# INFLUENCE OF THE OPERATIONAL CONDITIONS USED DURING SUPERCRITICAL $CO_2$ EXTRACTION ON THE YIELD AND ANTIOXIDANT ACTIVITY OF TOMATO JUICE LYCOPENE

**Juliana A. Egydio[1], Ângela M. Moraes[1], Paulo T.V. Rosa[2]**

[1]Departamento de Processos Biotecnológicos - Faculdade de Engenharia Química - Universidade Estadual de Campinas – Campinas – SP – Brazil
[2]Departamento de Físico-Química - Instituto de Química - Universidade Estadual de Campinas – Campinas – SP – Brazil
e-mail – paulorosa@iqm.unicamp.br ; fax – 55 19 3521-3023

There is an increasing interest in the use of lycopene due to its antioxidant capacity, which can prevent some kinds of cancers and heart diseases. The applications of this compound are mostly related to the nutraceutical food field. In this paper a lycopene extraction process from tomato juice using supercritical $CO_2$ as solvent is presented. The juice was centrifuged to collect the particles containing the lycopene. The water in the particles was removed by rinsing with ethanol. The extractions where carried out with temperatures from 40 to 80 °C and pressures from 200 to 350 bar. During the experiments, from 2.9 to 34.5% of the lycopene were extracted from the solid particles. The temperature had a strong influence in the extraction yield, but the pressure showed no effect on it. An antioxidant activity of up to 15.1%, expressed in terms of percentual $DPPH^{\bullet}$ reduction, was achieved for the extract obtained at 60°C and 275 bar.

## INTRODUCTION

Lycopene (ψ,ψ-carotene) is a carotenoid, from the same family as β-carotene. It is responsible for the red color in some fruits such as tomato, watermelon, papaya, and guava. Figure 1 shows the lycopene chemical structure. This compound can be found in nature in the *cis* and *trans* forms, comprehending up to 72 geometrical isomers [1]. The *trans* form is the most stable, and consequently, it is the most frequent structure found in tomato, corresponding to more than 90% of the total lycopene [2].

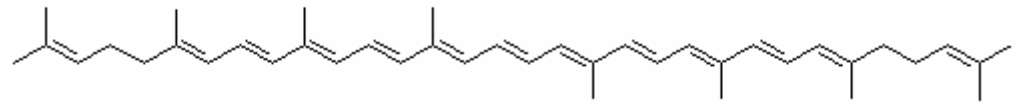

**Figure 1 – All *trans* lycopene chemical structure.**

The lycopene antioxidant capacity can be attributed to the 11 conjugate double bounds present in its structure. This carotenoid can inhibit the growth of prostate, breast, endometrial, and promyelocylic leukemia cancer cells [3-5], decrease the risk of developing

heart problems [6-7], and induce the apoptosis of immortalized fibroblast cells exposed to tobacco smoke [8].

The supercritical fluid extraction of lycopene from tomato was discussed by several authors [9-13]. In these works, the optimal operational conditions, the influence of raw material pre-treatment (humidity and particle size), and the use of co-solvents such as ethanol and vegetable oil, were studied.

For extractions carried out with pressures higher than 200 bar, the lycopene extraction yield increases with temperature [10-11]. About 85% of the lycopene present in the raw material can be extracted at 80°C and 280 bar [10], with higher extraction yields being achieved for smaller particles [13]. The use of hazelnut oil [13] and ethanol [9] as co-solvents can also increase the extraction yield. This behavior can be explained in terms of lycopene solubility variation in the supercritical phase with the use of the co-solvents [14].

Before the extraction step, the tomato particles humidity is frequently reduced in tray driers using low temperature (i.e. 40 °C) and long exposure periods. The raw material is dried to allow the $CO_2$ access in the regions where the lycopene is located. The drying operation represents an extra step in the extraction process that can both increase the final product cost and cause structural changes in the lycopene molecule.

One alternative approach for the drying process can be the partial replacement of the water present in the particle by a solvent soluble in supercritical $CO_2$, such as ethanol. This operation can be faster than the traditional drying process and in addition, causes less lycopene structural changes. In this context, the main goal of this work was to determine the effect of the operational conditions on the recovery of lycopene from tomato juice without the drying process and evaluate the antioxidant activity of the resulting extract.

## MATERIAL AND METHODS

### Raw Material Preparation

Commercial tomato juice (Milano, lot 99, Brazil) was used in the experiments. The juice was centrifuged for 30 minutes at 4000 rpm to separate the serum from the particles containing lycopene. The precipitated material was resuspended in absolute ethanol and centrifuged for 15 minutes at 4000 rpm. This procedure was repeated for four times. The last precipitate was used as the raw material in the extraction experiments.

### Determination of Lycopene Concentration

Lycopene was quantified using the colorimetric method proposed by Fish et al. [15]. From 0.1 to 0.7 g of sample was added to 20 mL of an extraction solution (25% ethanol:50% hexane:25% acetone+0.05% of BHT) in a dark flask. The flask was agitated at 180 rpm for 15 minutes. After that, 3 mL of deionized water is added. The flask is agitated at 180 rpm for 5 minutes. The absorbance of the hexane layer at 503 nm was determined and the lycopene content was calculated using an extinction coefficient of $17.2 \times 10^4$ /M cm.

### Supercritical Fluid Extraction

Approximately 18 grams of the raw material was packed in a semi-preparative stainless steal HPLC column (10 mm x 25 cm). The column was set into the oven of a Spe_ed SFE System (Applied Separations). It was used a static period of 5 minutes. After that, the extraction was conducted using 1.7 g $CO_2$/min for 90 minutes. A complete factorial experimental design with repetition in the central point was used to evaluate the

influence of temperature and pressure on the extraction yield. The limits of temperature used were 40 and 80°C and the pressure ranged from 200 to 350 bar.

### Antioxidant Activity of the Extracts Using the DPPH$^{\bullet}$ Method

It was used the Sanchez-Moreno et al. method [16] to determine the antioxidant activity of the extracts. A 0.1 mL of a ethanolic solution of the extract (obtained at 60°C and 275 bar) at several concentrations was added to a 2 mL of a methanolic DPPH solution ($6x10^{-5}$ mol/L). The mixture was sonicated for 30 seconds and its absorbance at 515 nm was measured right away ($ABS_0$). The absorbance was measured again at the same wavelength after 30 and 60 minutes ($ABS_f$) of reaction carried out in the absence of light. The percentual DPPH$^{\bullet}$ reduction ($G_R$) in 60 minutes was calculated using the following equation:

$$G_R = 100\left(1 - \frac{ABS_f}{ABS_0}\right) \quad (1)$$

The antioxidant capacity (AC) is defined as the absorbance measured at any time (ABS(t)) divided by the absorbance of the solution without extract, or:

$$AC = 100\left(1 - \frac{ABS(t)}{ABS(C=0)}\right) \quad (2)$$

## RESULTS AND DISCUSSION

### Supercritical Fluid Extraction

The lycopene concentration in the tomato juice was 84.1 μg/g of juice. The obtained value is comprehended into the range of 68.7 and 121.4 μg/g presented in the literature for tomato juice [2, 17]. The mean lycopene concentration in the raw material was 253.8 μg/g of wet material. The water substitution by ethanol caused only a small loss of lycopene due to the low solubility of the carotenoid in this solvent at room temperature. The lycopene recovery data obtained during the supercritical fluid extraction are shown in Table 1.

Table 1 – Influence of pressure and temperature on lycopene recovery.

| Pressure (bar) | Temperature (°C) | Lycopene Recovery (%) |
|---|---|---|
| 200 | 40 | 2.9 |
| 200 | 80 | 34.5 |
| 275 | 60 | 14.1 |
| 275 | 60 | 14.4 |
| 350 | 40 | 6.1 |
| 350 | 80 | 34.1 |

The lycopene recovery varied from 2.9 to 34.5%, depending on the operational condition. The lycopene recoveries obtained by Vasapollo et al. [13] were lower than 35%

for extractions conducted at 450 bar and 66°C. Thus, the water replacement by ethanol seems to have no effect on lycopene recovery.

The total mass of wet raw material used to fill the extraction column was 18 g. After extraction the mass of solid particles collected was approximately 1.1 g. Thus, a large amount of ethanol was present at the beginning of the extraction and it could have behaved as a co-solvent, at least at the first minutes of extraction, when most of the ethanol was removed from the column. Larger recoveries probably can be obtained increasing the solvent to raw material ratio.

Figure 2 shows the Pareto chart of the effects of extraction temperature and pressure on lycopene recovery. The temperature had a statistically significant effect on the extraction process ($p<0.05$), while the pressure had practically no effect.

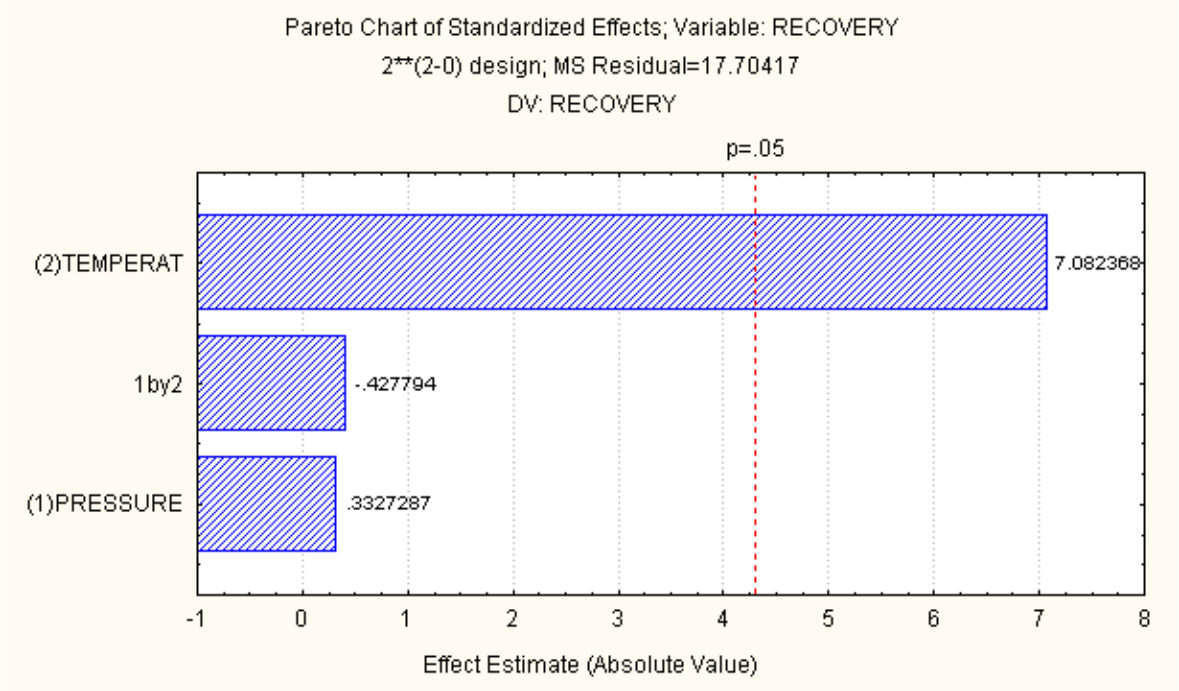


Figure 2 – Pareto Chart of the extraction pressure and temperature effects on lycopene recovery.

**Antioxidant Effect of the Extract**

Table 2 presents the DPPH$^{\bullet}$ analysis for the extract obtained at 275 bar and 60°C. It can be observed that the $G_R$ values increase with the extract concentration, being relatively low at the tested range. However, the $G_R$ values reported in the literature for pure lycopene with concentrations from 2.80 to 8.39 μg/mL range from 1 to 8% [18]. Its is also reported in the literature that the $G_R$ values decrease with the increase of lycopene concentration [18], an abnormal result that was not observed in the present work, since the tomato extract is a more complex system in which phenomena that could contribute to reduce the antioxidant activity (such as aggregation) could have been prevented.

Figure 3 shows the variation of the antioxidant capacity of the extract as a function of time and extract concentration. The antioxidant capacity is concentration-dependent and also increases with time.

Table 2 – Results of DPPH$^{\bullet}$ analysis of the extract obtained at 275 bar and 60°C.

| Extract Concentration (μg/mL) | $G_R$ (%) |
|---|---|
| 1.06 | 0.92 |
| 2.13 | 2.17 |
| 4.26 | 2.58 |
| 6.51 | 4.66 |
| 13.02 | 8.17 |

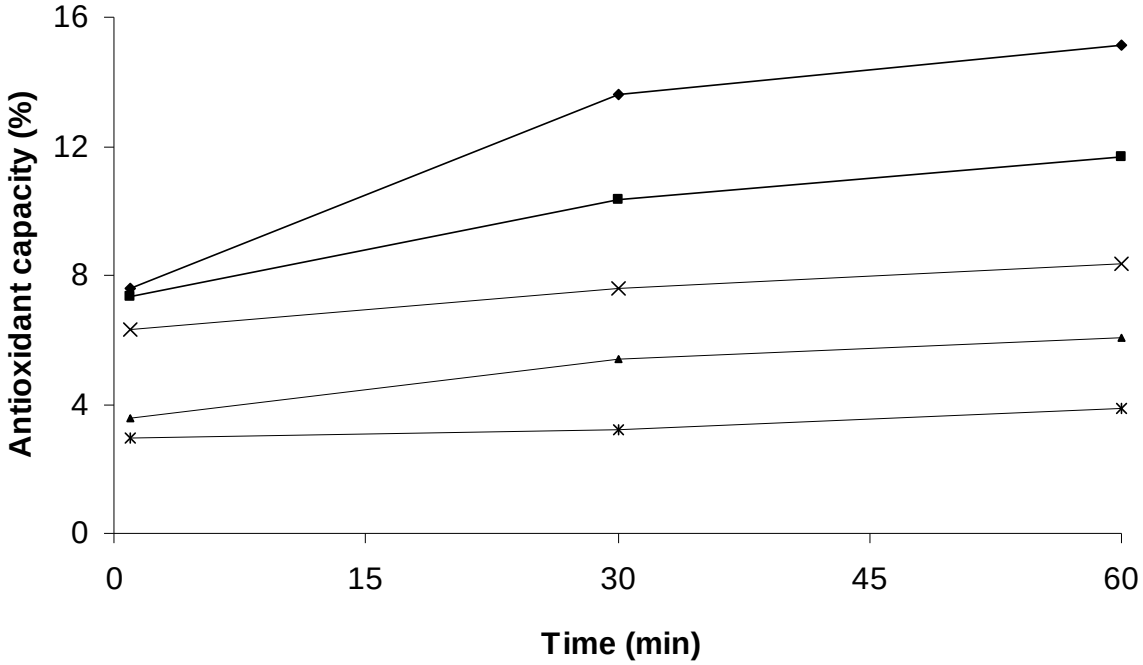


Figure 3 – Antioxidant capacity of the tomato juice extract obtained at 275 bar and 60°C. * 1.06 μg/mL; ▲ 2.13 μg/mL; × 4.26 μg/mL; ■ 6.51 μg/mL; ♦13.02 μg/mL

## CONCLUSION

In this work, the lycopene extraction recovery from tomato juice was directly comparable to the values reported in the literature for extraction employing dried particles. The antioxidant activity of the extract was also in accordance to reported values. Therefore, the water replacement by ethanol seems to be a technically viable process to recover lycopene from tomato juice.

## ACKNOWLEDGMENT

The authors would like to acknowledge FAPESP (proc. No. 06/04243-1) and CAPES for the financial support to this work.

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