when the samples were kept at higher temperatures. This is important when considering the use of complexes for food fortification. Generally, degradation of the xylan-based CAR complex was correlated with loss of AA with increasing temperature. At 80°C, the relative thermal stability of the CAR–XYL complexes prepared in CAR:XYL ratios of 1:3 and 1:5 was approximately 50%; the AA of the complexes also decreased by half at this temperature. Analysis of the AA of the complexes confirmed that the optimal ratios of CAR:XYL are 1:3 and 1:5. At a higher amount of XYL, the AA of the complexes is lower because of a larger mass portion of XYL in the complex. Xylan itself has low

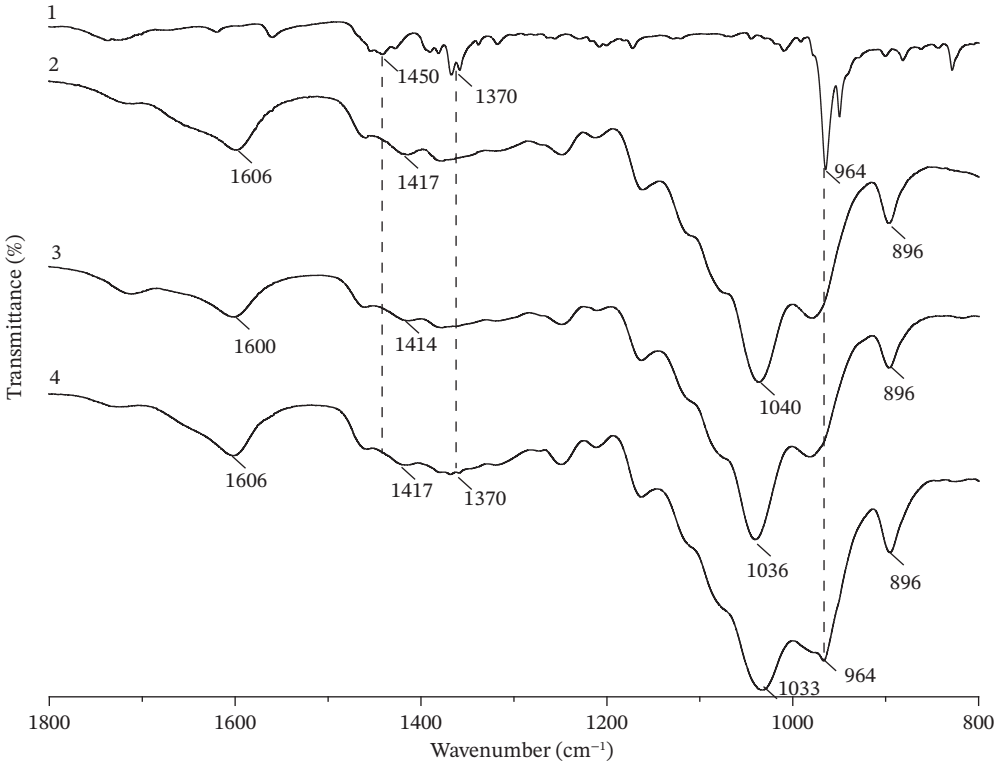

FIGURE 4 | FTIR spectra of pure β-carotene (1), xylan (2), their complex (3) and physical mixture (4). CAR–XYL complex and the physical mixture were prepared at the CAR:XYL ratio of 1:1.

AA. Furthermore, AA depends on the molecular mass of xylan. It decreases with increasing molecular mass [44]. As previously published, the AA of Megazyme xylan is 1.1 mg TE/g [45], while that of fabricated CAR complexes is 30–35 times higher (Figure 8) at the CAR:XYL ratios of 1:1, 1:3 and 1:5.

3.3 | Analysis of CAR Bioaccessibility

The water solubility and AA of the prepared CAR–XYL complexes are significant factors when considering their use in functional foods. An equally important, if not the most

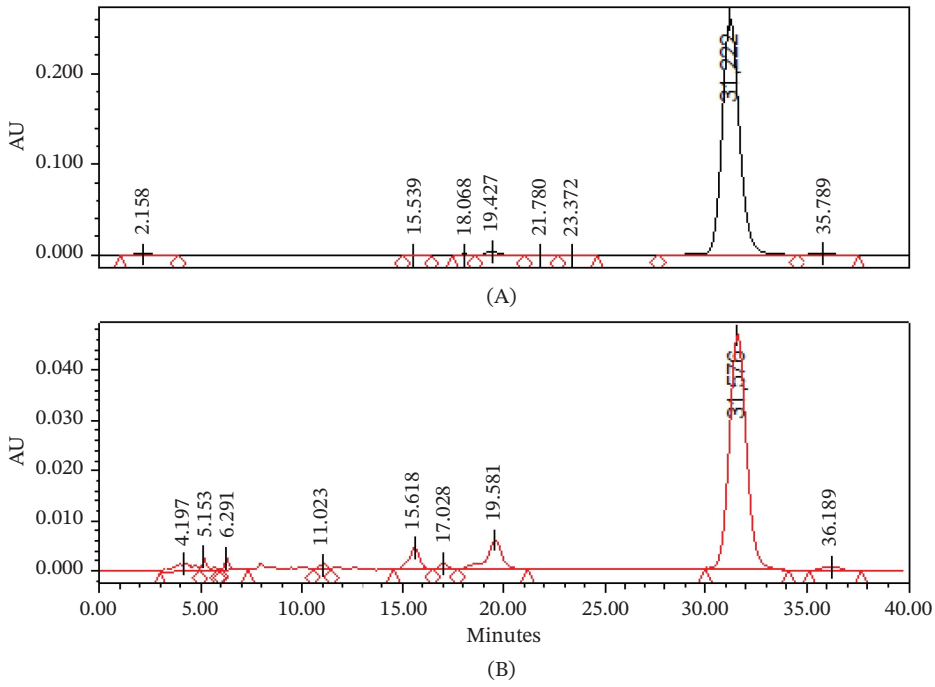

FIGURE 5 | HPLC analysis of β-carotene prior to complexation (A) and β-carotene extracted from the complex (B).

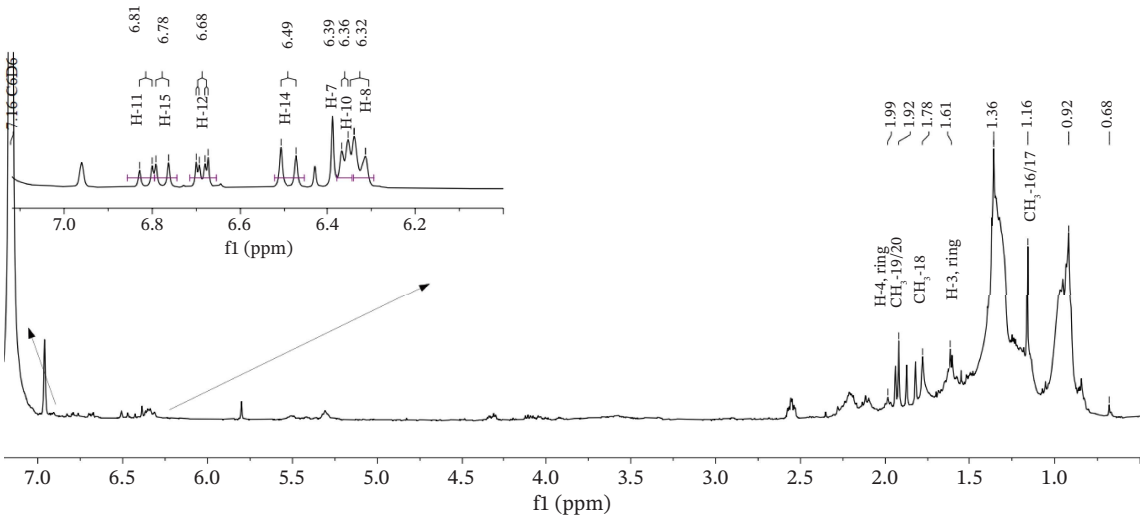

FIGURE 6 | ^{1}H NMR spectrum of β-carotene extracted from the complex.

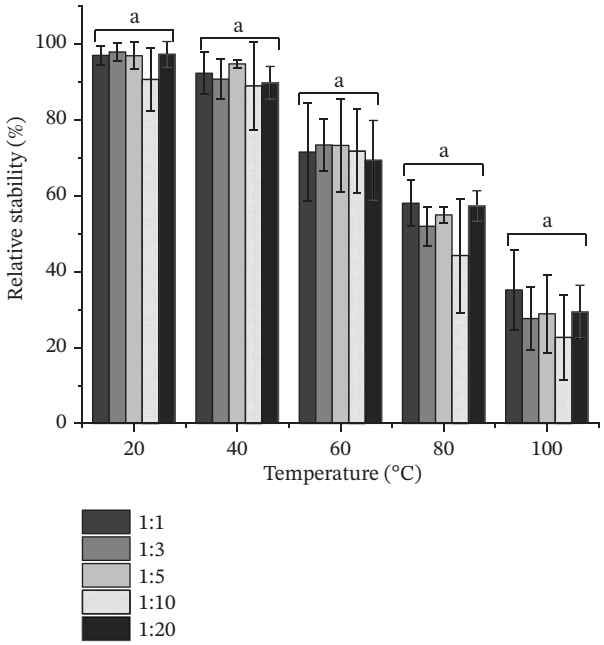

FIGURE 7 | Thermal stability of β-carotene complexed with xylan at different temperatures for 30 min. Data are shown as mean ± SD ($n = 3$).

TABLE 2 | Kinetic parameters of complexed β-carotene degradation under different storage conditions.

CAR:XYL ratio (w/w)	$k \times 10^2$ (day^{-1})			$t_{1/2}$ (days)		
	4°C (dark)	22°C (dark)	22°C (light)	4°C (dark)	22°C (dark)	22°C (light)
1:1	4.25 ± 0.70^{ab}	7.27 ± 1.24^{a}	14.59 ± 4.70^{a}	16.76 ± 3.45^{ab}	9.78 ± 1.88^{a}	5.01 ± 1.62^{a}
1:3	3.26 ± 0.37^{a}	6.88 ± 0.85^{a}	14.02 ± 1.02^{a}	21.50 ± 2.40^{b}	10.20 ± 1.22^{a}	4.96 ± 0.38^{a}
1:5	5.20 ± 1.27^{b}	8.45 ± 3.09^{a}	29.58 ± 3.12^{a}	14.17 ± 4.11^{a}	9.12 ± 3.34^{a}	2.36 ± 0.25^{b}
1:10	5.77 ± 1.11^{b}	9.42 ± 1.38^{a}	31.93 ± 10.76^{a}	12.34 ± 2.32^{a}	7.47 ± 1.10^{a}	2.40 ± 0.94^{b}
1:20	5.74 ± 0.98^{b}	10.12 ± 2.83^{a}	28.71 ± 9.03^{a}	12.35 ± 2.14^{a}	7.13 ± 1.99^{a}	2.54 ± 0.80^{ab}

Note: Data are shown as mean ± SD ($n = 3$). Different letters show significant differences ($p < 0.05$) in the mean within each column.

important, parameter is the bioaccessibility and bioavailability of complexed CAR. However, bioaccessibility depends on various factors such as encapsulation methods, CAR carriers and the food matrix, and it has to be thoroughly studied to increase the biological activities of the final food product [46–48]. Taking this into account, the bioaccessibility of CAR complexed with xylan was investigated using the INFOGEST protocol [33]. This is an internationally standardised in vitro digestion method that

TABLE 3 | Kinetic parameters of complexed β-carotene degradation under UV irradiation.

CAR:XYL ratio (w/w)	$k \times 10^2$ (min^{-1})*	$t_{1/2}$ (min)*
1:1	3.45 ± 0.8^a	21.7 ± 3.1^a
1:3	3.49 ± 0.9^a	21.4 ± 4.3^a
1:5	3.92 ± 0.6^a	18.0 ± 2.9^a
1:10	5.92 ± 0.9^b	11.7 ± 1.7^b
1:20	5.14 ± 0.7^b	13.5 ± 2.0^b

*Data are shown as mean ± SD ($n = 3$). Different letters show significant differences ($p < 0.05$) in the mean within each column.

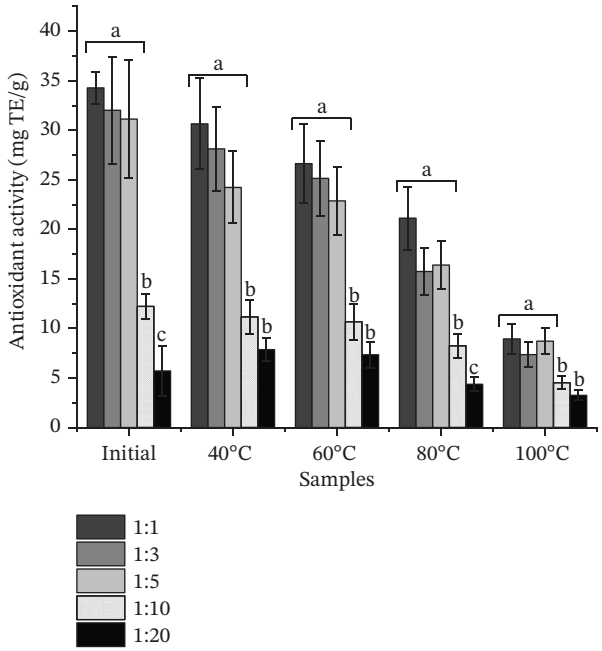

FIGURE 8 | Antioxidant activity of the complexes after incubation at different temperatures for 30 min. Data are shown as mean ± SD ($n = 3$). Different letters show significant differences ($p < 0.05$) in the mean of antioxidant activity at the same temperature, but at different CAR:XYL ratios.

enables the comparable evaluation of the breakdown of food components in the human gastrointestinal tract. The bioaccessibility of CAR complexed with different amounts of xylan was similar and found to be $54.3 \pm 5.6\%$ and $59.03 \pm 3.3\%$ for CAR–XYL complexes fabricated at CAR:XYL ratios of 1:3 and 1:5, respectively. Moreover, the recovery of CAR, which means the stability of CAR during the in vitro digestion process, was also similar and equal to $61.5 \pm 7.9\%$ and $58.6 \pm 4.1\%$ for CAR:XYL ratios of 1:3 and 1:5, respectively. The data obtained in this study are consistent with those in previous publications. For example, the bioaccessibility of CAR encapsulated in a κ-carrageenan-based oil-in-gel emulsion was almost 60% [49]. CAR encapsulation in an oleogel-in-water Pickering emulsion resulted in 68% bioaccessibility [50]. However, there is no linear relationship between enhanced bioaccessibility and bioavailability [51]. Therefore, further studies are required on the latter parameter.

4 | Conclusion

A new method based on immediate solvent evaporation was developed and optimised for the fabrication of the CAR–XYL complex. The analysis of the physical and chemical properties of the obtained complex was performed. ^{1}H NMR spectroscopy and HPLC analysis were used to confirm the structure of CAR extracted from the complex. Based on the complex solubility, EE, LC, AA and shelf life of CAR under UV irradiation and long-term storage under different conditions, the CAR:XYL ratios of 1:3 (w/w) and 1:5 (w/w) were found to be the most effective for the CAR formulation. The bioaccessibility of CAR–XYL complexes was analysed using the INFOGEST protocol and was found to be $54.3 \pm 5.6\%$ and $59.03 \pm 3.3\%$ for CAR–XYL complexes fabricated at CAR:XYL ratios of 1:3 and 1:5, respectively.

Author Contributions

Rūta Gruškienė: investigation and writing–original draft preparation. Tatjana Kavleiskaja: investigation and writing–original draft preparation. Jolanta Sereikaitė: supervision and writing–review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available on request from the authors.

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