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# The effects of carrot shape and oral processing behaviour on bolus properties and β-carotene bioaccessibility of raw carrots

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ABSTRACT

The aim of this study was to investigate the effects of carrot shape (cube vs. julienne) and oral processing behaviour, specifically chewing time, on bolus properties and bioaccessibility of β-carotene in raw carrots. Participants (n = 20) consumed raw carrot cubes (15 × 15 × 15 mm, 4.2 g/bite) and raw carrot julienne (2 × 3 × 90 mm, 4.2 g/bite) with normal (cube: 20 s/bite; julienne: 28 s/bite) and short (cube: 10 s/bite; julienne: 14 s/bite) chewing time. Expectorated boli were collected and characterized for number and mean area of carrot bolus particles. The proportion of easily extractable β-carotene of the carrot bolus was taken as an approximate indicator of the potentially bioaccessible β-carotene. Longer chewing time resulted in significantly more and smaller carrot bolus particles, larger particle surface area ($p < 0.01$) and higher proportion of easily extractable β-carotene than shorter chewing of raw carrots of both shapes (Cube_Normal vs. Cube_Short: 29 ± 7 % vs. 23 ± 7 %; Julienne_Normal vs. Julienne Short: 31 ± 8 % vs. 26 ± 6 %, $p < 0.05$). Carrot shape significantly influenced number and size of bolus particles ($p < 0.01$) with carrot julienne generating more and smaller carrot bolus particles than carrot cubes. These differences in bolus properties between carrot julienne and cubes did not influence the proportion of easily extractable β-carotene ($p > 0.05$). We conclude that differences in oral processing behaviour and the corresponding differences in bolus properties produce only modest differences in β-carotene bioaccessibility of raw carrots regardless of carrot shape.

## 1. Introduction

Carrots are one of the most widely consumed vegetables in the world, and are known as an excellent source of β-carotene, a carotenoid that plays an essential role in human (Palmero et al., 2014). β-Carotene is a precursor to vitamin A, which is essential for epithelial function, embryonic development, and immune system function (Palmero et al., 2014; Low, D'Arcy and Gidley, 2015). Although β-carotene is abundant in raw carrots, the bioaccessibility of β-carotene in raw carrots, i.e. the amount of β-carotene that is released from raw carrots during digestion and made accessible for absorption, is quite limited due to various exogenous and endogenous factors including the cellular structure of the raw carrot matrix (Hedrén, Diaz and Svanberg, 2002; Moelants et al., 2012; Capuano, 2017). It is well-established that enhancing the extent of carrot matrix disintegration is closely associated with an increase in the proportion of released β-carotene (Lemmens et al., 2010, 2011; Moelants et al., 2012; Palmero et al., 2014; Verrijssen et al., 2016; Bot et al., 2018). Oral processing behaviour has been suggested to modulate nutrient release from the cellular matrix by disintegrating intact plant tissues leading to an increased bioaccessibility of nutrients (Suzuki et al., 2005; Ranawana et al., 2010; Chen et al., 2022).

Oral processing behaviours are affected by food properties resulting in differences in matrix disintegration pathways and food bolus properties which may further impact nutrient release and digestion (Ellis et al., 2004; Capuano, 2017; Capuano et al., 2018; Do et al., 2019; Golding, 2019). Raw carrots can be consumed in a variety of shapes, for instance as cubes or stripes (julienne) of different lengths as typically done in carrot salads. The shape of carrots (cubes vs julienne) influences oral processing behaviour of raw carrots. Kohyama et al. (2007) reported that fine strips of raw carrots (1.0 × 1.5 × 30 mm, 7 g) required longer chewing time than raw carrot cubes (20 × 20 × 20 mm, 7 g). Van Eck et al. (2019) explored oral processing behaviour and bolus properties of raw carrot cubes (15 × 15 × 15 mm, 4.2 g) and carrot julienne pieces (2 × 3 × 90 mm, 4.2 g). The results confirmed that carrot julienne increased oral processing effort and generated more and smaller raw carrot bolus particles than carrot cubes. These studies demonstrated that

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altering the shape of raw carrots affects oral processing behaviour and modulates bolus properties. However, what remains unknown and forms the main research focus of our study, is whether these changes in oral processing behaviour and bolus properties caused by differences in carrot shape are sufficient to cause tangible changes in β-carotene release from carrots. If that would be the case, then changing the shape of raw carrots would be a convenient and easy to implement strategy to impact β-carotene release from raw carrots.

The aim of this study was to investigate the effects of carrot shape (cube vs. julienne) chewed for different times (short vs. normal) on bolus properties and bioaccessibility of β-carotene in raw carrots. We hypothesize that carrots julienne are consumed with longer chewing time leading to more and smaller carrot bolus particles resulting in higher bioaccessibility of β-carotene than carrot cubes. It is also hypothesized that longer chewing time (normal chewing) of the same shape of carrots leads to higher bioaccessibility of β-carotene by increasing carrot bolus surface area than short chewing time. It is worth highlighting that this study explores a potential strategy to enhance β-carotene release by simply cutting raw carrots differently and consequently altering oral processing behaviours and bolus properties.

## 2. Materials and methods

### 2.1. Materials

Fresh, raw carrots (Jumbo Wortelen, winter carrots) were purchased from a local supermarket (Jumbo Supermarkets BV, Wageningen, The Netherlands), stored in the refrigerator at 4 °C and used within 3 days. All chemicals used in this study were of analytical grade.

### 2.2. Preparation of carrots

A preliminary study was performed to explore the effect of various carrot shapes on oral processing behaviour and bolus properties in order to select the carrot shapes used in the main study. Five carrot samples of uniform weight (approx. 3 g) differing in shape including cube, slice, chip, brunoise, and julienne were prepared. In a preliminary study n = 10 participants (6 females, 4 males, 23 ± 1 y) were instructed to chew naturally on the carrots differing in shape. Chewing time (s), chewing frequency (chews/s) and bolus properties, number (no./g) and mean area ($mm^2$) of carrot bolus particles, of each shape of carrot were captured and analysed (Supplementary Table 1). Cube and julienne carrots differed the most in chewing time, number and mean area of carrot bolus particles and were therefore chosen as carrot shapes for the main study.

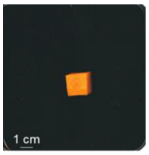
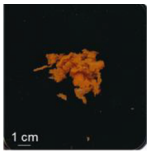
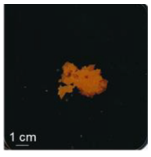
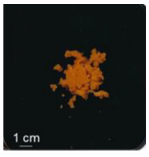
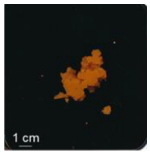
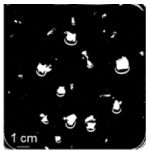
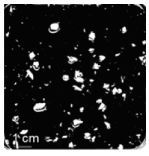
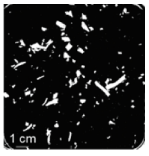
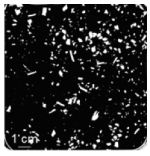
For the main study, each carrot was peeled and cut in half. One half was cut with a knife into cubes of 16 × 16 × 16 mm. The other half of the carrot was cut with a spiral slicer (Gefu Spiral Slicer Spirelli 2.0) and a knife into julienne pieces of 2 × 3 × 90 mm. All samples were freshly prepared in the morning and served at a fixed portion weight of around 4.2 g per sample. One portion of carrot cube contained 1 cube and one portion of carrot julienne contained 9 julienne pieces. Each participant received the raw carrot cubes and raw carrot julienne prepared from the same single carrot.

### 2.3. Characterization of oral processing behaviour of carrot cubes and carrot julienne

The chewing time of carrot cube (1 cube of 16 × 16 × 16 mm, 4.2 g) and carrot julienne (9 pieces of 2 × 3 × 90 mm, 4.2 g) was determined based on a preliminary trial with n = 17 participants (10 females, 7 males, 24 ± 1 y). Participants were given instructions to chew the carrot samples in their natural manner and then swallow. Participants signalled the moment of swallowing by raising their hand. The consumption process was video recorded. By analyzing the recorded videos, we extracted chewing time (s) as the time from when the participants started the first bite until they swallowed. Number of chews and chewing frequency were also determined. For the carrot cubes an average chewing time of 23 ± 4 s (ranging from 14 to 30 s), and chewing frequency of 1.4 ± 0.1 chews/s (ranging from 1.31 to 1.58 chews/s) were found. For the carrot julienne, an average chewing time of 27 ± 7 s (ranging from 13 to 39 s), and chewing frequency of 1.5 ± 0.1 chews/s (ranging from 1.36 to 1.74 chews/s) were found. These preliminary findings are in agreement with previous studies (van Eck & Wijne, 2019).

In the present study, we merged the results of our preliminary study

**Table 1**
Representative pictures of raw carrot cubes and raw carrot julienne samples, expectorated boli after short and normal chewing times, and scan pictures of boli fragments. Carrot cubes were chewed for 10 s or 20 s at a chewing frequency of 1.4 chews/s. Carrot julienne were chewed for 14 or 28 s at a chewing frequency of 1.4 chews/s.

| Sample | Cube_Short | Cube_Normal | Julienne_Short | Julienne_Normal |
|---|---|---|---|---|
| Carrot sample |  | |  | |
| Expectorated boli |  |  |  |  |
| Boli fragments |  |  |  |  |

and van Eck's study (van Eck & Wijne, 2019), referring to the average chewing time of carrot cubes and carrot julienne as normal chewing time. The shorter chewing time was defined as half of the average normal chewing time and is referred to as short chewing time. Consequently, the instructed oral processing behaviour which participants had to follow for the short and normal chewing condition for carrot cubes were 10 and 20 s chewing time, and for carrot julienne were 14 and 28 s chewing time. Chewing frequency were similar for carrot cube and carrot julienne so a chewing frequency of 1.4 chews/s was used for the instructed chewing behaviour.

### 2.4. Experimental design and carrot bolus collection

Expectorated boli of raw carrot cube and raw carrot julienne chewed for short and normal chewing times were collected from n = 20 participants (13 females, 7 males, 25 ± 2 y). Demographic information of participants, including gender, age, nationality, body weight and height, food intolerances, self-reported dental and oral health status, as well as smoking habits, was gathered via questionnaires and used as selection criteria. Participants had the ability to chew and swallow normally, without history or undergoing treatment for chronic medical illness, no smoking, alcohol or drug use, and no self-reported food allergies or intolerances to the tested foods (Ketel et al., 2019; van Eck & Fogliano, 2019). All participants (n = 20) completed this study. Carrot samples were randomly served to the participants. All participants were asked to chew one portion of carrot cube (1 cube of 16 × 16 × 16 mm, 4.2 g) or carrot julienne (9 pieces of 2 × 3 × 90 mm, 4.2 g) for short or normal chewing times (cube: 10 s and 20 s; julienne: 14 s and 28 s) at a chewing frequency of 1.4 chews/s. Carrot samples are referred to as Cube_Short (carrot cube chewed for 10 s), Cube_Normal (carrot cube chewed for 20 s), Julienne_Short (carrot julienne chewed for 14 s) and Julienne_Normal (carrot julienne chewed for 28 s). Chewing instructions were provided to participants by prompting a tone every time they needed to chew. After chewing, the carrot boli were expectorated into containers (Korff, Alu container round, 64 mm, 28 mL, Germany). Each collected carrot bolus was divided into three parts for further bolus analysis including dry matter (approx. 1.0 g), bolus particle number and size (approx. 1.0 g), and β-carotene extraction (approx. 2.0 g). All participants gave written informed consent prior to the study and were financially compensated for their participation. The study does not fall within the remit of the 'Medical Research Involving Human Subjects Act' of The Netherlands (WMO in Dutch) by the Medical Research Ethical Committee of The Netherlands. The study was conducted in agreement with the WMA Declaration of Helsinki about Ethical Principles for Medical Research Involving Human Subjects.

### 2.5. Dry matter content

Dry matter content (%) of expectorated carrot boli was measured by subjecting a known amount of expectorated carrot bolus to overnight drying in an oven at 105 °C to a stable weight.

### 2.6. Characterization of number and size of carrot bolus particles

Image analysis was performed to determine particle number and size of carrot bolus fragments as described previously (Chen, Capuano and Stieger, 2020; Chen et al., 2022). Approximately one gram of expectorated carrot bolus was manually separated in a Petri dish (120 × 120 × 17 mm) with the addition of 25 mL of Milli-Q water. Bolus fragments were gently manually separated. All measurements (n = 20 participants) were done in duplicate using a flatbed scanner (Canon CanoScan 9000F MarkII). ImageJ (version 1.52a, National Institute of Health, USA) was used for image analysis (Chen, Capuano and Stieger, 2020; Chen et al., 2022) to obtain number of bolus particles (no./g) and bolus particle mean area ($mm^2$). Number of carrot bolus particles was normalized by the corresponding wet weight of the carrot matrix.

### 2.7. In vitro digestion

To explore the feasibility of using *in vitro* digestion of expectorated carrot boli to assess β-carotene bio-accessibility, a preliminary study was performed on expectorated carrot boli collected from n = 3 participants. Enzymes types and activities, composition of the simulated digestive fluids, and pH values were based on the harmonized INFOGEST 2.0 protocol and modified as described before (Brodkorb et al., 2019; Chen et al., 2022). The digesta from *in vitro* intestinal digestion were centrifuged (Beckman Coulter, Optima XE-90 Ultracentrie, USA) at 160,150*g* for 67 min at 4 °C to separate the micelles containing β-carotene. The supernatant containing micelle fraction was collected for further extraction and β-carotene quantification (see section 2.8).

This preliminary *in vitro* digestion study revealed that the concentration of β-carotene released from the carrot bolus after *in vitro* digestion was too low to be quantified using high-performance liquid chromatography (HPLC) following the procedure described in section 2.8. Since the bolus size for each sample generated by each participant could not be increased or pooled together, we decided to discontinue with the *in vitro* digestion protocol and continued instead with the protocol described in section 2.8.

### 2.8. β-carotene extraction and quantification of estimated bioaccessibility of β-carotene

β-Carotene extraction from raw carrots and expectorated raw carrot bolus was performed based on the Khachik et al. (1991) and Sadler (Sadler, Davis and Dezman, 1990) extraction method which was slightly modified. The extraction procedures for carrots and expectorated carrot bolus differed. All extractions were performed under subdued light without ultraviolet light to prevent β-carotene degradation and isomerisation.

For the β-carotene extraction from raw carrots, unchewed carrot cube or carrot julienne were freeze dried and milled using a mixer mill (Retsch, Mixer Mill MM 400, Germany) with a frequency of 25 times/s for 30 s. 0.1 g of freeze dried carrots was weighed in a 50 mL Greiner centrifuge tube and 0.5 g NaCl, 15 mL Mill-Q water and 25 mL Sadler extraction solvent (Hexane:Acetone:Ethanol, 2:1:1 v/v/v) were added. The mixtures were vortexed for 10 min (Heidolph, Multi Reax, Germany). Then the mixtures were centrifuged (Thermo Scientific, Heraeus-Multifuge X3R, USA) at 4700 rpm for 5 min. The apolar phase (hexane phase) containing β-carotene was collected in an Greiner 50 mL standing tube for further rotary evaporation. The liquid part of the rest mixture was removed and the pellet was extracted an additional four times following the Sadler extraction protocol. For the β-carotene extraction from raw carrots in total five extraction rounds were applied. The hexane phases of all five extraction rounds were combined in the same Greiner standing tube and subjected to rotary evaporation.

For the β-carotene extraction from raw expectorated carrot bolus, carrot bolus were freshly extracted with a combination of extraction with hexane and Sadler solvent (Fig. 1). The first extraction round of carrot bolus was performed with hexane only. 2.0 ± 0.1 g of carrot bolus was weighed in a 50 mL Greiner centrifuge tube and 0.5 g NaCl, 15 mL Mill-Q water, and 15 mL hexane were added. The mixture was vortexed for 10 min and centrifuged at 4,700 rpm for 5 min. The hexane phase was collected in a separate Greiner tube for further rotary evaporation. The liquid part of the rest mixture was removed. The remaining carrot bolus particles were extracted with 25 mL Sadler solvent and additional four rounds of extraction following the Sadler extraction protocol as described above were performed. In total, expectorated raw carrot bolus were extracted for five rounds (one round with hexane and four rounds with Sadler solvent). The hexane phase of the Sadler extractions were combined and subjected to rotary evaporation.

In this study, β-carotene from the first extraction represents the easily extractable β-carotene and is referred to as easily extractable β-carotene hereafter. Easily extractable β-carotene is considered as an approximate

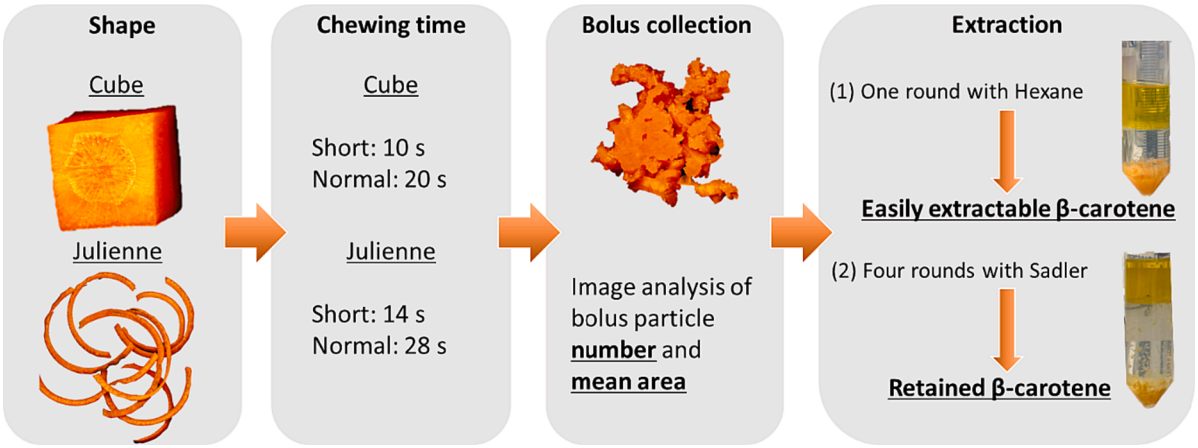



**Fig. 1.** Illustration of the experimental approach and β-carotene extraction procedure of raw carrot bolus chewed short and normal. Easily extractable β-carotene is considered as an approximate indicator of the potentially bio-accessible β-carotene fraction released from the carrot bolus. Retained β-carotene is considered as an approximate indicator of the not bio-accessible β-carotene fraction remaining in the carrots.

indicator of the potentially bio-accessible β-carotene fraction released from the carrot bolus. β-carotene from the remaining four extractions represents the fraction of β-carotene remaining in the carrot matrix and is referred to as retained β-carotene hereafter, an approximate indicator of the not bio-accessible β-carotene fraction remaining in the carrots.

Although the concentration of released β-carotene from the digested carrot bolus was insufficient to allow for quantification by HPLC. The description of this unsuccessful method is still reported to provide transparency in the research process and an opportunity for researchers to adjust methods. For the extraction of β-carotene from digested expectorated carrot bolus, the supernatant containing mixed micelles obtained as described in section 2.7 was mixed with two extraction rounds with hexane (1:1 v:v), and a third extraction round with tetrahydrofuran (1:1 v:v). The phase with β-carotene was collected and subjected to rotary evaporation.

For the easily extractable β-carotene, β-carotene extract was concentrated under vacuum (BÜCHI, Vacuum Pump V-300, USA) with nitrogen flow using a rotary evaporator (BÜCHI, Rotavapor R-200, USA) at 35 °C (BÜCHI, Heating Bath B-490, USA) until all hexane was evaporated. β-carotene concentrates were re-dissolved in 0.8 mL buffer (1:1 methanol:tetrahydrofuran + 0.01 % butylated hydroxytoluene, v:v + w/v) for three times. The re-dissolved sample (2.4 mL) was filtered (Phenex, Regenerated Cellulose syringe filter, 0.20 μm pore size, 15 mm diameter, Germany) and transferred to an amber HPLC vial. The headspace of the sample vial was flushed with nitrogen for 10 s to prevent β-carotene degradation.

β-Carotene quantification was subsequently performed by high-performance liquid chromatography (HPLC). The HPLC analyses were performed on a Ultimate 3000 Rapid Separation LC System (Thermo Scientific, USA) equipped with a Ultimate 3000 RS Diode Array Detector (DAD, Thermo Scientific, USA). β-carotene was separated at 35 °C on a reversed-phase C18 column (100 × 4.6 mm, particle size 2 μm, Phenomenex, Onyx Monolithic C18, Germany) with an isocratic method of single eluent (6:3:1 acetonitril:methanol:ethyl acetate + 0.1 % triethylamine, v:v:v + w/v). The injection flow was at a rate of 1.00 mL/min for 10 min with an injection volume of 20 μL. β-carotene was identified based on retention time and spectral characteristics compared to the calibration curves. HPLC with Diode-Array Detection (HPLC-DAD) responses were measured at 445 nm for β-carotene. β-Carotene content (μg/g) was calculated based on β-carotene concentration and further standardized as β-carotene (μg) per gram of carrot wet weight. The proportion of easily extractable β carotene (%) was calculated as:

$$Easily\ extactable\ \beta\ carotene(\%) = \frac{Easily\ extractable\ \beta\ carotene\ content}{Easily\ extractable\ \beta\ carotene\ content\ + Retained\ \beta\ carotene\ content} \times 100\%$$

### 2.9. Statistical data analysis

Data are expressed as mean ± standard deviation (SD) unless otherwise stated. A significance level of $p < 0.05$ was chosen. IBM SPSS Statistics (version 25.0) was used to perform all statistical analysis.

To investigate the influence of carrot shape (cube, julienne) and chewing time (short, normal) on bolus properties (number of bolus particles, mean area of bolus particles), and the proportion of easily extractable β carotene (%), linear mixed models were used with shape, chewing time, and the interaction of shape * chewing time as fixed factors and participant as random effect. The significant main effects were compared using *post hoc* Bonferroni tests.

Total β-carotene content of raw carrot cubes and raw carrot julienne was analysed by One-way ANOVA and *post hoc* Bonferroni tests. Normality was checked using Shapiro-Wilk tests and variances were checked using Levene's tests.

Bivariate Pearson correlation tests (two-tailed) were used to examine the relationships between bolus properties (number of bolus particles, mean area of bolus particles) and released β-carotene (%). All samples differing in shape (cube, julienne) and chewing time (short, normal) were merged together.

## 3. Results

Table 1 shows representative pictures of raw carrot samples cut into cube and julienne shapes before oral processing, expectorated boli of cube and julienne carrots after short and normal chewing, and scanned pictures of separated boli fragments. Carrot cubes were chewed for 10 s or 20 s at a chewing frequency of 1.4 chews/s. Carrot julienne were chewed for 14 or 28 s at a chewing frequency of 1.4 chews/s. Fig. 2

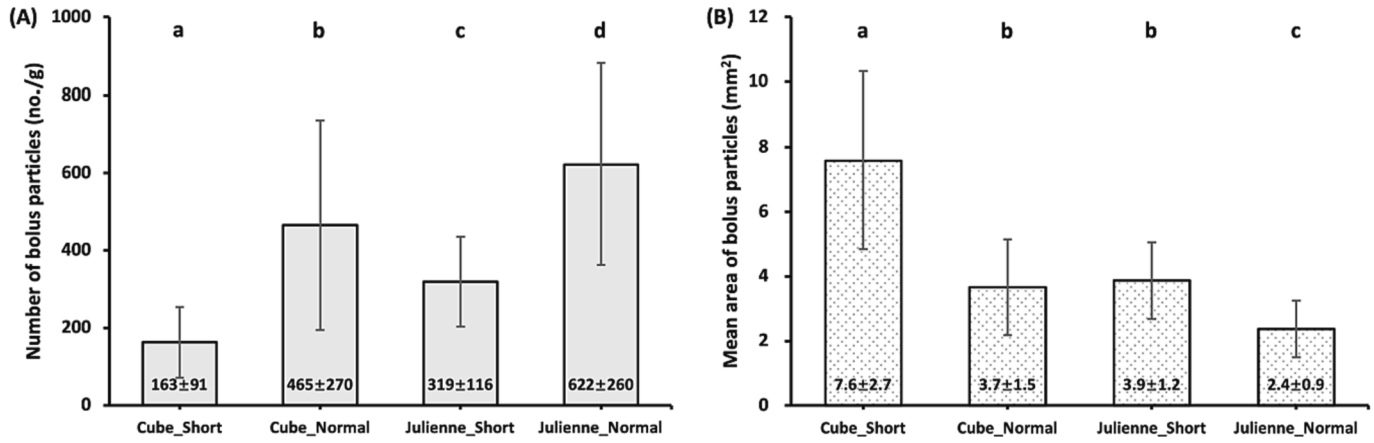



**Fig. 2.** Bolus properties of raw carrot cubes and raw carrot julienne masticated for short and normal chewing times. (A) Number of bolus particles (no./g) and (B) Mean area of bolus particles ($mm^2$) are shown as mean ± SD (n = 20, duplicate). Different letters indicate significant differences between means ($p < 0.01$).

**Table 2**
Effects of shape and chewing time on number of carrot bolus particles (no./g), mean area of bolus particles ($mm^2$), and the proportion of easily extractable β-carotene (%) derived from linear mixed models (n = 20, duplicate).

| | Shape | | Chewing time | | Shape*Chewing time | |
|---|---|---|---|---|---|---|
| | F | *p* | F | *p* | F | *p* |
| Number of particles | 12.1 | <0.001 | 45.1 | <0.001 | 0 | 0.993 |
| Mean area | 42.0 | <0.001 | 49.4 | <0.001 | 9.8 | 0.002 |
| Easily extractable β-carotene % | 1.6 | 0.217 | 10.8 | 0.002 | 0.0 | 0.855 |

shows bolus properties of raw carrot cubes and raw carrot julienne masticated for short and normal chewing times, including number of bolus particles (no./g) and mean area of bolus particles ($mm^2$).

Longer chewing resulted in significantly more (Fig. 2, Table 2, $F = 45.1$, $p < 0.001$) and significantly smaller (Fig. 2, Table 2, $F = 49.4$, $p < 0.001$) carrot bolus particles than shorter chewing for both carrot shapes (cubes and julienne). Carrot julienne generated significantly more (Fig. 2, Table 2, $F = 12.1$, $p < 0.001$) and smaller (Fig. 2, Table 2, $F = 42.0$, $p < 0.001$) carrot bolus fragments than carrot cubes. Shape and chewing time had a significant interaction effect on the mean area of carrot bolus particles (Table 2, $F = 9.8$, $p < 0.01$). Longer chewing of carrot julienne resulted in significantly smaller carrot bolus particles than longer chewing of carrot cubes.

In Fig. 3, easily extractable β-carotene content and retained β-carotene content (μg/g) for carrot cube and carrot julienne bolus after short or normal chewing are shown. The sum of easily extractable and retained β-carotene content was considered as total β-carotene content (μg/g) for each carrot bolus.

As expected, β-carotene content exhibited large variations among all carrot samples (Fig. 3). For the unchewed carrot matrix, carrot julienne (193.9 ± 23.1 μg/g) tended to show a higher amount of total β-carotene content than carrot cubes (175.0 ± 18.6 μg/g), despite that it did not show a significant difference ($p = 0.604$). Within the carrot bolus sample, carrot julienne displayed an overall higher β-carotene content than carrot cubes regardless of chewing time. The results above can be attributed to the preparation the carrot julienne and cube samples (session 2.1), and an overall heterogeneous distribution of β-carotene with a main concentration in the secondary phloem tissue and periderm of carrots (Gonzalvez et al., 2014). Therefore, unchewed carrot matrix and carrot bolus of carrot julienne tended to contain higher concentration of β-carotene than carrot cubes in this study. Prolonged chewing time also showed an effect of increasing the content of easily extractable β-carotene in both carrot cube and carrot julienne bolus samples (Fig. 3, $F = 12.4$, $p < 0.001$).

In order to assess the effect of carrot shape and chewing time on the estimated bioaccessibility of β-carotene, the proportion of easily extractable β-carotene (%) relative to the total β-carotene content (the sum of easily extractable and retained β-carotene) (section 2.8) is shown in Fig. 4.

Normal chewing time resulted in a significantly higher percentage of easily extractable β-carotene (Fig. 4 and Table 2, $F = 10.8$, $p < 0.01$) than short chewing time for both carrot shapes (cube and julienne). Regarding carrot cubes, extending chewing time from 10 s to 20 s resulted in a significant 6 % absolute increase in the proportion of easily extractable β-carotene (Cube_Short vs. Cube_Normal: 23 ± 7 % vs. 29 ± 7 %, $p < 0.05$). Similarly, for carrot julienne, prolonging chewing time from 14 s to 28 s led to a significant 5 % absolute increase in easily extractable β-carotene (Julienne_Short vs. Julienne_Normal: 26 ± 6 % vs. 31 ± 8 %, $p < 0.05$). The shape of carrots did not have a significant main effect on the proportion of easily extractable β-carotene (Table 2, $p = 0.217$). However, based on the *post hoc* Bonferroni test, cube carrot chewed for short time (Cube_Short) had a significantly lower proportion of easily extractable β-carotene compared to julienne carrot chewed for short time (Julienne_Short) ($p = 0.04$), while cube carrot chewed for normal time (Cube_Normal) and julienne carrot chewed for normal time (Julienne_Normal) were not significantly different ($p = 0.141$) from each other. The effect of shape on easily extractable β-carotene was only observed after short chewing and not for normal chewing. There was no significant interaction effect of shape and chewing time on the proportion of easily extractable β-carotene (Table 2, F = 0.0, $p = 0.855$).

Furthermore, we investigated the correlations between bolus properties and the proportion of easily extractable β-carotene (%) across all carrot samples. A strong positive and significant correlation was found between the number of bolus particles (no./g) and the proportion of easily extractable β-carotene (%) ($r = 0.600$, $p < 0.001$). A strong negative and significant correlation between the mean area of carrot bolus particles ($mm^2$) and the percentage of easily extractable β-carotene (%) ($r = -0.553$, $p < 0.001$) was found. Carrot bolus properties were therefore significantly influenced by chewing time as expected, and influenced by carrot shape in agreement with previous studies (van Eck, et al., 2019).

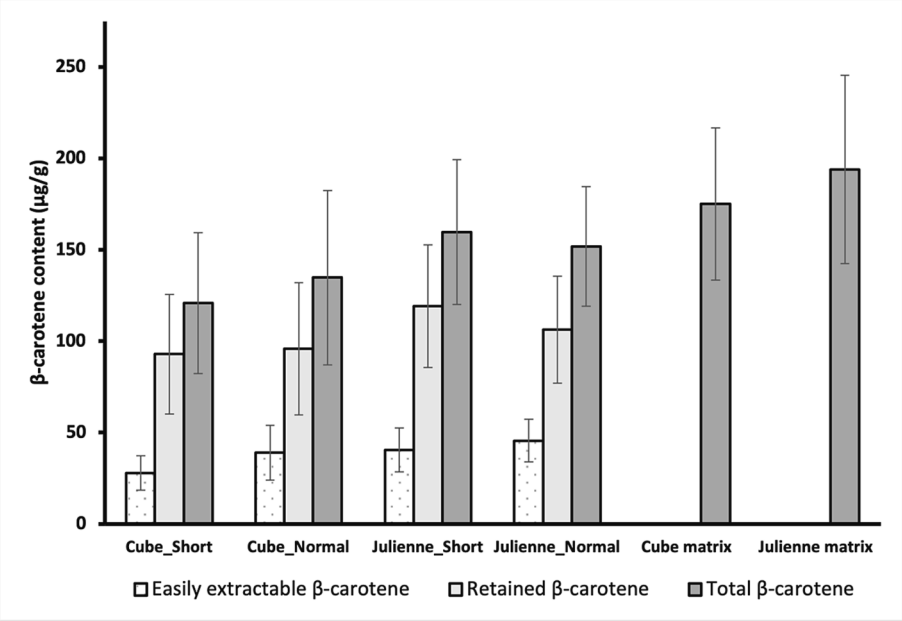



**Fig. 3.** Easily extractable β-carotene content (μg/g) and retained β-carotene content (μg/g) for expectorated raw carrot bolus after short and normal chewing. Carrot cubes were chewed for 10 s or 20 s at a chewing frequency of 1.4 chews/s. Carrot julienne were chewed for 14 or 28 s at a chewing frequency of 1.4 chews/s. Total β-carotene content (μg/g) for the raw carrots before oral processing (Cube matrix and Julienne matrix), and for expectorated raw carrot bolus after short and normal chewing (Cube_Short, Cube_Normal, Julienne_Short, Julienne_Normal) are shown. Total β-carotene content (μg/g) for expectorated raw carrot bolus is the sum of easily extractable β-carotene and retained β-carotene. Data are reported as mean ± SD (Carrot bolus sample: n = 20; Carrot matrix sample: n = 5. In duplicate).

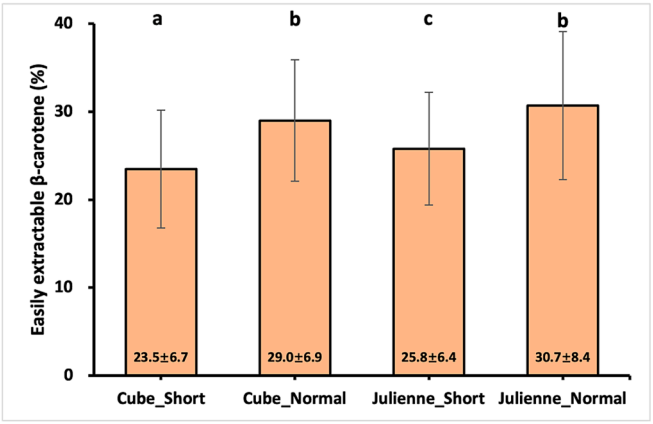



**Fig. 4.** Proportion of easily extractable β-carotene (%) of raw carrots differing in shape (cube, julienne) after short and normal chewing. Carrot cubes were chewed for 10 or 20 s at a chewing frequency of 1.4 chews/s. Carrot julienne were chewed for 14 or 28 s at a chewing frequency of 1.4 chews/s. Data are reported as mean ± SD (n = 20). Different letters indicate significant differences between means ($p < 0.05$).

## 4. Discussion

The aim of this study was to investigate the effects of carrot shape and oral processing behaviour on bolus properties and bioaccessibility of β-carotene in raw carrots. The proportion of easily extractable β-carotene of raw carrot bolus was taken as an approximate indicator of the potentially bioaccessible β-carotene. Carrot shape (cube, julienne) and chewing time (short, normal) influenced number and size of bolus fragments. Chewing time but not carrot shape influenced the proportion of easily extractable β-carotene.

To understand the effects of carrot shape and oral processing behaviour on β-carotene bioaccessibility of individual carrot bolus we first used a consensus *in vitro* model of gastrointestinal digestion. *In vitro* bioavailability is usually determined as the ratio of the amount of β-carotene incorporated into mixed micelles to the initial amount of β-carotene in the bolus being digested. However, as stated in section 2.7, β-carotene concentration in the micellarized phase collected after the intestinal phase of digestion was too low to be quantified using HPLC. There are several reasons for this outcome. First, the aim of quantifying the bioaccessibility of β-carotene in each individual bolus limited the amount of dry matter of the carrot bolus which could be digested (e.g. 5 g carrot cube bolus material corresponding to 0.63 g dry matter). Secondly, the β-carotene content of the raw carrots used in this study ranged from 175 to 194 μg/g fresh weight. This is in the similar magnitude of β-carotene content in raw carrots compared to previous studies reporting the range from 42 to 74 μg/g fresh weight (Biswas, Sahoo and Chatli, 2011; Koca Bozalan and Karadeniz, 2011), but such content generated a mass ratio of β-carotene to the fresh weight of raw carrots lower than 0.01 % (w/w). Thirdly, the need for standardization of the amount of β-carotene released from each individual carrot bolus after chewing to the corresponding initially total amount of β-carotene further limited the amount of beta-carotene that could be incorporated in mixed micelles. Indeed, we had to define both the β-carotene concentration in micelles and the total initial β-carotene concentration in the same individual bolus. Therefore, each bolus had to be split into at least two parts, one being subjected to *in vitro* digestion to determine the released β-carotene content, the other bolus part being analysed for the total β-carotene content to be used as internal reference of the initial β-carotene content for the bolus. Since the original weight of each carrot bolus was approximately 5 g, splitting the carrot bolus further reduced the amount of carrot bolus dry matter. This standardization procedure is necessary given the large variations in β-carotene content between different batches of carrots, between individual carrots within the same batch and between different positions within the same raw carrot (Gonzalvez et al., 2014). We argue that this variability in β-carotene content is a serious and challenging issue in studies like the present one where the initial content of a target compound cannot be standardized properly and varies largely between and within samples. This is especially problematic with nutrients and bioactive compounds with known low

bioaccessibility like β-carotene. Lemmens et al. (2010) reported that only 34.9 ± 2.1 μg of all β-carotene per gram of dry matter of raw carrots was bioaccessible in raw carrots which would make the determination of β-carotene bioaccessibility even more difficult. All in all, *in vitro* digestion was later no longer considered to assess β-carotene bioaccessibility and we chose to express β-carotene bioaccessibility in alternative ways.

Given the difficulties in quantifying the amount of micellarized β-carotene, an experimental approach was followed as described in section 2.8 where β-carotene bioaccessibility was quantified indirectly through its extractability from the carrot matrix. Proposing extractability as an indicator of bioaccessibility is based on the assumption that an increase in extractability corresponds to an increase in bioaccessibility. This assumption is substantiated by previous studies demonstrating that nutrients more readily extracted from food matrices tend to be more bioaccessible (Mæhre, Jensen and Eilertsen, 2016; Larabia, Welti-chanes and Cano, 2021). Higher extractability typically signifies enhanced potential for bioaccessibility. It has been reported that β-carotene bioaccessibility indicates the released β-carotene fraction from the matrix (Bot et al., 2018). However, to ensure that each measurement of β-carotene extractability could be related to the initial content in β-carotene in each carrot bolus, we decide to use the ratio between easily extractable β-carotene and the sum of easily extractable β-carotene and the less easily extractable β-carotene (retained β-carotene). The amount of β-carotene extracted with the first extraction was taken as a measure of the easily extractable β-carotene whereas the amount of β-carotene extracted with four subsequent extractions was taken as a measure of the less easily extractable β-carotene (retained β-carotene). It was acknowledged that the extractability of β-carotene was just an indirect indicator of β-carotene bioaccessibility. As a recommendation for future studies, the amount of micellarized β-carotene should be increased by modifying the digestion protocol or adding lipids to enhance β-carotene uptake in mixed micelles (Moelants et al., 2012).

The number and mean area of carrot bolus particles were significantly influenced by the carrot shape (cubes, julienne) and chewing time (short, normal). In general, carrot julienne generated more and smaller carrot bolus fragments than carrot cubes (Fig. 2 & Table 2). These results are in agreement with previous studies (Kohyama et al., 2007; van Eck & Wijne, 2019), demonstrating that cutting of raw carrots into julienne shapes increased oral processing effort of consumers. It has been suggested that carrot julienne resulted in more carrot pieces to be positioned in the oral cavity and a higher surface area than carrot cubes, so carrot julienne would require more effort to place the carrot pieces between teeth and more saliva secretion to lubricate the bolus. Kohyama et al. (2007) reported that pre-cutting raw, hard carrots increased the chewing effort during mastication whereas pre-cutting of softer foods such as cucumber, roast pork and surimi gels did not impact chewing effort. The difference in chewing effort between the different foods may indicate that oral processing behaviour are predominantly influenced by the mechanical properties of the food matrix rather than food shape. As expected, chewing for normal mastication times produced more and smaller carrot bolus particles than shorter chewing for both carrot shapes (cubes and julienne) (Fig. 2 & Table 2). Numerous studies have reported that for a variety of foods prolonged chewing increases the number and decreases the size of bolus fragments (Chen, 2012; de Lavergne et al., 2015; Boland, 2016; Golding, 2019; Chen, Capuano and Stieger, 2020; Chen et al., 2022). Our study also showed an interaction effect of carrot shape and chewing time on the surface area of raw carrot bolus, i.e. longer chewing time for carrot julienne could amplify the comminution of carrot bolus fragments.

A significant effect of chewing time on the proportion of easily extractable β-carotene was observed in this study. To the best of our knowledge, this is the first time that the effects of food shape and its corresponding oral processing behaviour on potential micronutrient bioaccessibility were investigated. Our findings support the notion that extended chewing by doubling the chewing time increases the potential bioaccessibility of β-carotene of raw carrots by 6 % and 5 % (in absolute terms). Our study shows that longer chewing leads to carrot bolus breakdown into more and smaller fragments thereby increasing the total surface area of the bolus fragments, which likely enhanced the release of β-carotene and promoted the bioaccessibility of β-carotene. The study conducted by Lemmens et al. (2010) also supported our finding that longer chewing would indeed significantly improve the bioaccessibility of β-carotene of raw carrots mainly by reducing the carrot particle size. Only one study investigated the effect of chewing time on the release of β-carotene from other fruits and vegetables. Low et al. (2015) found that longer chewing times (fine chewers vs. coarse chewers) significantly released larger absolute amounts of β-carotene from mango. Furthermore, it is important to note that the effect of chewing time may vary depending on the food matrix and the processing method used. For example, Lemmens et al. (2010) found, in contrast to raw carrots, no correlation between carrot particle size and β-carotene bioaccessibility in cooked carrots which means the contribution of chewing time to β-carotene bioaccessibility was mitigated by cooking. This discrepancy between raw carrots and cooked carrots was attributed to the fact that cooking softened the carrots tissues, reduced the carrot cell breakdown due to mastication and made the cell walls more porous facilitating the contact between β-carotene and digestive enzymes (Waldron, Parker and Smith, 2003; Lemmens et al., 2010).

It has been widely proven that β-carotene bioaccessibility is efficiently increased by the presence of fats and oils (Huo et al., 2007; Colle et al., 2012; Palmero et al., 2014). One study reported that the addition of rapeseed oil (20 % of dry weight of carrot) increased the *in vitro* bioaccessible β-carotene in cooked pulped carrots by 11 % (in absolute terms) compared to without oil (Hedrén, Diaz and Svanberg, 2002). In our study, prolonging the chewing time of raw carrot increased the easily extractable β-carotene content by 5–6 % (in absolute terms, Fig. 4) which is comparably limited and only half the effect size compared to the addition of oil to cooked pulped carrots. It should be noted that this increase in β-carotene bioaccessibility by prolonged chewing can be as big as 20 % (in absolute terms) for individual participants. We suggest that processing methods used for food preparation such as adding oil for carrot consumption have a stronger impact on release of β-carotene than increasing mastication. Additionally, one study investigated the release of β-carotene from different food matrices such as carrots, tomatoes, and papayas. The authors reported higher bioavailability of β-carotene in papayas compared to tomato and carrots and suggested that the morphological and physical characteristics of the chromoplasts where the carotenoids are stored would play a crucial role (Schweiggert et al., 2014). Selecting plants where carotenoids are stored in organelles wherefrom they can be more easily released might be an easier alternative way to increase mastication to increase absorption of β-carotene.

In the current study, a significant effect of carrot shape was observed on bolus properties but this effect did not translate to a significant effect on the proportion of easily extractable β-carotene (Fig. 4 & Table 2). Strong correlations were found between bolus properties and the proportion of easily extractable β-carotene from raw carrots in our study. This is in agreement with the previous findings that increasing bolus surface area could enhance the release of nutrients thereby facilitate the bioaccessibility of nutrients (Lemmens et al., 2010, 2011; Zhu, Hsu and Hollis, 2013, 2014; Golding, 2019; Chen, Capuano and Stieger, 2020). However, carrot shape only had a significant effect on the proportion of easily extractable β-carotene for carrots chewed for short times. Carrots julienne chewed shortly displayed a significantly higher easily extractable β-carotene proportion than carrot cubes chewed shortly but this effect of shape on the proportion of easily extractable β-carotene was not found in carrots chewed normally. This result showed that the effect of carrot shape on the release of β-carotene was limited compared to the effect of chewing time. The increase in released β-carotene due to prolonged chewing was larger than the increase due to changing carrot shapes. The extractability of β-carotene should be proportional to the surface area of bolus (Moelants et al., 2012), oral processing behaviour

could directly influence on bolus properties by breaking down the matrix, while carrot shape could only alter the bolus properties indirectly by influencing on the factors such as texture, and oral processing behaviour thereby weakening the effect of carrot shape on the extractability of β-carotene (Kohyama et al., 2007; van Eck & Wijne, 2019). This result suggested that larger differences in oral processing behaviour and subsequent bolus properties induced by the changes in carrot shape are needed to lead to a substantial improvement of β-carotene bioaccessibility.

## 5. Conclusions

The present study investigated for the first time the effects of carrot shape and oral processing behaviour on bolus properties and the potential β-carotene bioaccessibility of raw carrots. Prolonging chewing increased surface area of carrot bolus resulting in a 5–6 % increase (in absolute terms) in β-carotene release. The effect of prolonging chewing time on the proportion of potentially bioaccessible β-carotene was larger than the effect of changing carrot shape, but was overall modest. The findings of the current study emphasize the importance of understanding the effects of food preparation and oral processing behaviour on micronutrient bioaccessibility, particularly for plant-based foods that are rich in micronutrients such as β-carotene and highlight possible serious technical and practical challenges when investigating this, mostly related to the high variability of micronutrients content in plant tissues.

## Author contributions

YC, EC, MS: Study design. YC: Data collection and Data analysis. YC, EC, MS: Writing. YC, EC, MS: Reviewing and editing. YC: Overall responsibility for final manuscript.

## CRediT authorship contribution statement

**Yao Chen:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Edoardo Capuano:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Markus Stieger:** Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

## 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.

## Data availability

Data will be made available on request.

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