



In Vitro Bioaccessibility Assessment of Lycopene in Raw Tomato and Kuwaiti Tomato Sauce (Daqous)

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Abstract:

The objective of this study is to assess lycopene bioaccessibility in raw tomato and Kuwaiti tomato sauce (Daqous) with or without olive oil by using *in vitro* digestion model. Lycopene contents of raw tomato, tomato sauce, tomato sauce added olive oil, digestion-simulated and micelle-simulated samples were quantified using high performance liquid chromatography (HPLC). Lowest lycopene contents among the micelles were observed in fresh tomato samples ($2.5\mu\text{g/g}$). Lycopene contents of tomato sauce (Daqous) were $10.2\mu\text{g/g}$ while the samples of Daqous added olive oil had $16.5\mu\text{g/g}$ lycopene ($p < 0.05$). Cooking increased the bioaccessibility of lycopene to 21%. Addition of olive oil to the cooked tomato further increased lycopene's bioaccessibility to 34%. In conclusion, the addition of olive oil in the preparation of Kuwaiti tomato sauce (Daqous) significantly increased the bioaccessibility of lycopene.

Introduction

Carotenoids exhibit diverse metabolic effects and diets rich in carotenoids are associated with reduced risk of chronic diseases. An antioxidant carotenoid, lycopene, found in the reddish fruits and vegetables including tomatoes, watermelon, pink grape fruit, guava and papaya, is

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implicated for anti-carcinogenic properties and curative efficacies in cardiovascular diseases, neurological disorders, hypertension, osteoporosis, male infertility and many other complications (Bose and Agarwal 2007; Gartner et al. 1997; Donaldson 2004; Kong et al. 2010; Kim et al. 2011).

As tomato provide about 85% of lycopene in the diet, tomato and tomato products such as ketchup, tomato paste, and tomato sauce are considered to be the richest sources of lycopene in food. Skin of tomato contains higher contents of lycopene than the pulp (Hedges and Lister 2005). Contents of lycopene in the various fruits range from 0.005 to 7.2 mg/100g that can be enhanced by food processing to bring it in the range of 3.7 to 13.4 g/100g (Chauhan et al. 2011). Lycopene is mainly found in the chromoplasts and its synthesis increases several folds during fruit ripening (Singh and Goyal 2008). Tomato processing enhances the bioavailability of lycopene. Olive oil addition to diced tomato during cooking significantly increases bioavailability of both cis- and trans- isomers of lycopene after five day food intake (Fielding et al. 2005). Chilomicra contents of lycopene are reported to become double after consumption of processed tomato as compared to raw fruit (Gartner et al. 1997).

Lycopene is a lipophilic carotenoid that possesses twice antioxidant activity than its precursor, β -carotene (Fish and Davis 2003). Chemically, Lycopene is an acyclic highly unsaturated hydrocarbon with 11 conjugated and 2 non-conjugated double bonds (Bose and Agarwal 2007) which provides significant antioxidant capacity. This pigment which imparts red color to fruits is hydrophobic but readily dissolves in organic solvents, and its cis- isomers possess maximum antioxidant activity (Chauhan et al. 2011). Lycopene accounts for about 50% of the carotenoids in our blood (Kohlmeier et al. 1997) and the identification of oxidative metabolites of lycopene in human serum (Khachik et al. 1991) supports the evidence for its antioxidant mechanisms of action (Lenzi et al. 1994).

Whereas, bioavailability can be defined as the fraction of a particular ingested food nutrient available for absorption, metabolism and storage in the body (Jackson 1997), bioaccessibility is the fraction of a nutrient available for absorption into mucosa after being digested

in the gut (Hedren et al. 2002). A number of studies have addressed various aspects of lycopene bioavailability and bioaccessibility to reveal useful information (Zhou et al. 1996; Paetau et al. 1998; Paetau et al. 1999; Erdman 2005; Palafox-Carlos et al. 2011).

An important component of the Kuwaiti diet is the consumption of a traditional tomato sauce (Daqous) which is prepared by cooking fresh tomatoes, olive oil, garlic, salt and spices on medium heat. No studies, so far, have investigated lycopene bioaccessibility of Daqous and, therefore, the present study investigates lycopene bioaccessibility in raw tomato and Kuwaiti tomato sauce (Daqous) with and without olive oil addition using an *in vitro* digestion model. *In vitro* digestion models simulate digestion process and helps in understanding the bioaccessibility of nutrients.

Materials and Methods

Fresh fruit sampling

One batch of tomatoes (Rengo) cultivated on Wafrah in Kuwait was purchased in a local store in Kuwait City, Kuwait.

Kuwaiti tomato sauce (Daqous) preparation

About 13.72g of extra virgin olive oil was heated in a pan. Approximately 12.74g of garlic (crushed) and a 170.37mg of salt were added to the heated oil. Peeled and chopped tomato samples (250g) were then added to the pan and the mixture was heated under medium heat for 30 minutes.

Chemicals

Lycopene and β -apo-8'-carotenal standards (HPLC) were purchased from CaroteNature GmbH (Lupsingen, Switzerland). Pepsin (porcine) and pancreatin (porcine) were purchased from Fluka BioChemika (Germany). All other reagents used in the method were purchased from Sigma Aldrich (Germany).

Sample preparation and in vitro digestion procedure

The experiment methodology was performed using the method of O'Connell et al. (2007) with some modifications. All procedures were

performed under room temperature (25°C) and under dim light to reduce the chances of photodestruction of lycopene.

Approximately 2g of intact samples (raw tomato and tomato sauce; daqous) were placed in glass tubes with saline (0.9% NaCl containing 0.066 g/L butylated hydroxytoluene) to prevent oxidation and then were frozen at °C after adding 1 ml of saline to utilize for extraction and analysis later.

For the *in vitro* digestion, samples were prepared by homogenizing raw tomato and tomato sauce (dacous) of which 2g each were placed in amber bottles (to preserve from light exposure). In the next step, 18 ml of saline was added to end up with a total volume of 20 ml. To mimic the gastric phase, the acidity of the samples was maintained at pH 2 by the addition of 1 ml of prepared pepsin (0.04 g pepsin in 1 ml 0.1mol/l HCl) and drops of HCl. The samples were incubated at 37°C in a shaking water bath (Julabo labortechnik GMBH, Germany) at 95 rpm for 1 hour after flushing the ambers' headspace with nitrogen gas to prevent oxidation.

For mimicking intestinal phase, acidity of the samples was maintained at pH 5.3 by adding 0.9 mol/l sodium bicarbonate followed by the addition of 0.2 ml of fresh bile extract (sheep) to form micelles. Afterwards, 0.1 ml of prepared pancreatin (0.04 g in 0.5 mL saline) was added to the samples and then pH of the medium was changed to 7.4 by adding 1 mol/l NaOH. Samples were then incubated at 37°C for 2.5 hours in shaking water bath (95 rpm) after flushing the headspace with nitrogen gas.

The resulting digestate was divided into two portions. About 3 ml of the digestate was frozen at °C for extraction and HPLC analysis. The remainder of the digestate was centrifuged (Sigma 3K30, Germany) in a 12155 rotor at 20,000 rpm for 4 hours at 4°C to isolate the micellar fraction. Supernatant was drawn with syringe and filtered (0.2µm) to ensure that only aqueous phase is collected from the samples. The collected fractions were frozen at -80°C for extraction and HPLC analysis.

Extraction procedure and HPLC analysis:

Intact, digestate and micellar fractions of both raw and tomato

sauce were thawed and vortexed. For extraction, 2 ml of each sample were mixed with 0.7 ml of internal standard (1 μg β -apo-8'-carotenal in 100 ml methanol) in order to assess extraction loss and then each sample was extracted twice with 1 ml of hexane/ethanol/acetone (50:25:25) followed by centrifugation and separation of upper layer. Combinations of the two extracts from each sample were dried at room temperature in the presence of nitrogen stream and were frozen at $^{\circ}\text{C}$ before HPLC analysis.

The HPLC system (Shimadzu, Japan) consisted of an LC10-AD pump connected to an SIL-20A autoinjector, SCL-10AVP system controller, M10A-visible detector, and SPD-10AV UV-visible detector. The column system consisted of a Spherisorb ODS-2 C18 5- μm PEEK guard column connected to a Vydac 201TP54 (250x 4.6 mm) reverse-phase C18 column. Column temperature was maintained at 25°C using a column water jacket with athermostatically controlled water bath. The visible detector was set at 450 nm for lycopene estimation and had a flow rate of 1.5 mL/min. The injection volume was 45 μL ; samples were eluted using isocratic mobile phase composed of acetonitrile-methanol dichloromethane (75:20:5, vol/vol/vol) containing 10 mmol/l ammonium acetate, 4.5 mmol/l butylated hydroxytoluene, and 3.6 mmol/l triethylamine. The mobile phase was filtered through a 0.5 μm filter and degassed by ultrasonic agitation. Results were generated and analyzed using Shimadzu class-VP software. Lycopene levels in raw tomato and tomato sauce (daquous) were extrapolated from pure carotenoid standard curves. Lycopene contents of the whole food, digested food and micellar fractions were determined. The percent bioaccessibility of lycopene was determined by calculating change in levels of lycopene from digestate to micelles. All samples were performed in triplicates and replicated once or twice.

Statistical analysis

Data Analysis was performed using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 19. Differences in mean values of lycopene's bioaccessibility and p-values were tested by analysis of variance (ANOVA) and Tukey's HSD test, respectively.

Results

Total lycopene contents of intact samples (fresh tomato, tomato sauce (daqous) and daqous added olive oil) were almost similar (49.5-52.2 $\mu\text{g/g}$). Almost same results were obtained for the digestate fractions of these three categories of samples (48.4-51.1 $\mu\text{g/g}$) and there was no statistical difference ($p > 0.05$) between the three patches (Fig. 1). Lycopene contents of micelle-simulated samples differed significantly ($p < 0.05$) not only from intact and digestate samples but also among fresh tomato, tomato sauce (daqous) and tomato sauce with oil. Lowest lycopene contents among the micelles were observed in fresh tomato samples (2.5 $\mu\text{g/g}$). Lycopene contents of tomato sauce (daqous) were 10.2 $\mu\text{g/g}$ while the samples of daqous added with olive oil had 16.5 $\mu\text{g/g}$ lycopene. Percent bioaccessibility of lycopene was 5% for fresh tomato, 21% for tomato sauce (daqous) and 34% for tomato sauce (daqous) with oil addition (Fig. 2).

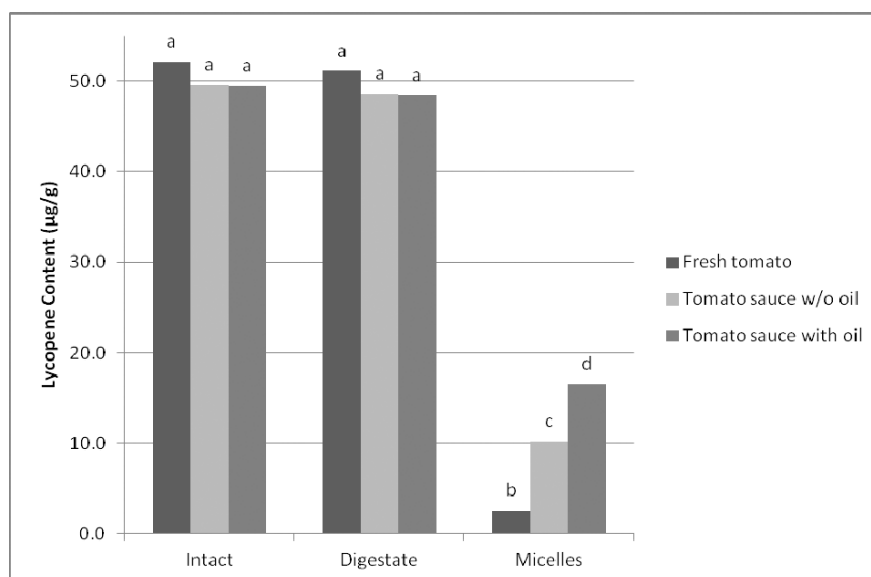


Figure 1 - Lycopene content ($\mu\text{g/g}$) of intact, digestate and micelles of fresh tomato, tomato sauce w/o oil and tomato sauce with oil (Values in the same column not sharing the same superscript were significantly different ($P < 0.05$)).

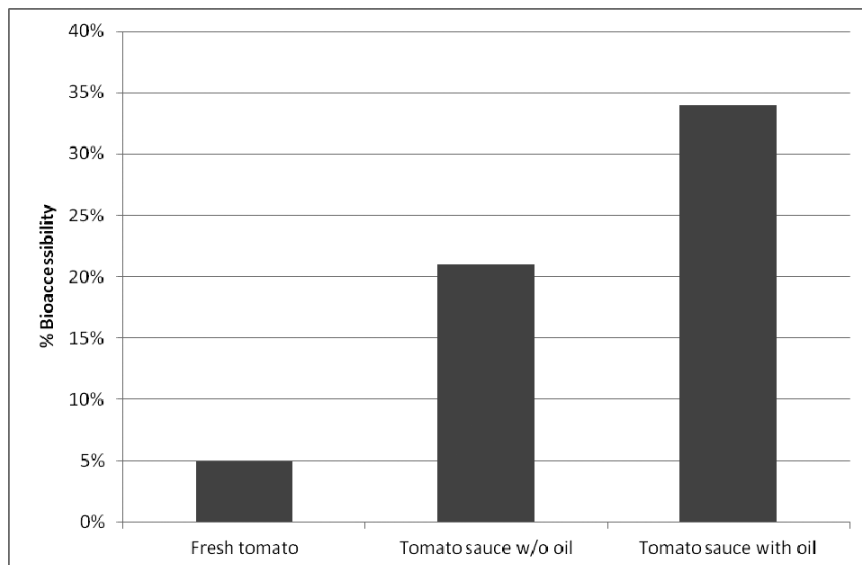


Figure 2 - Lycopene bioaccessibility (%) of raw tomato, tomato sauce w/o oil and tomato sauce with oil.

Discussion

Our study aimed at investigating the bioaccessibility properties of dacous (a traditional Kuwaiti tomato sauce) by using an *in vitro* digestion model. The salient finding of this study is that cooking increased the bioaccessibility of lycopene up to 21% and addition of olive oil to tomatoes during cooking increased lycopene bioaccessibility up to 34%. Our results are consistent with the generally accepted dogma that processed tomato food offers more lycopene than the raw fruit. Support to this principle has gained by many previous studies which have shown that processing and heat treatments of food improve the bioavailability and bioaccessibility of lycopene and carotenes (Gartner et al. 1997; Stahl and Sies 1992; Rao and Agarwal 1999; Van het Hof et al. 2000).

Our finding of increased lycopene contents of the tomato sauce added with oil in the samples that mimicked micelles is consistent with previous studies which report enhanced absorption of lycopene by the

gut mucosa from the foods containing tomatoes cooked with oil (Gartner et al. 1997; Fielding et al. 2005). Increased bioaccessibility of lycopene of processed tomatoes with olive oil is presumably due to the lipophilic nature of lycopene. It is postulated that sufficient fats are needed in the food to stimulate bile secretion and thus micelles to form. Heating and chopping makes more lycopene available physically by tissue disintegration and cooking is presumed to increase the intimacy between lycopene and lipids (Fielding et al. 2005). The transfer of lycopene from chyme to mixed micelles during *in vitro* digestion requires a minimal of 0.5-1% lipid milieu. This may be affected by the length of fatty acyl chains rather than the degree of unsaturation (Huo et al. 2007).

Studies on lycopene bioavailability have generated quite useful but variable findings. Lycopene concentrations in the plasma usually do not increase in the post-dose period of 12-24 hours, rather persistently increased lycopene concentrations are noted after chronic dosage of many days to a few weeks in the plasma (Paetau et al. 1998) as well as in the tissues (Paetau et al. 1999). Tissue uptake of lycopene may explain the variability seen in various studies addressing the bioavailability of lycopene by measuring its concentrations in blood. Furthermore, dietary fiber reduces the bioavailability of nutrients including antioxidants like β -carotene and lycopene (Palafox-Carlos et al. 2011). Crystallization of lycopene molecules in the tomato extracts is one of the most important causes of its low bioavailability (Zhou et al. 1996). Other factors that can affect the bioavailability of lycopene are amount of fats in the diet, fat substitutes, and cholesterol lowering drugs that can interfere with lycopene-micelle assembly (Singh and Goyal 2008). An inverse relationship has also been observed in the androgen status and lycopene accumulation in the tissues (Erdman 2005).

It has been reported in a number of studies that lycopene curative efficacy increases if it is applied in combination with its precursor molecules, phytoene and phytofluene (Engelmann et al. 2001). Lycopene bioavailability is increased when it is taken in combination with β -carotene (Johnson et al. 1997) and even a single tomato-

oleoresin dose is reported to significantly increase plasma lycopene concentrations after 24 hours (Grag et al. 1994). These findings suggest existence of synergistic relationships among carotenoids and offer a potential area of future research.

Conclusion

By the virtue of our *in vitro* digestion simulated experiment, we conclude that tomato processing increases bioaccessibility of lycopene that can be further increased by mixing oil while cooking. It will be quite useful to further explore the factors that affect bioaccessibility and bioavailability of lycopene.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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