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Isolation of high pure lycopene from ripened tomato and its Nano preparation with corn zein protein for improved stability and bioaccessibility

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Abstract
Carotenoid (lycopene) research is growing significantly, as several epidemiological and clinical trials demonstrate consumption of tomato-rich diet reduces the risk of cardiovascular diseases and prostate cancer. The aim of the study was to isolate and analyse high pure lycopene from ripened tomatoes to target enhanced lycopene bioavailability through nano-delivery using phytoconstituents like zein corn protein and alginate. Standardized procedure yield high pure (93.3%) lycopene and developed UPLC/MS method for lycopene analysis is rapid and simple. As free lycopene is unstable, we synthesised lycopene encapsulated zein-alginate nanoparticles (LYZA) and characterized its physicochemical properties. LYZA nanoparticle produced a cumulative 195 ± 31 nm particle size, stable zeta potential (-30.4 ± 3 mV), PDI (0.09 ±0.02), and amorphous in nature. Further, the release kinetics exhibited inhibition of lycopene release at acidic conditions and sustained controlled release at intestinal pH. Lycopene bioaccessibility of LYZA was significantly higher than digestion of synthetic lycopene (LYMM) and tomato (TMM) supplements. The results demonstrate high stable and sustainable lycopene supplements as a promising application in nutraceuticals/pharmaceuticals.

Keywords: Tomato, lycopene, bio accessibility, zein, biopolymer

Introduction

Lycopene is a red pigment carotenoid generally present in coloured fruits and vegetables like tomato, watermelon, guava, papaya, grapefruit, wolfberry, apricots, seabuckthorn, and rosehip. Chemically lycopene possesses C40 tetraterpenoids built with eight C5 isoprenoid units with alternative conjugated double bonds assembling it as a superior antioxidant [1, 2]. This characteristic conjugated polyene structure imparts deep red colour to lycopene with the absorbance maxima at 444, 470, and 502 nm [3]. Dietary lycopene is a potent bioactive compound among dietary carotenoids associated with various health benefits like reduced risk of prostate cancer and other cardiovascular diseases [4-6]. Lycopene is a robust singlet oxygen-quencher having superior antioxidant activity like immune system stimulator, cell cycle regulator, gap junction communication enhancer, antitumor immune response, mutagenesis reducer, tumor cell antiproliferation, and anti-inflammation [7]. Lycopene is highly unstable when exposed to environmental (light, oxygen, high temperatures) and physiological conditions (gastrointestinal enzymes) inclined to various modifications such as hydrogenation/dehydrogenation, double-bond migration, chain shortening, extension, rearrangement, isomerization, oxidation or combinations of these processes, thus obtaining high pure intact lycopene from natural sources is challenging [8]. The unique chemical structure of lycopene reacts with free radicals and interrupts polyene chain by cleavage/addition to one of the double bonds making it highly unstable which adversely affect its biological properties [2]. Further, lycopene content in tomatoes is influenced by several factors like agronomic, nature of cultivar, climate, environment, harvesting, and ripening [9, 10]. Tomato fruits are classically harvested at diverse stages of ripeness (green/pink/red colour). The content of bioactive compounds, predominantly carotenoid lycopene, varies over tomato ripening and governs its food quality or nutritional/pharmaceutical value. Morphological, physiological, biochemical (lycopene biosynthesis) and molecular changes (chlorophyll degradation) are associated with increased tomato ripening [10, 11].

Lycopene bioaccessibility and bioavailability from tomato might be influenced by dietary and physiological factors like lycopene content, presence of dietary matrix or antinutritional factors and food processing steps [12-14]. Also, the ascribed reasons for low bioaccessibility and bioavailability are high non-polarity, susceptible chemical structure, and sensitivity to gastrointestinal enzymes/physiological conditions [15]. Clinical studies suggest a lack of improvement of lycopene bioavailability from its rich sources. Therefore, stable and consistent delivery of high pure lycopene may requisite the supplementation of bio fortified food or through biocompatible nano-delivery to prevent isomerization and degradation of the native structure. This processing seems to enhance lycopene bioaccessibility as it disrupts the food matrix and eases the release and absorption of lycopene during digestion [16, 17]. Therefore, biopolymers from plants and natural sources which are biodegradable non-toxic, non-allergic, and non-immunogenic is important in nutraceutical and pharmaceutical supplements. Hence, the delivery of intact carotenoids/nutrients/drugs requires safe delivery with increased solubility and control release at the target site prerequisite the essentiality of biopolymers.

Biopolymer delivery systems containing biodegradable food-grade ingredients are best suited for the deliveries of encapsulated molecules are highly biocompatible. In natural biopolymers, zein phytoconstituent is documented safe by FDA for oral nutrient/drug delivery to encapsulate, protect,

and release nutraceuticals. Zein protein is a phytoconstituent of corn (*Zea mays L*.) comprising non-polar leucine, proline and alanine amino acids resulting in hydrophobic biopolymer [18- 20]. Zein is soluble in 60-90% aqueous ethanol solution. Previously, zein has been explored for encapsulation of various nutrients like lutein [21], quercitin, curcumin [22, 23], gitoxin, quercetagetin [24], β-carotene [25, 26], vitamin D3 and retinol [27]. Zein exhibits brick-like structure in aqueous solution and has advantage of not necessitating additional cross-linking/ modifications to stabilize as nanoparticles. Zein when used alone aggregates and exposes the encapsulant for hydrolysis by acidic enzymes in GI tract, so when used in combination with polysaccharide improves its stability. Polysaccharide (chitosan, alginate) addition may provide better protection against action of gastrointestinal enzymes. Kim *et al*. (2013) [27] synthesized zein-chitosan nanoparticles loaded with retinol to explore its release properties. Li *et al*. (2012) [28] produced thymol-zein-caseinate nanoparticles for antimicrobial film characterization. Patel *et al*. (2010) [22] prepared curcumin zein colloidal nanopreparations to prove the mucoadhesion of zein nanoparticles. In the present study, alginate is used with zein to form electrostatic complex at pH below the protein pI. Alginate, an anionic aqueous soluble linear polysaccharide composed of alternating monomers (α-L guluronic acid and β-D-mannuronic acid) is extracted from brown marine algae. Alginate can be used for crosslinking with other biomaterial and due to its pH sensitivity, low cost has significant application in drug delivery [30]. Alginate when used with zein forms a protein-polysaccaride complex that can protect encapsulant against enzymatic digestion [31].
Few studies attempted to evaluate the bioaccessibility of lycopene from various fruits, vegetables, and plants, but detailed mechanisms arbitrated by specific dietary factors are not known [32, 33]. Previously, we synthesised lutein chitosan nanoparticles for increased bioaccessibility and Caco-2 cellular uptake [34]. Tyssandier *et al*. (2001) [35], demonstrated the variable bioaccessibility of carotenoids based on matrices found in the green leafy vegetables and the influence of other dietary sources. With this background, we aimed to isolate high pure lycopene from tomato and encapsulate it in zein (protein from maize) and alginate (polysaccharide from brown seaweed) to evaluate its influence on the physicochemical properties and bioaccessibility versus tomato puree supplement using simulated *in vitro* digestion. The phytoconstituents like zein used for pure lycopene supplementation in present study found to overcome the downsides of pure lycopene, like solubility, stability, and bioavailability.

Materials and Methods

Extraction of Lycopene from Ripened Tomatoes

Lycopene was extracted from Indian hybrid ripened tomatoes as per our method Arathi *et al*. (2015) [36]. In brief, known quantity of tomatoes was taken, washed with deionized water; and only epicarp portions were taken for the preparation of tomato puree using a grinding mixer. In brief, a portion of the tomato puree (n=5) (2.5 g) was mixed with solvent- methanol: acetone: hexane, 25:50:25, v/v/v (25 mL) in the ratio of 1:10 with 0.1 % BHT (w/v) in ethanol added to minimize isomerization/ oxidation. The sample extract was vortexed vigorously and kept in the dark at 4 °C for 20 min. Then, the top hexane layer was separated by adding an equal volume of deionized water in a separating funnel. These procedures were repeated three times otherwise until the hexane layer becomes colourless. The pooled hexane layers were collected and evaporated to dryness under N_2 gas, and the residue was re-dissolved in a known volume of THF (3 mL) for further aliquots. Extraction and preparation of samples and standards were carried out under dim yellow light to prevent isomerization and degradation to obtain high pure lycopene. The peak identity, absorption maxima (λ_{max}), and characteristics UV-spectra of purified high pure lycopene were confirmed by our earlier established UPLC analysis. Lycopene was analysed by ACQUITY UPLC® CSH Phenyl-Hexyl column (100 ×2.1 mm; 1.7 µm) with solvents-methanol/acetone/hexane (25:50:25, v/v/v)in ACQUITY UPLC® system (Waters Corp., Milford, MA) as per previously established procedure [36].

Preparation of Lycopene Encapsulated Zein-Alginate Nanoparticles (Lyza)

LYZA were prepared by antisolvent precipitation and then stabilized with alginate shell by electrostatic deposition as per Hu *et al*. (2014) [37]. Briefly, 1% of zein solution (pH 5.7) was prepared in 85% (v/v) ethanol solution. Lycopene (0.5 mg/mL) in 0.1% ethyl acetate was added dropwise to the zein solution at a ratio of 1:1 under mild stirring conditions. Then 0.05% (w/v) tween 80 (pH 4) was added under mild stirring conditions at room temperature. Further 0.03% (w/v) alginate solution was added drop wise under 600rpm magnetic stirring conditions. The LYZA prepared was collected and kept at 4 °C for further analysis.

Encapsulation Efficiency

Encapsulation efficiency of lycopene in LYZA was measured by quantifying the entrapped lycopene using the previously reported procedure of extraction and UPLC analysis [36]. The encapsulation efficiency of LYZA was determined by using the following formula.
Encapsulation efficiency (%) = (Total concentration of lycopene used for encapsulation- Concentration of free form of lycopene)/ (Total concentration of lycopene used for encapsulation) X 100.

Preparation of Mixed Micelles

Standard lycopene solubilised mixed micelles were prepared as per Garrett *et al*. (1999) [32] for physiological reference standard. Briefly, aliquots of the standard compounds were transferred to tubes to obtain mono-olein (0.3 mM), oleic acid (0.5 mM) and lysophosphatidylcholine (0.16 mM), phosphatidylcholine (0.04 mM), cholesterol (0.1 mM), lycopene (8.3 μg/ml).The mixture obtained was nitrogen dried, sterilized and lycopene concentration was determined by UPLC [36].

Physicochemical Characterization of LYZA.

Particle size, PDI, and zeta potential of LYMM, TMM, and LYZA and (n=3) were measured using Zetasizer Nano ZS (Malvern, South borough MA). The viscosity of LYMM, TMM, and LYZA were measured on DHR-S rheometer (TA Instruments, USA) at 37 °C. 0.1 to 100 s−1 (up curve) and 100 to 0.1 s−1 (down curve) range was used to monitor shear rate. The physical (crystalline or amorphous) state of LYZA was analysed by XRD. The diffractogram of samples was recorded using X-ray diffractometer (Philips X'Pert XRD, Philips, Netherlands). The X-ray generator optimized

with the tube voltage at 40 kV, tube current of 40 mA using Kα lines of copper as the radiation source and scanning angle set from 1 to 60° for 1 h in a step scan mode with the step width of 1°/min. LYZA was subjected for atomic force microscope (Nanosurf AG, Switzerland) to analyse typical morphological structure and appearance. Further, thin film of sample coated on a glass slide by dropping 100 µL suspension (10 min) at RT for drying. The dried slides scanned using Nanosurf Easyscan-2 software and used for AFM analysis. The Morphological characteristics of LYZA were also analysed using Transmission Electron Microscopy (Tecnai^{TM} T20- ST, Thermo Fisher, Massachusetts, USA). The FTIR was executed to study the interaction of lycopene with the zein and alginate. Briefly, 5 mg of powdered LYZA were finely mixed with KBr using motor and pestle and made into pellets. The FTIR spectra in the range of 4000 to 400 cm^{-1}were obtained in transmission mode using Nicolet 5700 FTIR spectrometer (Thermo electron corporation, USA).

Release Kinetics

The release of lycopene from LYZA was assessed at gastric (pH 2) and intestinal (pH 7.4) conditions. LYZA (1 mg) was dispersed in 10 mL of 0.01 M Tris HCl buffer (pH 2) and PBS (pH 7.4) in the dark. Aliquot of 1mL LYZA suspension was taken in eppendorf, incubated in dark conditions with100 rpm shaking conditions in the water bath at 37 °C. The solution was centrifuged at 8000 rpm for 10 min after the specified time intervals of incubation. The released lycopene in the supernatant was extracted and quantified by UPLC as per our previous report [36].

Bioaccessibility

Tomato puree (0.5g), LYZA and lycopene (8.3 µg/ml) disseminated in olive oil were exposed for *in vitro* digestion method as per Garrett *et al*., (1999) [32] with slight modification for lycopene bioaccessibility/micellisation measurement. Briefly, samples were homogenized in 32 mL HBSS. Followed acidification (pH. 2) with 0.04 g/mL in 0.1 mmol/L HCl porcine pepsin for 1hr at 37 ºC in an orbital shaking water bath at 120 strokes/min. Further, the pH increased to 5.3 by the addition of 0.8 mmol/L glycodeoxycholate, 0.75 mmol/L taurocholate, 0.45 mmol/L taurodeoxycholate, 0.08 g mL/L porcine pancreatine, and 1 U/ mL cholesterol esterase. The sample pH increased to 7.4 and incubated 2 h for intestinal digestion. This digest was further centrifuged at 194000 g for 60 min, and separated micelles. These micelles aspirated in syringe and filtered through a sterilized 0.2 µM cellulose acetate filter. The lycopene content in micelles was considered bio accessible and was quantified by UPLC [36].

Statistical Analysis

Values represent the mean ± SD of three samples. The data was analysed using ANOVA (GraphPad Prism 6. software). Tukey's test evaluated the differences between the treated and control groups. Significant differences between the experimental samples were considered at $P < 0.05$.

Results and Discussion

The chemical property and integrity of high pure lycopene isolated from ripened tomato was confirmed by its retention time, peak identity (λ_{max}), UV pattern, and MS and compared to reference standard. The UPLC profile demonstrated that the isolated lycopene from tomato was found to be high pure 93.3% pure (Fig. 1). The results were compared with standard lycopene. Among natural plant produce, carotenoids exist as geometrical isomers or are formed during food processing. Hence supercritical chromatographic techniques are involved in purifying high-quality lycopene and this process is laborious and expensive. In our present study combination of solvents used to obtain pure lycopene by simple steps and advanced UPLC-PDA-MS/MS was adapted for lycopene detection and quantification. The established method is a better tool to address genetic variability, environmental factors and edaphic properties on carotenoid profile [38, 39].

Lycopene extracted from tomato is very sensitive to light, heat, air, pH, and degradation in the gastrointestinal tract. Hence, combination with biocompatible biopolymers enhances the physical stability, solubility, functional properties, targeted delivery, and bioavailability of encapsulant [26]. Previously reports have shown encapsulation of lycopene by different biopolymers from plants and animals to increase its physical stability and bioavailability [21]. Several biopolymers are used for targeted nutrient delivery but their chemistry of interaction at the physiological level is limited. Among biopolymers corn derived, zein is most suitable for encapsulation, as it is recognized as safe by FDA and exhibit mucoadhesive nature [22]. Further, zein-alginate nanoparticles are biodegradable and biocompatible. This substitute delivery approach using plant derived biopolymer benefits over dietary supplementation of poorly bioavailable nutrient like lycopene. In the present study, we focus on plant-derived protein-polysaccharide complex like zein-alginate for the encapsulation of purified lycopene. LYZA prepared in this study was explored for desired particle size, morphology, and biochemistry as they are the determining factors of bioavailability of lycopene. LYZA synthesized by the anti-solvent method and electrostatic deposition yielded 90 ± 1% (LYZA) lycopene encapsulation efficiency.

The physicochemical properties- size, charge, PDI and viscosity of standard micellar lycopene, micelles obtained by simulated *in vitro* digestion of tomato and lycopene encapsulated zein-alginate nanoparticles are shown in Table 1. Particle size is vital characteristic for delivery vehicle such as micelles or nanoparticle, indicating the capability for efficient nutrient delivery. Particle size results showed significant difference in LYZA (195 ± 31 nm), LYMM (964 ± 40) and TMM (2191± 257). The particle size of the TMM was larger as compared to LYMM. Whereas, LYZA yielded significantly smaller nanoparticles than LMM (4.9-fold) and TMM (11.2-fold). This data suggests that encapsulation of lycopene in zein-alginate biopolymers resulted in smaller particle size. The PDI was better in LYZA (0.09±0.02) indicating narrow distribution of particle size than LMM (0.18± 0.15) and TMM (0.47± 0.07). Zeta potential is one more important characteristic indicating the stability of micelles or nanoparticles. Zeta potential values was found stable in LYZA (-30.4± 3 mV), than LYMM (-12.2± 0.1 mV) and TMM (-3.61± 0.1 mV). The LYZA zeta potential was 19 times lower than LYMM and 28.5 times lower than TMM. The viscosity of LYZA was lowest of LYMM and TMM. These physicochemical properties of desired smaller particle size, stable zeta potential, monodispersed and less viscous properties than LYMM and TMM indicate appropriate physicochemical properties.

The LYZA morphological characterizations are shown in Fig.2. The representation of particle size intensity showed the size range from 100-450 nm as confirmed by DLS (Fig. 2A). The zeta potential charge of LYZA -30.4± 3 mV indicates the particle stability and disaggregation (Fig. 2B).The apparent viscosity of zein-alginate nanoparticle (ZA) and LYZA decreased as the shear rate increased. This tendency was obvious at shear rates below 10 s^{-1} (Fig. 2C). XRD is a dynamic tool to inspect the physical state of the lycopene in zein-alginate biopolymer matrix. Encapsulated drugs/nutrients can be in amorphous form or crystalline state that depends upon drug's/nutrient's properties and its interaction with the biopolymer matrix. XRD diffractogram revealed that lycopene present in an amorphous /disordered state in zein-alginate biopolymer matrix (Fig. 2D). Further, the absence of a crystalline peak in the LYZA designates and confirms these nanoparticles were in amorphous form. This could be a major reason for the dispersibility and bioavailability of lycopene from LYZA compared to micellar lycopene [34]. The LYZA nanoparticles yielded spherical with smooth surface area, as documented by TEM micrographs (Fig. 2E). The AFM images demonstrate the lycopene encapsulation in zein-alginate biopolymer spherical in shape and confirm nanometer in size formed are in the range from 80 to 600 nm (Fig. 2F).The FTIR spectra of LYZA are showed in Fig. 2G. Comparison between the nanoparticles revealed the shift in wavenumber at 3285, 2930, 1643, 1514 cm−1, 1238 cm−1 and 1031 cm−1. The peak identities of interaction between zein, alginate, and lycopene are documented. The peaks at 1028 cm^{-1} assigned to a trans CH deformation vibration, 3285 cm^{-1} assigned to CH (sp2), 2960 cm^{-1} corresponds to C-H (sp3) stretch, other bands occur at (1620- 1680) -C=C stretching vibration, C-H bends alkene confirmed the presence of lycopene in LYZA.

Lycopene release at gastric and intestinal pH from LYZA is shown in Fig. 3. The release of lycopene from LYZA was 15% at 6 h and 54% in 42 h time point in gastric pH (pH 2). In contrast, the release rate of lycopene was significantly higher at pH 7.4 in lycopene encapsulated zein-alginate nanoparticles. Lycopene release at intestinal pH (pH 7.4) from LYZA was 10% at 6 h and 71.8% in 42 h time point. The inhibition of release at acidic condition and sustain controlled release at intestinal pH of lycopene from zein-alginate complex could be the reason for higher bioaccessibility of lycopene.

Images of light microscopy images (40X) of LYMM (Fig 4A) and micelles obtained from *in vitro* digestion of tomato (Fig 4B) exhibited spherical shape. The bioaccessibility by *in vitro* digestion of tomato, LYMM and LYZA are shown in Fig. 4C. The stimulated gastric and intestinal digestion showed lycopene percentage micellization after the digestion process of LYZA obtained was significantly higher by 45% in LYMM and 51.9 % in TMM. The bioaccessibility revealed lycopene was more accessible from LYZA as compared to combination *in vitro* digested tomato and micellar lycopene. Many research groups measured the bio accessible lycopene content from tomato and other sources [16]. Few reports also discussed the strategies to improve lycopene bioaccessibility like thermal processing and synthesizing excipient emulsions [40, 41]. But bioaccessibility from encapsulated bioactives are always found higher as they protect the encapsulant's form and properties in natural biomaterials.

Decreased particle size (136 ± 31 nm), stable zeta potential (-30. ± 3 mV), PDI (0.15 ±0.2), amorphous form and inhibition of release at acidic condition and sustain controlled release at intestinal pH of lycopene from zein-alginate complex could be the reason for higher micellization after simulated digestion. This could also lead to higher transport of lycopene across the intestinal epithelial cells leading to higher bioavailability and tissue distribution.

Table 1: Physical properties of LYMM, TMM vs. LYZA.

Samples	Particle size (nm)	PDI	Zeta potential (mV)	Viscosity (Pa.s)
LYMM[#]	964 ± 40[a]	0.18±0.15[a]	-12.2± 0.1[a]	0.007±0.02
TMM[$]	2191± 257[b]	0.47±0.07[b]	-3.61± 0.1[b]	0.006±0.01
LYZA	195± 31[c]	0.09±0.02[c]	-30.4± 3[c]	0.005±0.02

[#]Lycopene mixed micelles prepared using standard chemicals.
[$]Lycopene mixed micelles derived from *in vitro* digestion process of tomatoes.

Values are mean of ±SD of three samples. Values not sharing common superscript letters are significantly ($p< 0.05$) different as analysed by one-way ANOVA followed by Turkey's test.

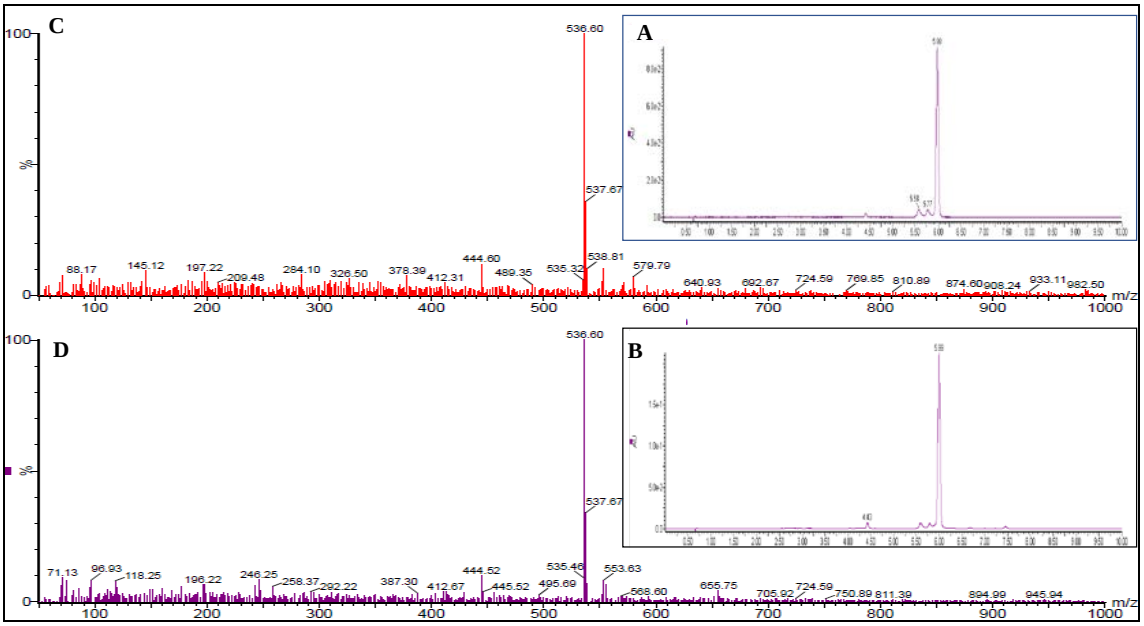

Fig 1: UPLC profile of standard lycopene (A) and high pure lycopene isolated from ripened tomato (B). APCI^{+ve} Tandem Mass spectra profile of commercial standard lycopene (C) and high pure lycopene isolated from ripened tomato (D).

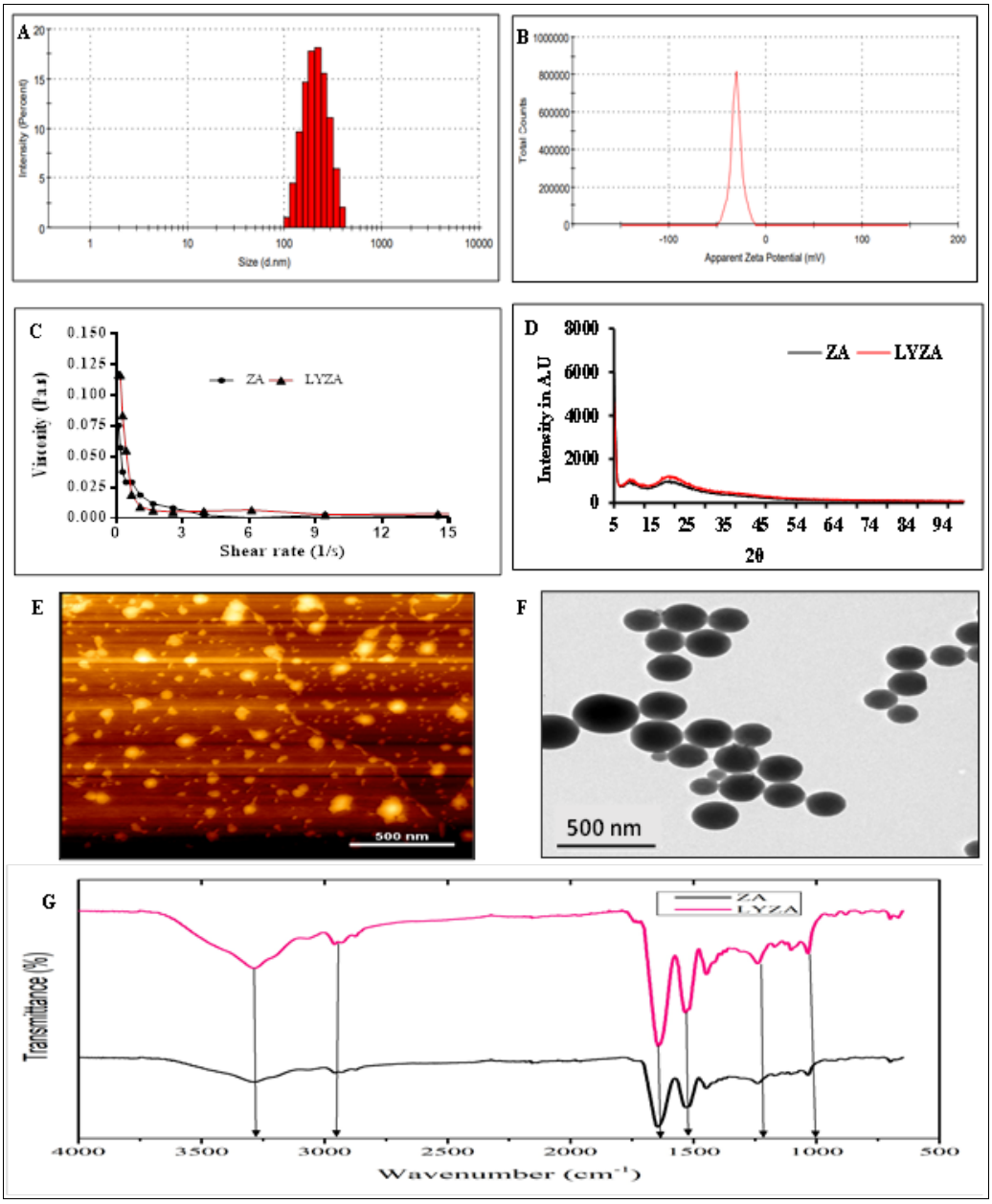

Fig 2: Particle size (A), zeta potential (B), viscosity (C), XRD (D), AFM (E), TEM (F) and FTIR spectral patterns (G) of LYZA nanoparticles.

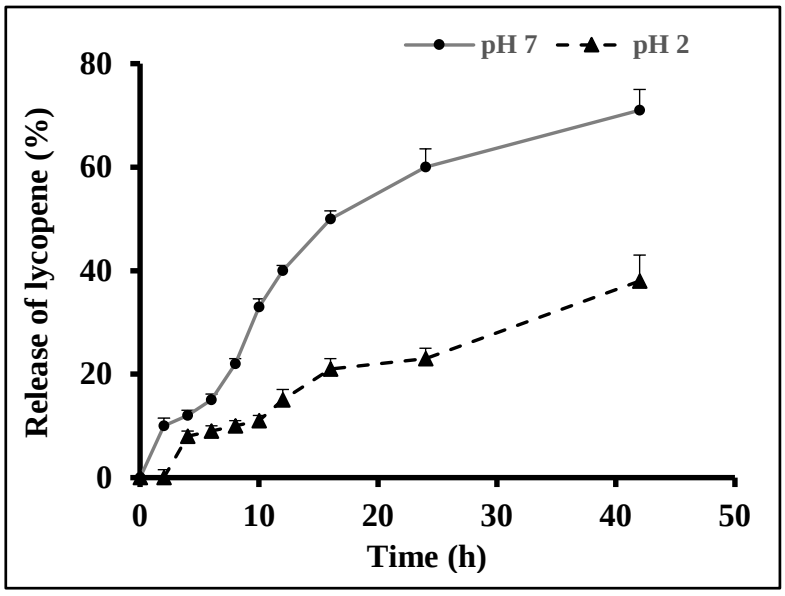

Fig 3: *In vitro* release kinetics of lycopene from LYZA at gastric (pH 2) and intestinal (pH 7) conditions.

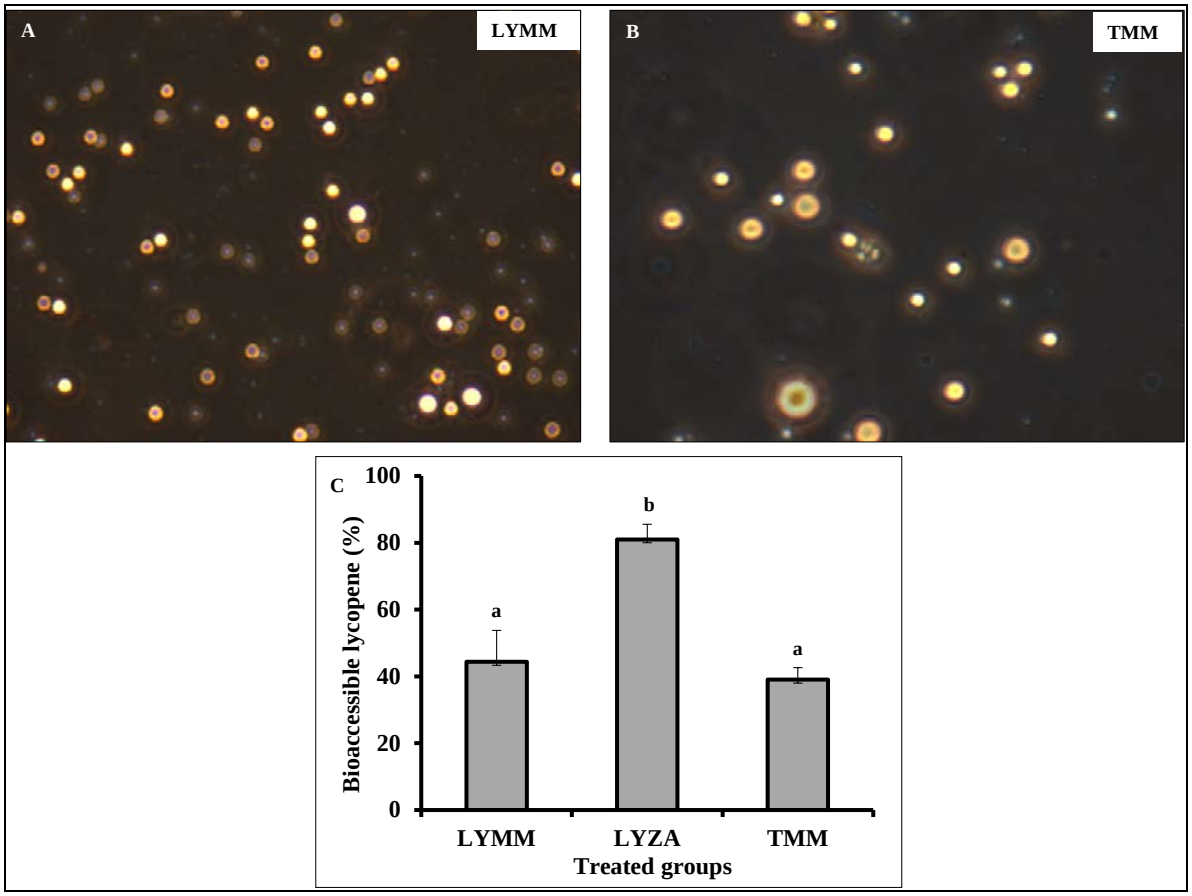

Fig 4: Microscopic images (40×) of LYMM (A) and TMM (B) Percent bioaccessibility of lycopene after simulated digestion process of tomato (TMM), LYMM and LYZA. Values are mean of ±SD of three samples. Values not sharing common superscript letters are significantly ($p < 0.05$) different as analysed by one-way ANOVA followed by Turkey's test (C).

Conclusion

Isolation of high pure lycopene in its native form is tough due to its vulnerable chemical structure which leads to low solubility, stability, and bioavailability. Further the isolated lycopene is highly suspectable for isomerization/degradation when exposed to environmental and physiological conditions. Hence formulation of nanopreparations from plant derived zein protein is best strategy to protect its native form. The lycopene-zein-alginate nanoparticles prepared found to overcome the downsides of pure lycopene. LYZA displayed appropriate physicochemical paraments like size, shape, lycopene encapsulation efficiency, release kinetics and increased bioaccessibility. These desired properties may be due to the encapsulation of lycopene in natural biopolymers zein and alginate. Further, this study demonstrated importance of analytical techniques for purification of potent nutraceutical lycopene, and sensitivity of method helps to determine the plant physiological changes, modification or improvement of fruit/vegetable quality and assessment of nutritive value of organically grown food respect to high value nutrients /phytochemicals. Also using botanical sources provide wide scope for agricultural products and adaptation to nutraceutical supplements/ food biofortification to eradicate malnutrition and related deficiency health problem.

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References

1. Britton G. Structure and properties of carotenoids in relation to function. FASEB J. 1995; 9:1551-1558.
2. Goodwin TW. Biogeochemistry of Carotenoids. In the Biochemistry of the Carotenoids, 1980, 346-349.
3. Clinton SK. Lycopene: Chemistry, biology, and implications for human health and disease. Nutr. Rev. 1998; 56:35-51.
4. Rao AV, Agarwal S. Role of antioxidant lycopene in cancer and heart disease. J American College Nutr. 2000; 19:563-569.
5. Nahum A, Hirsch K, Danilenko M, Watts CK, Prall OW, Levy J *et al*. Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27 Kip1 in the cyclin E-cdk2 complexes. Oncogene. 2001; 20:3428-3436.
6. Etminan M, Takkouche B, Caamaño-Isorna F. The role of tomato products and lycopene in the prevention of prostate cancer: a meta-analysis of observational studies. Cancer Epidemiol. Biomarkers Prev. 2004; 13:340-345.
7. Mascio P DI, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys. 1989; 274:532-8.

8. Anguelova T, Warthesen J. Lycopene Stability in Tomato Powders. 2000; 65:67-70.
9. Riahi A, Hdider C. Bioactive compounds and antioxidant activity of organically grown tomato (*Solanum lycopersicum* L.) cultivars as affected by fertilization. Sci. Hort. 2013, 90-96.
10. Ilahy R, Hdider C, Lenucci MS, Tlili I, Dalessandro G. Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. J. Food Composit. Anal. 2011; 24:588-595.
11. Ilahy R, Siddiqui MW, Tlili I, Montefusco A, Piro G, Hdider C *et al*. When color really matters: horticultural performance and functional quality of high-lycopene tomatoes. CRC Crit. Rev. Plant Sci. 2018; 37:15-53.
12. John Shi, Laura-Mac Naughton, Yukio Kakuda, William Bettger. Bioavailability of lycopene from Tomato Products. Prev. Nutr. Food Sci, 2004, 9.
13. Stahl W, Sies H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. J Nutr. 1992; 122(11):2161-6.
14. Van het Hof KH, West CE, Weststrate JA, Hautvast JG. Dietary factors that affect the bio availability of carotenoids. J. Nutr. 2000; 130:503-506.
15. Yonekura L, Nagao A. Intestinal absorption of dietary carotenoids. Mol. Nutr. Food Res. 2007; 51:107-115.
16. Isabel Goñi, José Serrano, Fulgencio Saura-Calixto. Bioaccessibility of β-Carotene, lutein, and lycopene from fruits and vegetables. Agric. Food Chem. 2006; 54(15):5382-5387.
17. Lu Y, Mu K, McClements DJ, Liang X, Liu X, Liu F *et al*. Fermentation of tomato juice improves *in vitro* bioaccessibility of lycopene. J. Funct. Foods. 2020; 71:104-020.
18. Shukla R, Cheriyan M. Zein: the industrial protein from corn. Ind. Crop Prod. 2001; 13:171-92.
19. Lawton JW. Zein: a history of processing and use. Cereal Chem. 2002; 79:1-18.
20. Liu X, Sun Q, Wang H, Zhang L, Wang JY. Microspheres of corn protein, zein, for an ivermectin drug delivery system. Biomaterials. 2005; 26:109-15.
21. Hu D, Lin C, Liu L, Li S, Zhao Y. Preparation, characterization, and *in vitro* release investigation of lutein/zein nanoparticles via solution enhanced dispersion by supercritical fluids. J. Food Eng. 2012; 109:545-552.
22. Patel A, Hu Y, Tiwari JK, Velikov KP. Synthesis and characterisation of zein–curcumin colloidal particles. Soft Matter. 2010; 6:6192-6199.
23. Dai L, Li R, Wei Y, Sun C, Mao L, Gao Y *et al*. Fabrication of zein and rhamnolipid complex nanoparticles to enhance the stability and *in vitro* release of curcumin. Food Hydrocoll. 2018; 77:617-628.
24. Sun C, Wei Y, Li R, Dai L, Gao Y. Quercetagetin Loaded Zein-Propylene Glycol Alginate Ternary Composite Particles Induced by Calcium Ions: Structure Characterization and Formation Mechanism. J. Agric. Food Chem. 2017; 65:3934-3945.
25. Wang M, Fu Y, Shi Y, Chen G, Li X, Zhang H. *et al*. Fabrication and characterization of carboxymethyl chitosan and tea polyphenols coating on zein nanoparticles to encapsulate β-carotene by anti-solvent precipitation method. Food Hydrocoll. 2017; 77:577-587.
26. Wei Y, Sun C, Dai L, Zhan X, Gao Y. Structure, physicochemical stability and *in vitro* simulated gastrointestinal digestion properties of β-carotene loaded zein-propylene glycol alginate composite nanoparticles fabricated by emulsification-evaporation method, Food Hydrocoll. 2018; 81:149-158.
27. Kim BK, Kim JC, Kim MH, Park CE, Lee EJ. Physicochemical and release properties of retinol-loaded zein chitosan nanoparticles. FASEB J. 2013; 27(1):251.
28. Li KK, Yin SW, Yang XQ, Tang CH, Wei ZH. Fabrication and characterization of novel antimicrobial films derived from thymol-loaded zein–sodium caseinate (SC) nanoparticles. J. Agric. Food Chem., 2012; 60:11592-11600.
29. Draget KI, Skjåk-Bræk G, Smidsrød O. Alginate based new materials. Int. J. Biol. Macromol. 1997; 21:47-55.
30. Reis CP, Neufeld RJ, Vilela S, Ribeiro AJ, Veiga F. Review and current status of emulsion/dispersion technology using an internal gelation process for the design of alginate particles. J Microencapsul. 2006; 23:245-57.
31. Wee S, Gombotz WR. Protein release from alginate matrices. Adv. Drug Deliv. Rev. 1998; 31(3):267-85.
32. Garrett DA, Failla ML, Sarama RJ, Craft N. Accumulation and retention of micellar b-carotene and lutein by Caco-2 human intestinal cells, J. Nutr. Biochem. 1999; 10:573-581.
33. Reboul E, Borel P. Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes. Prog. Lipid Res. 2011; 50(4):388-402.
34. Shwetha HJ, Shilpa S, Mousumi BM, Ambedkar R, Raichur AM, Lakshminarayana R. Fabrication of chitosan nanoparticles with phosphatidylcholine for improved sustain release, basolateral secretion, and transport of lutein in Caco-2 cells. Int. J. Biol. Macromol. 2020; 163:2224-2235.
35. Tyssandier V, Bernard Lyan, Patrick Borel. Main Factors Governing the Transfer of Carotenoids from Emulsion Lipid Droplets to Micelles. Biochimica Biophysica Acta. 1533:285-92
36. Arathi BP, Sowmya PR, Vijay K, Dilshad P, Saikat B, Gopal P, Lakshminarayana R An improved method of UPLC-PDA-MS/MS analysis of lycopene isomers. Food Anal Method. 2014; 8:1962-1969.
37. Kun Hu, David Julian McClements. Fabrication of biopolymer nanoparticles by antisolvent precipitation and electrostatic deposition: Zein-alginate core/shell nanoparticles. Food Hydrocoll. 2015; 44:101-108.
38. Barba AO, Hurtado MC, Mata MS, Ruiz VF, De Tejada ML. Application of a UV–vis detection-HPLC method for a rapid determination of lycopene and β-carotene in vegetables. Food Chem. 2006; 95:328-36.
39. Shwetha HJ, Shilpa S, Arathi BP, Raju M, Lakshminarayana R. Bio fortification of carotenoids in agricultural and horticultural crops: A promising strategy to target vitamin a malnutrition. Vitamins and minerals bio fortification of edible plants. Wiley's Publisher, New Jersey, USA, 2020, 123-161.
40. Salvia-Trujillo L, McClements DJ. Enhancement of lycopene bioaccessibility from tomato juice using

excipient emulsions: Influence of lipid droplet size. Food Chem. 2016; 210:295-304.
41. Jayathunge KG, Stratakos AC, Cregenzán-Albertia O, Grant IR, Lyng J, Koidis A. *et al*. Enhancing the lycopene *in vitro* bioaccessibility of tomato juice synergistically applying thermal and non-thermal processing technologies. Food Chem. 2017; 221:698-705.