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ORIGINAL ARTICLE

Compositions for health products obtained by treatment of tomato with beta-cyclodextrin

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Abstract The aim of this work was to produce lycopene-containing powders from tomato products by a solvent-free method making use of β-cyclodextrin (βCD). Powders were prepared by spray-drying a tomato concentrate (TC), one of the most bioavailable form of lycopene, after mechanical treatment with βCD in different weight ratios. The obtained product was centrifuged to eliminate partly food matrix and characterized for the amount of lycopene hydrodispersed/hydrosolubilized in the aqueous fraction. The chemical antioxidant activity of sera was evaluated too. Powders obtained by spray-drying sera exhibited good flow properties, a lycopene content between 0.4 and 1.09 mg/g and excellent water dispersability. The process developed, which makes use of βCD for the treatment of tomato products, turns to be of great interest to obtain a bulk material for nutraceuticals displaying superior biovailability of lycopene.

Keywords Lycopene · β-cyclodextrin · Spray-drying · Tomato powders

Introduction

Several beneficial effects for human health have been attributed to diet supplementation with lycopene such as protection against cardiovascular risk, prostate cancer and neurodegenerative diseases [1]. The main source of lycopene is tomato, which contains also a number of compounds with a recognized activity as radical scavengers. As a polyene, lycopene undergoes *cis-trans* isomerization induced by light, thermal energy or chemical reactions [2]. In raw tomatoes, all-*trans* is the predominant isomeric form of lycopene but isomerization to *cis* occours during cooking, food processing or storage [3, 4]. Lycopene is highly lipophilic and absorbed in the GI tract after solubilization in bile salt micelles *via* passive diffusion [5]. Although *trans*-lycopene constitutes the predominant isomer in food sources, in human plasma 50% of the lycopene has been found as *cis* isomers [6]. Whether this is due to in vivo isomerization or preferential absorption of *cis*-lycopene is still unclear. However, a number of studies suggest that *cis*-isomers are more bioavailable than *trans*-form probably because *cis*-isomers are more soluble in bile salts micelles [7], may be preferentially incorporated in chylomicrons [8] and display a lower tendency to crystallize. Thus, lycopene solubility in GI contents plays a critical role in determining its oral bioavailability. Furthermore, lycopene from heat-processed food is more bioavailable than from fresh tomatoes, probably due to its prompter release from the matrix and heat-induced isomerization from all *trans* to *cis* conformation [6].

Synthetic lycopene is a red crystalline powder that is soluble in most organic solvents, but is insoluble in water. It is sensitive to light and oxygen and hardly suitable for commercial use. Powdered tomato extracts employed in nutraceutical products contain 5–10% of lycopene, are obtained by extraction of fresh tomato with different organic solvents and added with inert excipients such as maltodextrins. The use of organic

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solvents is needed to increase the amount of extracted lycopene, which is highly lipophilic and entrapped in the food matrix where it takes the form of elongated, needle-like crystals [1]. A branded form of powdered lycopene (lactolycopene®) has been recently developed and introduced in the formula of some nutraceuticals [9]. This composition consists in lycopene from a tomato oleoresin embedded in a whey protein matrix and is produced by aid of extraction solvents such as acetone, isopropanol or ethanol [9]. This compound displayed the same bioavailability of a tomato paste when administered to humans [10].

Another approach to improve the solubility of carotenoids has concerned the use of cyclodextrins (CD). It has been demonstrated that a number of CD can form complexes with lycopene in aqueous media through inclusion and non-inclusion mechanisms [11, 12]. Methyl-βCD has been successfully employed to improve aqueous solubility of different carotenoids and found to give solid complexes with superior biological availability and excellent stability when compared to other application forms such as organic solvents, mixed micelles, liposomes or beadlets [13]. Thus our working hypothesis was that CD could be useful to promote lycopene extraction from the food matrix without the use of organic solvents and provide lycopene hydrodispersed in an aqueous medium from which powders could be easily prepared by drying.

The aim of this work was to prove this hypothesis and produce tomato powders useful as bulk materials to prepare oral nutraceuticals displaying superior biovailability of lycopene. Powders were prepared from tomato concentrate (TC), one of the most bioavailable form of lycopene, by treatment with βCD, widely employed in the food and pharmaceutical industry due to its safety profile and limited cost.

Experimental

Materials

Tomato concentrate was obtained from a commercial source. β-cyclodextrin was a kind gift of Roquette Frères (France). HPLC-grade tetrahydrofuran (THF) and methanol, analysis-grade diethyl ether and potassium persulfate were purchased from Carlo Erba (Italy). ABTS [2,2′′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] and Trolox [(±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] were obtained from Sigma-Aldrich (USA). Milli Q water was used for the study.

Extraction of lycopene from fresh tomato

Extraction of lycopene from fresh tomatos (*lycopersicon esculentum*, cherry tomatoes) was accomplished by the method of Ishida et al. [14]. Crystalline lycopene was kept under nitrogen at –20°C and used as reference standard.

HPLC analysis of lycopene

The quantitative analysis of lycopene was performed by HPLC on a Shimadzu system equipped with a Phenosphere Next C_{18} column 5 μ (250 × 4.6 mm) (Phenomenex, USA) using as mobile phase a methanol/tetrahydrofuran mixture (80:20 v/v) run at 1 mL/min. Linearity of response was obtained in the concentration range 0.2–4 mg/mL. Peaks assignment of lycopene isomers was carried out according to Ishida et al. [14] on a YMC pack C_{30} column 5 μ (250 × 4.6 mm) (YMC, Japan) for carotenoids using as eluent a mixture ethyl acetate/methanol/methyl tert-butyl ether (10/50/40 v/v/v). For both methods flow rate was 1 mL/min and wavelength set at 472 nm.

Treatment of tomato concentrate with βCD

Tomato concentrate (100 g) was treated with βCD (15 and 50 g corresponding to TC/βCD ratios of 1:0.15 and 1:0.5 by wt) and diluted with water up to a final weight of 200 g. The sample was treated in an electric mortar for 30 min. The kneaded product was centrifuged at 5,000 rpm for 15 min to separate supernatant (serum) from pellets. As control, the same procedure on TC was performed without βCD.

Characterization of sera collected from TC treated with βCD

Centrifuged sera were collected and characterized for the amount of ''hydrodispersed lycopene'' as follows. Serum (0.1 mL) was diluted with water (0.9 mL) and completely extracted with diethyl ether (1 mL). The organic fractions were collected, dried under a nitrogen stream and the solid reconstituted in 0.75 mL of tetrahydrofuran. Twenty microlitres of the lipophilic extract were analysed by the HPLC method reported above to quantify lycopene. Centrifuged sera were characterized for the amount of ''solubilized lycopene'' after filtering 2 mL through 0.45 μm filters (RC, Chemtek, Italy). Lycopene was completely extracted from the aqueous solution (1 mL) with diethyl ether (1 mL) as reported for serum. Twenty microlitres of

the lipophilic extract were analysed by the HPLC method reported above to quantify lycopene.

Chemical antioxidant activity of sera was evaluated by an assay based on scavenging of long-lived radical anions $ABTS^{\bullet+}$. ABTS was dissolved in water (7 mM) and transformed in the radical cation by adding potassium persulfate (2.45 mM final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. $ABTS^{\bullet+}$was stable in this form for more than 2 days when stored in the dark at room temperature. The absorbance of the $ABTS^{\bullet+}$ solution at 734 nm was adjusted at 0.8 OD by dilution with a 5 mM phosphate buffer at pH 7.4 [15]. A 10 μL of the lipophilic extract in THF was added to 1.0 mL of $ABTS^{\bullet+}$ diluted solution and absorbance recorded after 1 min. Results are expressed as percentage of inhibition ($Inh\%$) according to

$$Inh\% = 1 - \frac{ABS_f}{ABS_i}$$

where ABS_f and ABS_i are sample absorbances after and before the addition of lipophilic extract containing antioxidants.

Lypophilic antioxidant activity was compared to that of the reference water-soluble vitamin E analogue Trolox and expressed as TEAC (Trolox Equivalent Antioxidant Capacity). Trolox standard solutions were prepared in methanol and their corresponding $Inh\%$ evaluated according to the method reported above. Linearity of response was verified in the range of Trolox concentrations 0.08–4 mM.

Preparation and characterization of tomato powders

Uncentrifuged or centrifuged TC treated products were spray-dried in a Buchi 190 system (Buchi, Switzerland) equipped with a 0.5 mm nozzle setting the following process parameters: feed rate 5 mL/min; aspirator setting 9; spray-flow 600 Nl/h; inlet temperature 152°C. Tomato/βCD powders were characterized for lycopene and cyclodextrin content. To this end, 25 mg of powders were suspended in 1 mL of water and lycopene extracted as reported for serum. Twenty microlitres of the lipophilic extract were analysed by the HPLC method reported above to quantify lycopene. Lycopene titre of the powders was evaluated also after storage at –20°C, 4°C and room temperature. To quantify βCD in tomato powders, an UV-colorimetric assay was used [16]. In this assay the fading of a phenolphtaleine solution due to the formation of a complex with βCD was evaluated. Tomato powder (25 mg) was suspended in 1 mL of water filtered through 0.45 μm filters, diluted 1:100 v/v with water, added to a phenolphtaleine solution and analysed on a UV–VIS spectrophotometer (Shimadzu UV-1204) at the wavelength of 553 nm. Linearity of response was obtained in the concentration range 0.02–0.38 mg/mL.

Results and discussion

As bulk material to produce tomato powders we used a TC (containing about 90% of water as evaluated after TC dehydration) available on the market, since it contains good amount of lycopene in a high bioavailable form. TC was treated as reported in Fig. 1. Processing consisted in kneading TC with different amounts of CD in an electric mortar. The final weight of treated TC was adjusted with water to 200 g in order to compare the results for all the samples. Then the kneaded product was centrifuged to separate the solid pellets from the serum. Powders were produced from the serum or serum + pellets by a following spray drying step. The process was structured as above because a fine grinding in the presence of CD could be expected to increase the bioavailability of lycopene by disrupting or softening plant cell walls and release lycopene from complex with proteins [1]. Furthermore, the use of CD could allow a better extraction of lycopene from the complex food matrix due to solubilization of lycopene in the serum where it could be maintained in its soluble form.

As a first step, we analysed the chromatograms of the lipophilic extracts obtained from sera after the TC treatment described above with or without βCD (Fig. 2). As it can be seen, two main signals are present, one with the highest intensity eluting at 11 min,

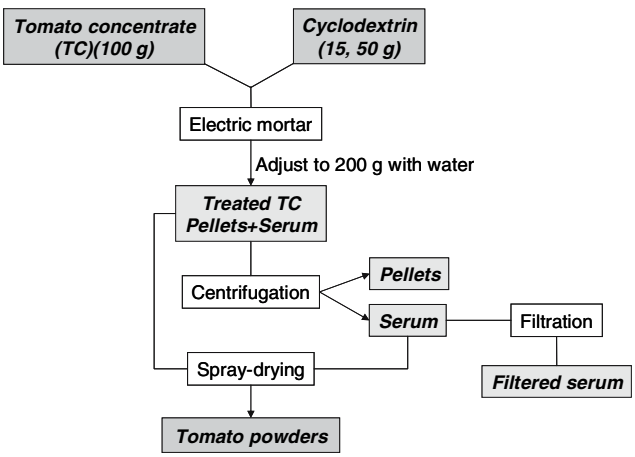

Fig. 1 Schematic representation of the treatment of tomato concentrate with βCD

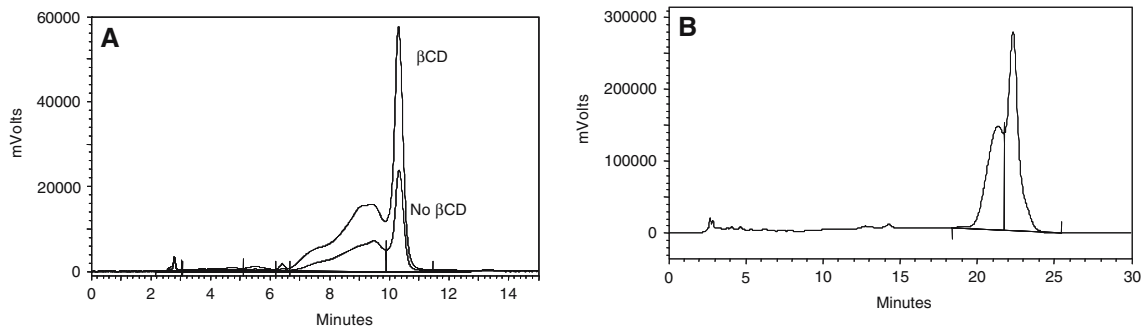

Fig. 2 Chromatograms of lipophilic extract of sera obtained by treatment of TC with or without *β*CD (1:0.5 by weight) according to the method reported in Fig. 1 on C_{18} (**A**) and C_{30} (**B**) columns (conditions described under materials and methods)

likely corresponding to all-*trans* lycopene and a second very broad one at about 9 min, probably attributable to a *cis*-isomer of lycopene. Peak assignment was confirmed by HPLC on a C_{30} column especially useful for carotenoid separation by employing the method developed by Ishida et al. [13]. According to their results, the first peak (at about 21 min) was attributed to all-*trans* lycopene, the second one (at about 22 min) at a *cis*-isomer of lycopene. The analysis also highlighted that the amount of hydrodispersed lycopene in serum highly increases when TC was treated with *β*CD.

The following step consisted in evaluating if the ratio between the starting materials (TC and *β*CD) could affect the amount of lycopene hydrodispersed/hydrosolubilized in sera. The amount of lycopene present in sera (hydrodispersed lycopene) (Fig. 3A) and filtered sera (hydrosolubilized lycopene) (Fig. 3B), obtained after treatment of TC with different amounts of *β*CD, was much lower than expected considering the content initially present in TC (42 mg of lycopene *per* 100 g of TC on a wet basis). This demonstrated that lycopene in TC is still entrapped in the food matrix (pellets) where it is present mainly in crystalline form. However, we found that the content of lycopene in the serum increased as compared to TC grinded without *β*CD and depended on the amount of *β*CD used for the treatment. An increase in the TC/*β*CD ratio up to 1:0.5 by wt (1:5 on a dry basis) was useful to obtain a progressive increase in the amount of hydrodyspersed lycopene. In parallel, the amount of lycopene in filtered serum, namely solubilized in the aqueous medium, progressively increased as *β*CD concentration used to treat TC did. This result well demonstrated that, besides a mechanical effect exerted by *β*CD on the food matrix which may contribute to promote lycopene capability to transfer in the aqueous medium, the complexation of lycopene by *β*CD is involved. Nevertheless, the amount of lycopene in aqueous suspension was significantly higher than that found in aqueous solution suggesting that lycopene in the serum is still entrapped in the food matrix (pellets).

Lycopene amount in sera was also evaluated after 2 weeks of storage at –20°C. In fact, lycopene can undergo degradation/isomerization as a function of temperature and light exposure [1, 17]. The results, shown in Fig. 3A, demonstrate that the amount of all-*trans* lycopene slightly decreases only at the highest TC/*β*CD ratio. Furthermore, no peak other than those present at time 0 in the chromatogram of lipophilic extracts was present. These results suggested also that more than an oxidation of lycopene in solution, a precipitation of lycopene in the sample occurs due to its very low

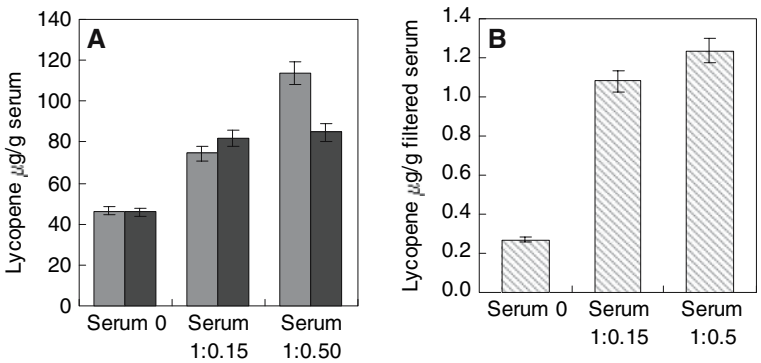

Fig. 3 Effect of TC treatment with *β*CD at different weight ratios on the amount of lycopene extracted from sera (**A**) and filtered sera (**B**). Panel A: lycopene content of sera immediately after TC treatment (light grey) and after 2 weeks of storage at –20°C (dark grey)

solubility in sera. Therefore, it is of fundamental importance that the sera are processed immediately after their preparation when high TC/βCD ratios are employed.

Chemical antioxidant activity of the lipophilic fraction containing lycopene extracted from sera after TC treatment is reported in Fig. 4. As it can be seen, sera displayed TEAC progressively higher as the amount of βCD used for TC processing increased. Furthermore, a direct relationship was found between TEAC and lycopene content of sera.

To produce tomato powders from treated TC, a spray-drying technique was selected. In fact spray-drying is a fast single-step process widely employed in pharmaceutical and food industry for the large scale drying of fragile molecules due to the use of mild conditions. As explained before, sera were dispersion of a hydrated solid in an aqueous medium. To understand if sera could be employed to obtain tomato powders by spray-drying, and thus pass through the 0.5 mm nozzle, a preliminary assessment of particle dimension was carried out. Size distribution of untreated TC and sera obtained after TC treatment with βCD at different ratios (Fig. 5) showed that sample is monodispersed and mean particle size is around 19 μm and unchanged upon βCD treatment. Therefore, particle size of the dispersed phase was compatible with the spray-drying process. Since we observed that a high amount of lycopene was still entrapped in the solid matrix, tomato powders were prepared by spray-drying the centrifuged and uncentrifuged TC (serum and serum + pellets, respectively) after treatment with βCD at 1:0.15 and 1:0.5 weight ratios (Table 1). When centrifuged or uncentrifuged TC were spray dried, a sticky powder was obtained whereas βCD treatment of

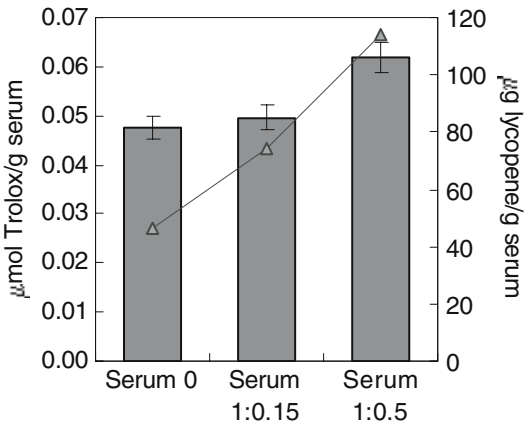

Fig. 4 Chemical antioxidant activity (bars) and lycopene content (triangles) of sera after TC treatment with βCD at different weight ratios

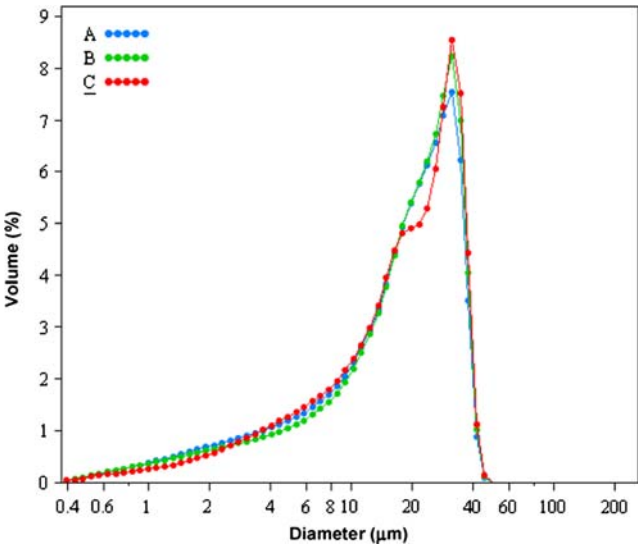

Fig. 5 Mean size distribution of sera produced by TC treatment with βCD at 1:0.15 by weight (**A**) and 1:0.5 by weight (**B**). Untreated TC suspended in water (**C**)

Table 1 Some properties of tomato powders prepared by spray-drying

	TC (g)	βCD (g)	Yield (%)	Lycopene mg/g	βCD mg/g
Powder 15S (serum)	100	15	91	0.47 ± 0.09	42 ± 3
Powder 50S (serum)	100	50	77	0.61 ± 0.11	127 ± 8
Powder 15SP (serum + pellets)	100	15	80	1.09 ± 0.19	169 ± 12
Powder 50SP (serum + pellets)	100	50	63	0.61 ± 0.12	325 ± 24

TC gave powders with good flow properties. A SEM micrograph of powder 15S (Fig. 6) clearly shows that it consists in regular, non-aggregated smooth particles. The good characteristics of the powder are also responsible for the good yield of the spray-drying process.

Lycopene content of the different powders was then evaluated and reported in Table 1. As it can be seen, lycopene content of the powders obtained from centrifuged serum increased as the amount of βCD used for the treatment increases. An opposite trend was observed for powders prepared from uncentrifuged treated TC (serum + pellets) where lycopene content decreased as the amount of βCD increased. We also quantified the amount of βCD present in the powder, which increased as its amount used for TC treatment did. We found that the amount of βCD in powders from uncentrifuged treated TC (SP powders) was higher than that found in centrifuged treated TC (S powders) confirming that βCD is only partly solu-

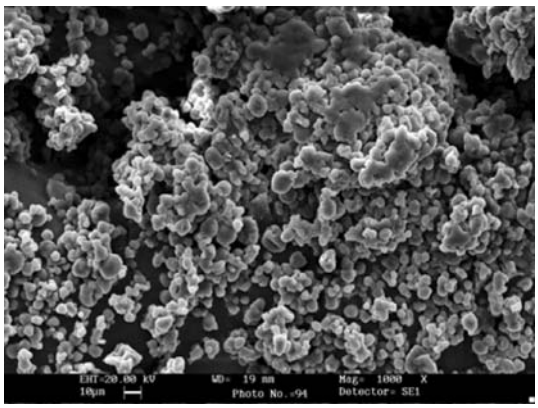

Fig. 6 SEM micrograph of powder 15S obtained by spray-drying the serum obtained from TC treatment with βCD at 1:0.15 ratio by weight

ble in the aqueous medium upon TC treatment. Furthermore, the slight increase in lycopene titre of powder 50S as compared to powder 15S is well correlated to the higher amount of hydrodispersed/hydrosolubilized lycopene in the serum (Fig. 3A and B). Lycopene content of powders prepared from the uncentrifuged treated TC was in the same order of magnitude of powders prepared from sera although these powders contained the dry pellets where the highest amount of lycopene was present. This result can be explained considering that powders of the SP series contain the highest content *per* gram of βCD and are therefore more diluted since all βCD used for the treatment is present in the spray-dried powders.

Conclusion

βCD was found to be an interesting additive for the treatment of tomato products. It was shown that the treatment of TC with βCD is useful to increase the fraction of hydrodispersed/hydrosolubilized lycopene and obtain powders suitable to produce oral solid dosage forms.

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