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Development of a lutein-fortified sports beverage and lutein-fortified cupcakes, and bioavailability study to evaluate their capacity to increase lutein levels in human blood

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ABSTRACT

The carotenoid lutein is a bioactive that protects the retina and supports brain health. However, lutein-rich foods such as leafy greens, maize and eggs are insufficiently consumed. We developed a lutein-fortified isotonic sports beverage (9.94 ± 0.17 mg lutein/500 mL) and cupcakes (10.57 ± 0.39 mg lutein/50 g) and conducted a 96-h, single-dose bioavailability study in two groups of healthy adults (younger adults: 29.00 ± 3.46 years; middle-aged adults: 53.00 ± 9.39 years, respectively) to assess the capacity of these foods to increase serum lutein levels. Lutein remained stable for two months in the refrigerated beverage and seven days in the refrigerated cupcakes. Consumption of 500 mL of beverage or a 50-g cupcake increased serum lutein area under the curve (ΔAUC(0–96 h)) by 956.9 ± 440.0 and 1257.6 ± 635.7 h·μg/L, respectively. These findings confirm that lutein-fortified foods offer a convenient and effective method to increase serum lutein levels.

1. Introduction

Carotenoids are yellow, orange and red pigments synthesized by plants, microalgae and some bacteria, archaea and fungi. Lutein, a prominent member of the carotenoid subclass of xanthophylls, is present in the photosynthetic apparatus of all plants, where it helps chlorophyll collect incident light for photosynthesis. In addition, lutein aids in dissipating excess energy when sunlight intensifies, thereby protecting the photosynthetic apparatus from damage (Liguori et al., 2017). Humans cannot synthesize carotenoids. However, we readily absorb these bioactives from the diet, from vegetables such as spinach, kale, broccoli, maize, pumpkin, fruits such as kiwi or red grapes, or egg yolk (Sommerburg et al., 1998) and accumulate them in tissues such as the retina (Bone et al., 1993), the brain (Vishwanathan et al., 2014) or the adipose tissue (El-Sohemy et al., 2002). The fraction of lutein released during digestion of these foods is largely determined by the food matrix in which this carotenoid is embedded (Chacón-Ordóñez et al., 2019). In spinach, for example, lutein bioaccessibility is relatively low, as the digestive system must first disrupt the plant cell walls to release it. Thermal processing (e.g., cooking) facilitates this release by breaking down cellular structures (Benítez-González et al., 2024). Subsequently, the extent to which this bioaccessible lutein enters the bloodstream through the digestive system (its bioavailability) is influenced by factors including lifestyle, age, sex, health status, and genetic background (Bohn et al., 2017).

Lutein accumulates in the human retina along its structural isomers zeaxanthin and *meso*-zeaxanthin (Bone et al., 1993), forming a yellow spot known as the macula lutea, where these three carotenoids protect the photoreceptors in the fovea from photooxidation (Bian et al., 2012). The study AREDS2 suggests that an adequate consumption of lutein helps prevent the progression of age-related macular degeneration (Chew et al., 2014). Furthermore, lutein supplementation has been reported to improve visual acuity and contrast sensitivity, as reviewed by Buscemi et al. (Buscemi et al., 2018). In the brain, lutein accumulates in the hippocampus and in the prefrontal, frontal, auditory, and occipital cortices (Johnson et al., 2013; Vishwanathan et al., 2014). Interestingly, lutein has been reported to lower the risk of depression (Lu et al., 2025), contribute to preventing cognitive decline (Li & Abdel-Aal, 2021; Nolan et al., 2022), and improve visual and cognitive performance in children (Parekh et al., 2024).

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Lutein remains under-consumed worldwide. This may be due to the increasing consumption of ultra-processed foods (Monteiro et al., 2013), driven by factors such as uneducated food choices, financial challenges (Chen & Antonelli, 2020), and the retail environment (Zhang & Huang, 2018), to name a few. The average intake of lutein by adults in the general population is 1–3 mg per day (Estévez-Santiago et al., 2016; Johnson et al., 2010; Ranard et al., 2017), while researchers recommend an intake of 6 mg per day (Seddon et al., 1994) and some governments recommend 10 mg of lutein per day (Chinese Nutrition Society, 2013; Korean Ministry of Food and Drug Safety, 2020). Lutein is extensively marketed as a dietary supplement (US Department of Health and Human Services - National Institutes of Health - Office of Dietary Supplements, 2025), mainly for adults aiming to delay the progression of AMD. This source of lutein is contributing to a higher lutein intake in the population. However, swallowing capsules *can be difficult for individuals with dysphagia, a condition whose prevalence increases with advancing age* (Serra-Prat et al., 2011).

Lutein, primarily obtained from the marigold flower (Lin et al., 2015), is permitted for use as a food colourant additive within the European Union (The European Commission, 2012). Subsequently, lutein is added to commercial dairy products, baked goods, confectionery and beverages to provide an appealing yellow hue (European Food Safety Authority, 2010). However, despite some products containing enough levels of lutein to be considered as a source of supplementation, food manufacturers only focus on the dyeing capabilities of this carotenoid. The potential of carotenoids as constituents of functional foods has been suggested (Meléndez-Martínez et al., 2021; Shi et al., 2024a), and there is an unmet need for developing palatable, convenient lutein-fortified foods that can effectively increase lutein intake in population groups at risk of deficiency. Several research groups, including our own, have prepared lutein-fortified foods and have reported that they increase lutein levels in the bloodstream of humans. Examples are a lutein-fortified fermented milk (Granado-Lorencio et al., 2010), a lutein-enriched egg-yolk-based buttermilk beverage (Kelly et al., 2014) and the lutein-fortified yoghurt developed by our group (Shi et al., 2024b).

Other foods suitable for lutein fortification include isotonic sports beverages and confectionery, since lutein prepared as an emulsion has the capacity to remain stable in both water-based and lipid-based food matrices (Shi et al., 2024a). Isotonic sports beverages are widely consumed by physically active individuals to replenish electrolytes, carbohydrates and fluids lost during exercise, although their consumption is expanding to all population groups (Park et al., 2013). Per capita sales volume of sports drinks in the United States (including energy drinks) increased tenfold between 2000 and 2010 (Park et al., 2013), and over a third of adolescents consumed sports drinks at least once a week, with consumption significantly associated with physical activity and organised sport participation (Larson et al., 2014), reflecting a rapid growth of this market. Regarding confectionery, this food category is of particular interest for *middle-aged and older adults, as the ability to prepare meals declines progressively with advancing age* (Whitelock & Ensaff, 2018), often resulting in compromised nutritional status (Brownie, 2006). Factors such as isolation, chronic illness, reduced mobility and difficulty shopping for groceries *make it increasingly challenging to follow a healthy diet from middle age onwards* (Brownie, 2006). *Accordingly, convenience becomes a progressively more* important driver of food choice (Chaffee & Ross, 2023). In *Ireland*, 94% of Irish adults aged 65 years and above are consumers of confectionery and snacks, with a mean daily intake of 65 g, including biscuits, cakes and pastries, chocolate, preserves and savoury snacks (Irish Universities Nutrition Alliance (IUNA), 2024).

In this work we have developed and produced a lutein-fortified isotonic sports beverage and lutein-fortified cupcakes, and we have assessed the shelf-life of lutein in these foods in different storage conditions. Then we conducted a 96-h, single-dose bioavailability study to assess how these lutein-fortified foods increased lutein levels in the bloodstream of humans.

2. Materials and methods

2.1. Materials

The lutein formulation, plantcol Lutein Extract WS3 (0.5% lutein), was provided by Cybercolors Ltd. (Fermoy, Ireland). Materials used to prepare the beverage were sugar (Nordic Sugar A/S, Denmark), maltodextrin (Tereos, Belgium), gum acacia (Kerry Ingredients & Flavours, Ireland), ascorbic acid (Shandong Luwei Pharmaceutical Co. Ltd., China), citric acid (Citribel NV, Belgium), lactic acid (Moorepark Technology Limited, Cork, Ireland), potassium sorbate (Nutrinova, Germany), trisodium citrate (Carbon Group, Cork, Ireland), Niacin (vitamin B3) (Solgar Supplements, USA), deionised water (Moorepark Technology Limited, Cork, Ireland) and lemon juice from fresh lemons (sourced locally from supermarket, Cork, Ireland). Materials used to prepare the lutein-fortified cupcakes were butter (Kerrygold, Ireland), caster sugar (Siúcra, Ireland), eggs (Class A free-range, sourced from local supermarket, Cork, Ireland), and self-rising flour (Odlum's, Ireland).

Tetrahydrofuran (THF) (Fisher Scientific, Loughborough, UK), n-hexane (VWR Chemicals, Gliwice, Poland) and methanol (VWR Chemicals, Paris, France) were of HPLC grade.

2.2. Preparation of lutein-fortified isotonic sports beverage

Cybercolors is accredited to the International Food Safety Standard (FSSC22000) and has elaborate HACCP/food safety systems in place. These standards were applied in the preparation of the foods described in this work. The recipe to prepare the lutein-fortified isotonic sports beverage was based on the ingredients of a commercially available isotonic sports beverage. The ingredients, including sugar (30.5 g per 500 mL), maltodextrin, gum acacia, ascorbic acid (15 mg per 500 mL), citric acid (265 mg per 500 mL), lactic acid (15 mg per 500 mL), potassium sorbate, trisodium citrate, Niacin (vitamin B3), water and lemon juice were weighed in a calibrated analytical balance (Mettler Toledo ME204T/00, Toledo, Ohio, United States). The electrolytes and carbohydrates were dissolved in water to create a concentrated solution in a 5 L sterile mixing vat (Kenwood KMIX, KMX750AC, Kenwood, Havant Hampshire, United Kingdom). Plantcol Lutein Extract WS3 (E 161b) was added at either 0.1, 0.5 or 0.9% (*v*/v) dose rates. These three concentrations of the lutein formulation were prepared to assess lutein stability across a dose range. The intermediate concentration, providing approximately 10 mg of lutein per serving, was selected for the bioavailability study as it aligns with the daily lutein intake recommended by some governmental bodies (Chinese Nutrition Society, 2013; Korean Ministry of Food and Drug Safety, 2020). The mixture was stirred for 2–3 min. The beverage pH was checked using a pH meter (WTW ProfiLine pH 3310 Pocket Meter, Xylem, Washington DC, United States) and adjusted to 3.2 using lemon juice and citric acid. The beverage was then sterilised by heating the mixing vat on a hot plate at 72–75 °C for 10 min. After pasteurisation, the mixing vat containing the isotonic beverage mixture was transported to an ice bath where the beverage was cooled to 15 °C. Then, the mixture was carefully weighed and packed into sterile opaque cardboard (polyethylene-lined) beverage containers, each one containing 500 g of the beverage. Once filled, each bottle was sealed and closed with a lock cap.

2.3. Preparation of lutein-fortified cupcakes

The preparation and procedure for the lutein-fortified cupcakes followed a recipe described previously (Abdel-Aal et al., 2010) with slight modifications. The ingredients (including 13.25 g of sugar per 50 g cupcake) were weighed using a calibrated analytical balance. Using an electric whisk/food mixer (Kenwood KMIX, KMX750AC), softened butter and caster sugar were beaten together. The eggs were whisked into the mixture. Self-rising flour was then folded into the mixture. The

batter was mixed for 6 min until fully combined. Plantcol Lutein Extract WS3 was then added to achieve either 1.2, 5 or 9.6% (*v*/v) lutein concentration. Similarly to the beverage, three lutein concentrations were prepared to assess stability over a dose range, while the intermediate concentration, providing approximately 10 mg of lutein per serving, was selected for the bioavailability study (Chinese Nutrition Society, 2013; Korean Ministry of Food and Drug Safety, 2020). 50 g of the mixture was accurately weighed and spooned into each of the cupcake cases. The cupcakes were baked for 15–18 min at 180 °C until golden brown. The cupcakes were left to cool completely to 15–20 °C on a wire rack before being packed into sterile plastic packaging containers and placed in a refrigerator (4–6 °C).

2.4. Microbiological testing

Randomly selected bottles of prepared lutein-fortified isotonic sports beverage and randomly selected lutein-fortified cupcakes were sent to Eurofins Food Testing Ireland Ltd. (Dungarvan, Ireland) for microbiological testing.

2.5. Carotenoid extraction from lutein-fortified foods

The procedure used for carotenoid extraction from the functional foods was based on a method described previously (Breithaupt, 2004), with slight adjustments. All procedures for carotenoid extraction and HPLC analysis were carried out under orange lighting to reduce carotenoids' exposure to white light. For each food product, 0.5–8 g of sample was weighed in triplicate in 50 mL polypropylene tubes and brought up to 10 mL using deionised water. The samples were vortexed for 30 s and then 10 mL of methanol and 10 mL of n-hexane were added to the polypropylene tubes to extract the carotenoids. The polypropylene tubes were mixed by inversion three times, and centrifuged at 3500*g*, at 20 °C for 3 min to separate the layers. The upper organic phase was collected in a second 50 mL polypropylene tube and dried in the vacuum centrifuge at <30 °C for 5 min. The dry residue was resuspended in 10 mL of n-hexane.

2.6. Carotenoid quantification of lutein in prepared foods by HPLC

Carotenoid esters were quantified by HPLC (Agilent Technologies 1260 series system, Agilent Technologies, USA) using a chiral column (Daicel Chiralpak IA-3, 4.6 mm I.D. × 250 mm length, 3 μm particle size; Daicel Corporation, Japan). 30 μL of each sample was injected into the HPLC. The system had a binary pump, degasser, a thermostatically controlled column compartment and an autosampler. An isocratic program was applied with mobile phase A consisting of n-hexane, and mobile phase B consisting of isopropanol. The flow rate was 0.5 mL/min, and the column temperature was set to 20 °C. Solvent A was held at 90% and solvent B was held at 10%. The identification of the main lutein peak was based on the retention time and UV–visible spectrum compared with true standards.

Lutein quantification by HPLC was performed by constructing a calibration line using plantcol Lutein Extract WS3. Spectrophotometric quantification of the lutein standard was performed using a UV–Vis spectrophotometer (UV-1900i, Shimadzu, Kyoto, Japan) in THF at λ_{max} 444 nm. Six lutein calibrators at different concentrations were injected in duplicate, whereas the calibrator of the lowest concentration was injected 5 times to experimentally establish the lower limit of quantification (LLOQ). The upper limit of quantification (ULOQ) was allocated as the calibrator of the highest concentration of each calibration curve. The equation of the calibration line obtained to quantify lutein extracted from the lutein-fortified foods was $y = 0.04765\times + 0.44625$ (quantification range 3.37 ± 0.05 to 90.11 ± 0.12 ng/mL), with $R^2 = 0.9986$.

2.7. Assessment of lutein stability in the lutein-fortified foods

Lutein-fortified isotonic sports beverages were prepared at three concentrations (2, 11 and 20 mg per 500 mL) and bottled in quadruplicate in opaque polyethylene-lined cardboard containers. For each concentration, two bottles were allocated to refrigerated storage (4 °C, dark) and two to ambient temperature storage (dark). Lutein concentration was measured in the bulk beverage at day 0 prior to bottling, and subsequently in one unopened bottle per storage condition at month 2 and at month 6. In this way, all bottles remained sealed until their designated analysis time. Each bottle was analysed in triplicate by HPLC-DAD. Storage temperatures were recorded on data loggers (Comark Instruments RF11-T, Cruinn Diagnostics, Dublin, Ireland).

2.8. Assessment of colour in the lutein-fortified isotonic sports beverage

The colour of the lutein-fortified isotonic sports beverages was measured at day 0 and month 6 using a Konica Minolta colourimeter (CR-5) in reflectance mode. Samples were equilibrated to room temperature (20–22 °C) and placed in a glass cuvette on the measurement area. CIE L*a*b* colour space coordinates were recorded in triplicate for each sample. Colour differences between day 0 and month 6 were calculated as Delta E $(\Delta E) = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$.

2.9. Carotenoid extraction from serum

Blood serum carotenoid extraction and analysis was performed following methods described previously (Shi et al., 2024b).

2.10. Human single-dose bioavailability trial (ProFiLE)

ProFiLE was a single-dose, 96-h bioavailability human trial approved by the Ethics Committee, HSE, South East Technological University (registration number ISRCTN13108633). All researchers who were part of this study completed research integrity training. All participants provided written informed consent prior to their inclusion in the study. Participants were recruited through advertisement campaigns using online and social media platforms. Participants included in this trial were healthy adults aged between 18 and 65 years old. The ten younger participants were allocated to group 1 (lutein-fortified isotonic sports beverage), and the older nine participants were allocated to group 2 (lutein-fortified cupcake), reflecting the intended target populations of each product: younger adults for the beverage and *middle-aged* adults for the cupcake. "Healthy" was defined as currently/historically not having a chronic disease, having no history of gastrointestinal diseases that could inhibit lutein absorption, having no current severe illnesses or infections, and having not undergone major surgery within the last 6 months. Participants were required not to take any medications or dietary supplements that would interfere with lutein absorption. Pregnant or lactating women were excluded from the trial due to potential effects on lutein metabolism and nutrient demands. Participants taking medications or supplements known to impact lutein absorption were excluded from the trial. Individuals with allergies to gluten, eggs, and dairy were excluded from the study as the intervention required participants to ingest lutein-fortified foods containing gluten (flour), eggs, and dairy ingredients (butter).

This study recruited a total of 19 participants who were allocated into 2 groups: Consumption of lutein-fortified isotonic sport beverages (ten subjects) or consumption of lutein-fortified cupcakes (nine subjects). Each participant was asked to consume one single serving of their group's specified food product (Group 1: isotonic sports beverage – 500 mL serving containing 10 mg of lutein and Group 2: cupcakes – one 50 g cupcake containing 10 mg of lutein). The food products used in the trial were fully compliant with all EU food safety regulations and standards (European Food Safety Authority, 2010). The lutein-fortified food

products were produced in the applications laboratory of Cybercolors Ltd. under the supervision of personnel trained in HACCP/food safety and were microbiologically tested for safety before human consumption.

On the first day of the study, the following information was recorded from all participants: contact information, demographic information, smoking and alcohol intake, dietary habits, body mass index (BMI), and blood samples. Participant carotenoid skin levels were measured using a non-invasive carotenoid skin scanner (Ermakov et al., 2018). A baseline blood sample was obtained from the participants, and then they were asked to consume one single serving of their group's specified functional food product, as outlined above. On the same day, blood samples were taken again from the participants at three, six and ten hours after the lutein-fortified food consumption (Table 1). Days 2, 3, 4, and 5 entailed one brief morning visit by each participant (15–20 min), where a single fasting blood sample was drawn each day before breakfast was provided (Table 1).

Lutein and zeaxanthin concentrations were determined by HPLC.

For day 1, breakfast and lunch were provided for participants, and for days 2, 3, 4, and 5, breakfast was provided (Table 1). Throughout day 1 of the study, participants engaged in typical on-site activities, provided they were low-impact, such as desk work. Upon completion of day 1, participants received a list of foods they were allowed to eat for the following four days. This dietary allowance aimed to maintain a low lutein intake during the duration of the study.

2.11. Data and statistical analysis

HPLC carotenoid chromatograms generated were analysed using Agilent ChemStation (version 04.03.087). Statistical analysis was performed using SPSS statistics version 29.0.1.0 (IBM, USA). Paired *t*-tests were used to evaluate variations in serum carotenoid levels for each dose at each time point. Statistical significance was set at $p < 0.05$. Because of the limited sample size, the statistical power of this study was deemed insufficient for comparing the means between the groups.

3. Results

3.1. Lutein quantification in lutein formulation and lutein-fortified foods

The commercial lutein emulsion plantcol Lutein Extract WS3 (declaring 0.5% lutein (*w*/w)), contained 4.6 ± 0.2 mg of lutein per g of sample as lutein esters. The lutein-fortified isotonic sports beverage prepared at either 0.1, 0.5 or 0.9% (*v*/v) plantcol Lutein Extract WS3 contained a final lutein concentration of 2.21 ± 0.04, 11.35 ± 0.67, or 20.41 ± 0.79 mg of lutein per 500 mL of beverage, respectively. The lutein-fortified cupcakes prepared at either 1.2, 5 or 9.6% (v/v) plantcol Lutein Extract WS3 contained a final concentration of 2.70 ± 0.50, 11.25 ± 0.59 and 22.04 ± 0.90 mg of lutein per cupcake (50 g), respectively. Fig. 1 shows an example of the lutein-fortified sports beverage and the lutein-fortified cupcake prepared with 20.41 ± 0.79 and 22.04 ± 0.90 mg of lutein, respectively.

3.2. Lutein stability in the lutein-fortified isotonic sports beverages

The bottles of lutein-fortified isotonic sports beverages prepared at three different lutein concentrations were stored in refrigeration in the dark (6 ± 0.3 °C, range 0.3–6.9 °C) or ambient dark (19.2 °C ± 0.9 °C, range 16.7 °C – 22.2 °C). Table 2 shows the stability of lutein in the lutein-fortified isotonic sports beverages stored under these storage conditions. Under refrigeration, lutein remained stable after two months of storage for the three concentrations of lutein prepared and then declined at month six. For the beverages stored at ambient dark, the concentration of lutein was lower at month 2 and it further decreased at month 6 for the three concentrations prepared (Table 2). Given that each time point was represented by a single bottle analysed in triplicate, formal statistical comparison across time points was not performed; the values reported reflect analytical means ± SD.

3.3. Colour stability in the lutein-fortified isotonic sports beverage

Colourimetric analysis of the lutein-fortified isotonic sports beverages showed that beverages stored under refrigeration retained their colour over six months, with low ΔE values of 5.95, 3.68 and 3.85 for the 2, 11 and 20 mg/500 mL concentrations, respectively. In contrast, beverages stored under ambient dark conditions showed substantial colour loss, with ΔE values ranging from 70.59 to 178.89. These colourimetric results are consistent with the lutein degradation measured by HPLC (Section 3.2), confirming that colour loss paralleled carotenoid loss in the beverages.

3.4. Lutein stability in the lutein-fortified cupcake

The lutein-fortified cupcakes prepared with three different concentrations of lutein were stored at 6 °C ± 0.3 °C for seven days in the dark. Table 3 shows lutein concentration in the cupcakes at day zero and day seven.

3.5. Microbiology analysis of the lutein-fortified foods

The testing results for pathogenic microorganisms in the lutein-fortified foods complied with current guidelines (The European Parliament and the Council of the European Union, 2002). Therefore, the HACCP plan designed met the requirements for the safe preparation of lutein-fortified isotonic sports beverage and lutein-fortified cupcakes, and the foods produced were considered safe for human consumption in the single-dose bioavailability trial.

3.6. Bioavailability study

Thirty-two volunteers were assessed for eligibility for the ProFiLE human bioavailability trial, of which nineteen were recruited and distributed among the two intervention groups. The ten younger participants were allocated to group 1 (lutein-fortified isotonic sports

Table 1
Experimental design and blood sampling schedule for the single-dose bioavailability trial.

Pre-trial	Lutein stability assessed over six months in beverages (Section 3.2) and seven days in cupcakes (Section 3.4). Microbiological safety confirmed (Section 3.5).							
Day	1	1	1	1	2	3	4	5
Withdrawal No.	1st	2nd	3rd	4th	5th	6th	7th	8th
Time-points (h)	Baseline (0 h)	3 h	6 h	10 h	24 h	48 h	72 h	96 h
Suggested time	8 am	11 am	2 pm	6 pm	9 am	8 am	8 am	8 am
Actions	Fasting blood sample, lutein-fortified food administration after 1st withdrawal	Breakfast* provided after 2nd withdrawal	Lunch* provided after 3rd withdrawal		Breakfast* provided after each blood withdrawal			
Post-trial	Determination of serum lutein and zeaxanthin concentrations†							

* Specially formulated carotenoid-free breakfast and lunch were provided.



Fig. 1. Lutein-fortified foods. A Lutein-fortified isotonic sports beverage containing 20.41 ± 0.79 mg of lutein per 500 mL. B Lutein-fortified isotonic sports beverages in opaque polyethylene-lined cardboard storage containers. C Lutein-fortified cupcake containing 22.04 ± 0.90 mg of lutein per cupcake (50 g), showing the interior of the cupcake post-baking. D Lutein-fortified cupcakes containing 10.57 ± 0.39 mg of lutein per cupcake (50 g) in a sterile clear plastic storage container.

Table 2
Lutein concentration in lutein-fortified isotonic sports beverages prepared with 2, 11 or 20 mg of lutein per 500 mL serving, at day 0 and after two and six months of storage in the dark at two temperatures (refrigeration, 6 ± 0.3 °C; ambient, 19.2 ± 0.9 °C). The beverages were stored in opaque cardboard (polyethylene lined) containers, one bottle per storage condition and sampling time, and remained closed until analysis. Each bottle was analysed in triplicate by HPLC-DAD. Values are expressed as mean ± SD ($n = 3$).

		Lutein (mg per 500 mL)		
Target concentration		2 mg	11 mg	20 mg
Refrigeration (dark)	Day 0	2.21 ± 0.04	11.35 ± 0.67	20.41 ± 0.79
	Month 2	2.15 ± 0.10	11.66 ± 0.56	21.03 ± 0.37
	Month 6	1.34 ± 0.17	8.93 ± 0.23	15.34 ± 0.19
Ambient (dark)	Month 2	1.22 ± 0.02	7.55 ± 0.17	13.23 ± 0.22
	Month 6	0.86 ± 0.03	4.93 ± 0.15	8.68 ± 0.12

Table 3
Lutein concentration in cupcakes prepared with 2, 11 or 22 mg of lutein per 50 g of cupcake, at day 0 and after seven days of storage in refrigeration in the dark (6 °C ± 0.3 °C). The cupcakes were stored in transparent sterile plastic packaging containers. Each cupcake was analysed in triplicate by HPLC-DAD. Values are expressed as mean ± SD (n = 3).

	Lutein (mg per 50 g cupcake)		
Target concentration	2 mg	11 mg	22 mg
Day 0	2.70 ± 0.50	11.25 ± 0.59	22.04 ± 0.90
Day 7	2.71 ± 0.65	10.94 ± 1.08	22.00 ± 0.70

beverage), and the older nine participants were allocated to group 2 (lutein-fortified cupcake). All participants completed the study with 100% compliance. Participants in group 1 were significantly younger (29.00 ± 3.46 years) than participants in group 2 (53.00 ± 9.39) ($P <$ 0.001) and had a mean BMI significantly lower ($P < 0.05$) (Table 4). No statistically significant differences were found in weight or height between the two groups (Table 4). At baseline, before lutein-fortified food consumption, group 1 and group 2 had statistically similar skin carotenoid levels (424.1 ± 63.25 and 461.22 ± 101.07, respectively, $p >$ 0.05).

The participants consumed a single dose of either a lutein-fortified isotonic sports beverage (500 mL, containing 9.94 ± 0.17 mg of

Table 4
Baseline demographics, health, and lifestyle data of groups participating in the ProFiLE bioavailability trial. Group 1 (Lutein-fortified beverage), and group 2 (Lutein-fortified cupcake).

Variable	Group 1 Lutein-fortified isotonic sports beverage (n = 10)	Group 2 Lutein-fortified cupcake (n = 9)	*p* Value
Age (years)	29.00 ± 3.46 (23–35)	53.00 ± 9.39 (37–65)	< 0.001
Sex (female: n, %)	2 (20.0%)	5 (55.6%)	
BMI (kg/m2)	24.92 ± 2.71 (22.1–30.5)	29.4 ± 5.17 (21.9–37.6)	0.039
Skin Carotenoid levels	424.1 ± 63.25 (335–573)	461.22 ± 101.07 (305–671)	0.345
Weight (kg)	74.56 ± 10.46 (56.3–87.0)	86.98 ± 23.07 (59.9–122.5)	0.166
Weight status ([n]); [%])			
Normal	6 (60.0%)	3 (33.3%)	
Overweight	3 (30.0%)	1 (11.1%)	
Obese	1 (10%)	5 (55.6%)	
Height (cm)	173.00 ± 12.95 (150–188)	170.56 ± 11.11 (range: 158–185)	0.666
Smoking ([n]); [%])			
Current smoker	0	1 (11.1%)	
Ex-smoker	0	1 (11.1%)	
Never smoked	10 (100%)	7 (77.8%)	
Alcohol intake per week			
0 units	5 (50.0%)	3 (33.3%)	
1 unit	3 (30.0%)	2 (22.2%)	
2–5 units	1 (10.0%)	0	
6–10 units	0	1 (11.1%)	
>10 units	1 (10.0%)	3 (33.3%)	

Data displayed are mean ± SD for numeric data and percentages for categorical data, with ranges in brackets. BMI, body mass index; BMI category, 18.5–24.9 = normal, 25–29.9 = overweight, >30 = Obese. Smoking status: Current smoker = smoked ≥100 cigarettes in a lifetime and at least 1 cigarette in the last year; ex-smoker = smoked ≥100 cigarettes in a lifetime and none in the past year; never smoker = smoked <100 cigarettes in a lifetime. Alcohol consumption was measured in standard drinks per week where one standard drink contains 10 g of pure alcohol. One standard drink is equivalent to one 100 mL glass of 12.5% wine.

lutein) or a lutein-fortified cupcake (50 g, containing 10.57 ± 0.39 mg of lutein). The consumption of both the lutein-fortified isotonic sports beverage and the lutein-fortified cupcake produced a typical pharmacokinetic curve of increase and decrease for lutein, while zeaxanthin remained unchanged (Fig. 2). Both the beverage and the cupcake group experienced a small drop in lutein concentration three hours post-consumption of the fortified food product, which was not significant ($p > 0.05$). This drop was followed by a sharp rise in lutein serum levels in both intervention groups. The baseline concentration of lutein in the beverage group was 92.2 ± 49.0 μg/L and significantly increased to a C_{max} 114.33 ± 17.93 μg/L ($p < 0.05$) at 19.00 ± 15.90 h (Table 5). For the cupcake group, baseline serum lutein was 121.8 ± 68.7 μg/L, peaking post-consumption at a C_{max} of 158.65 ± 73.7 μg/L ($p < 0.05$) at 20.44 ± 12.48 h (Table 5). After reaching peak concentration, the serum lutein levels gradually declined in both groups and returned close to baseline at 96 h.

A summary of the pharmacokinetic parameters of the two intervention groups is shown in Table 5. Statistical comparison of pharmacokinetic parameters between the two intervention groups was not performed due to the small sample size ($n = 10$ and $n = 9$) and deliberate differences in the age of the participants between groups. AUC was slightly higher in the cupcake group. ΔAUC, calculated as the area under the curve from start to end of the trial, considering only the area from baseline concentration, was also higher in the cupcake group. Finally, ΔC_{max} (C_{max} after baseline correction) was also higher in the cupcake group. Zeaxanthin showed no significant changes in AUC, ΔAUC or ΔC_{max} in either of the two groups.

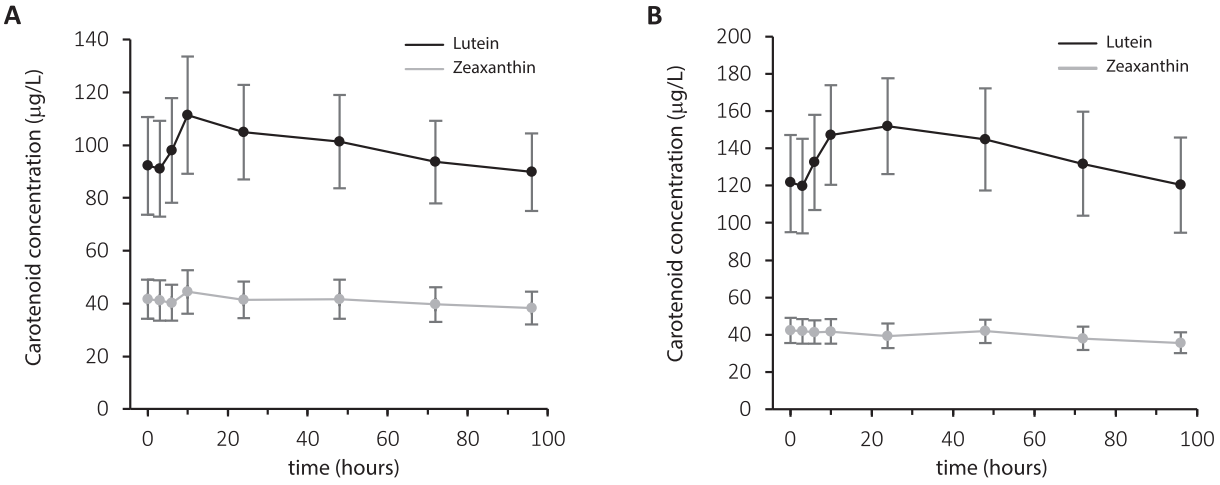


Fig. 2. Average lutein concentration in human serum after a single dose of lutein-fortified food consumption. A. Average lutein changes (black line) in serum after a single dose of lutein-fortified isotonic sports beverage (mean ± SEM). The grey line represents zeaxanthin evolution (mean ± SEM) over the same period of time. B. Average lutein changes (black line) in serum after a single dose of lutein-fortified cupcake. The grey line represents zeaxanthin evolution (mean ± SEM) over the same period of time.

Table 5
Lutein pharmacokinetic parameters after the ingestion of a single dose of lutein-fortified beverage and cupcake.

	Lutein-fortified isotonic sports beverage (n = 10)	Lutein-fortified cupcake (n = 9)
Dose (mg)	9.94 ± 0.17	10.57 ± 0.39
Baseline (μg/L)	92.19 ± 49.03	121.82 ± 68.74
C_{max} (μg/L)	114.33 ± 17.93*	158.65 ± 73.65*
T_{max} (h)	19.00 ± 15.90	20.44 ± 12.48
$AUC_{(0–96\ h)}$ (h·μg/L)	9511.19 ± 4374.16	13,292.26 ± 6719.66
$\Delta AUC_{(0–96\ h)}$ (h·μg/L)	956.86 ± 440.04	1257.55 ± 635.73
ΔC_{max} (μg/L)	22.15 ± 14.99	36.83 ± 17.00

Data are presented as mean ± SD. Values are standardised per mg of each carotenoid. Within-group comparisons for the difference from baseline were made using *t*-tests. * Statistically significant difference observed in comparison to baseline ($p < 0.05$).

4. Discussion

In this work, we prepared two lutein-fortified foods, tested the stability of lutein in these foods in different storage conditions, and conducted a bioavailability study to assess how the ingestion of one dose of these lutein-fortified foods increased lutein in the serum of human participants.

In the lutein-fortified sports isotonic beverage, lutein remained stable for two months under refrigeration in the dark (Table 2). At six months of storage, lutein showed marked degradation. In contrast, lutein degraded already at month 2 when the beverages were stored at room temperature in the dark (Table 2). Colourimetric analysis of the beverages corroborated these findings, with minimal colour change (ΔE < 6) under refrigeration and substantial colour loss (ΔE up to 178.89) under ambient dark conditions. This correlation between colour loss and lutein degradation suggests that colourimetry may serve as a rapid indicator of gross carotenoid degradation in fortified beverages, as previously demonstrated for β-carotene and β-cryptoxanthin in cashew apple juice (Zepka et al., 2009). A limitation of our study is that we did not sparge the headspace of the bottles before storage. As suggested previously, the presence of oxygen in the headspace of the bottle is likely promoting oxygen-consuming reactions and hence lutein degradation (Atencio et al., 2022; Vidal & Moutounet, 2006). On the other hand, it is possible that the presence of ascorbic acid in the beverage, at 15 mg per 500 mL of beverage might have contributed to lutein stability. It has been suggested that the use of antioxidants such as ascorbic acid might contribute to carotenoid preservation in foods due to its antioxidant capabilities (Choi et al., 2002; Wibowo et al., 2015). Therefore, to improve lutein preservation in a beverage, techniques such as opaque packaging with modified atmosphere that protect against oxygen, refrigeration and antioxidants such as ascorbic acid can be used.

In the lutein-fortified cupcakes, storage in refrigeration preserved lutein stable for one week. Lutein-fortified cupcakes have been previously produced (Xavier et al., 2018) using a commercial water-soluble lutein ester emulsion. However, Xavier et al. only tested lutein stability post-baking, as they froze the cupcakes until use for an in vitro bioaccessibility study. Lutein-fortified muffins, where lutein fortification was performed throughout flours naturally high in lutein or added free lutein have also been produced (Abdel-Aal et al., 2010). In this study, lutein remained stable in the muffins after storage for three days at room temperature. To complete the assessment of lutein stability in lutein-fortified cupcakes, conditions such as ambient temperature and ambient light over a longer period of time to simulate retail conditions should be considered.

In the single-dose bioavailability study with the lutein-fortified foods, the study participants started with a high variable basal serum lutein concentration. Within this variation, the participants that ingested the cupcake had higher basal serum lutein on average, but the difference with the beverage group was not statistically significant. Accordingly, total carotenoids in the skin of the participants were also higher in the participants of the cupcake group, but again, this difference was not statistically significant due to the high variability among participants. These non-significant differences in basal serum lutein and skin carotenoids may be due to the difference in age and BMI between the two intervention groups, and to the fact that in the study design, we did not request the volunteers to change their eating habits before starting the trial. These differences among individuals have been previously reported as determinators of variability of serum carotenoid concentrations among the population (Bohn et al., 2017).

Zeaxanthin is a minor carotenoid that is co-extracted with lutein from marigold flowers (Tagetes erecta) and is present in the lutein formulation at a ratio of 0.44 mg per 10 mg of lutein. Serum zeaxanthin was expected to remain unchanged, as observed previously (Shi et al., 2024b). Therefore, we measured zeaxanthin alongside lutein to serve as an internal control. The stability of serum zeaxanthin in the participants across the 96 h of the study supports the specificity of the observed lutein response. In the case of the cupcake group, lutein C_{max} and ΔAUC were descriptively higher than in the beverage group, which could suggest a higher overall absorption or bioavailability of lutein from the cupcake. However, these observations are purely descriptive, and no statistical comparison was performed, as the two groups differed deliberately in age, BMI and food matrix, precluding any meaningful inter-group statistical analysis. In any case, this trend is congruent with the presence of fats in the matrix of the cupcake, a factor that seems to facilitate a better absorption of lutein, as suggested previously (Moran et al., 2018). Furthermore, a bioavailability study carried out by Unlu et al. (2005) showed that adding avocado fruit (containing fat) to salad enhanced lutein absorption ($P < 0.01$), resulting in 5.1 times the mean AUC compared to the intake of avocado-free salad (Unlu et al., 2005). In the present study, at hour 96, the lutein serum concentration returned to very close to baseline concentrations in both groups. This suggests that the body returns to its homeostatic state after the significant change in blood serum lutein levels.

Our results are comparable to those of the bioavailability trial carried out by Shi et al. with a lutein-fortified yoghurt in our research centre (Shi et al., 2024b). This yoghurt facilitated a significant increase in both ΔAUC (1946.86 ± 1514.72 h μg/L) and ΔC_{max} (38.39 ± 22.41 μg/L) in the study participants, with a return to the baseline serum lutein concentration over the course of the study, similar to the pattern observed in the present work with the beverage and the cupcake. The absorption rate per unit mass (ARUM), defined as the ratio of ΔC_{max} to the amount of lutein ingested (μg/L mg^{-1}) (Serra et al., 2021; Shi et al., 2024b) was 2.6 μg/L mg^{-1} for the yoghurt (Shi et al., 2024b). In the present study, ARUM was 2.2 μg/L mg^{-1} for the beverage and 3.5 μg/L mg^{-1} for the cupcake. These values are in the same range as the ARUM of the lutein-fortified yoghurt, and of a lutein-fortified fermented milk developed by Granado-Lorencio et al. (Granado-Lorencio et al., 2010), (ARUM 2.7 μg/L mg^{-1}, as calculated by Shi et al.) (Shi et al., 2024b), suggesting that ARUM may be relatively consistent across lutein-fortified dairy and non-dairy matrices. They are, however, lower than ARUM for foods naturally rich in lutein: for example, consumption of broccoli and spinach (Riso et al., 2013) and cooked egg (Chung et al., 2004) yielded ARUMs of 5.0, 10.5 and 12.1 μg/L mg^{-1}, respectively (Shi et al., 2024b). This difference may reflect the higher bioaccessibility of lutein from a native food matrix compared with the exogenous lutein formulations used in fortified products.

Nonetheless, our results confirm that a single dose of the lutein-fortified sports beverage or cupcake, providing approximately 10 mg of lutein, can deliver measurable amounts of this carotenoid to the bloodstream. Foods naturally rich in lutein remain the preferred dietary source of this and other carotenoids, as they additionally provide vitamins, minerals and fibre. However, for population groups where additional lutein intake may be beneficial, such as physically active individuals or older adults with inadequate dietary intake, lutein-

fortified foods such as a sports beverage and a cupcake could contribute to addressing this shortfall.

5. Conclusion

Our results demonstrate the feasibility of fortifying a beverage and a cupcake with lutein, such that this carotenoid remains stable in these matrices for a significant shelf-life period when stored in refrigeration in the dark. We also demonstrated that the ingestion of a single dose of these lutein-fortified foods significantly increased lutein levels in the bloodstream of humans. However, the participants in the cupcake group (mean age 53 years) were younger than the elderly population ($\geq$ 65 years) for whom the product was designed. As ageing may affect gastrointestinal absorption and lutein metabolism, future studies should confirm these bioavailability findings in participants aged 65 years and above. Future research should also assess the sensory acceptability of these lutein-fortified functional foods from a consumer perspective in terms of appearance, colour, taste and texture, and explore the effects of repeated daily consumption on long-term lutein accumulation in serum and tissues, which would complement the single-dose bioavailability data presented here. Of special importance for future product development is reformulation to reduce the sugar content of both the cupcake and the beverage, or the use of alternative sweeteners, to ensure the nutritional profile of the fortified foods is appropriate for the target populations.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used Claude Sonnet 4.6 (Anthropic) to check the manuscript and the accompanying rebuttal letter for typographical errors, internal inconsistencies, and inconsistent reference formatting. The authors reviewed and edited the output as needed and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Alfonso Prado-Cabrero: Writing – original draft, Supervision, Methodology, Conceptualization. **Róisín Flynn:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Tommy Power:** Formal analysis. **Marina Green-Gomez:** Supervision, Methodology. **John M. Nolan:** Supervision, Methodology.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: A. P.-C. is a co-founder of a company dedicated to testing dietary supplements with macular carotenoids and omega-3 fatty acids for compliance with their label claim over shelf life. A.P-C. is a co-founder of a company dedicated to producing zooplankton as feed and food. R.F. is an employee of Cybercolors Ltd. T.P. is a technician of a company dedicated to testing the quality of dietary supplements. M.G.-G. declares no financial interests/personal relationships. J.M.N. does consultancy work as a Director of NOW Science Consultancy Ltd. for companies with an interest in vision and food supplements such as Heidelberg Engineering, Macuhealth LLC, Stauber Nutrition, Industrial Orgánica SA de CV. J.M. N. is a co-founder of a company dedicated to testing dietary supplements with macular carotenoids and omega-3 fatty acids for compliance with their label claim over shelf life. J.M.N. is a co-founder of a company dedicated to producing zooplankton as feed and food.

Data availability

The data that has been used is confidential.

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