SUPPLEMENTARY MATERIAL

Tunisian tomato by-products, as a potential source of natural bioactive compounds

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Abstract

Consumption of tomato and tomato products, is positively related to the reduction of cardiovascular disease and several types of cancer, thanks to the presence of natural compounds, such as antioxidants. Peels and seeds fractions of tomato, collected after industrial processing in Tunisian industries, were analyzed for nutritional and antioxidants composition in perspective of its utilization. Proximate composition, fatty acids profile, carotenoids, such as lycopene and beta-carotene, polyphenols contents, demonstrated the good potential of these residual products as a source of natural compounds, useful for food and nutraceuticals applications.

Keywords: *Solanum lycopersicum*; fatty acids; antioxidants; carotenoids; lycopene; polyphenols.

Experimental section

Samples collection

Tomato pomace was obtained from a production plant located in the north of Tunisia. The material was collected immediately prior to the disposal step. The different parts of the tomato residues, peels (TP) and seeds (TS) were separated by sedimentation in water. Most of

the seeds settle on the bottom, while the peels remain afloat on the surface. The tomato fractions were placed in air-tight plastic bags and stored immediately at -4°C until analyses.

All analyses were carried in duplicate aliquots; each aliquot was realized taking samples from three randomized batches of wastes; sampling were done five time.

Samples drying

Drying experiments were performed in a laboratory scale hot-air dryer. Tomato peels samples ($1 \pm 0.2\text{g}$) were placed and dried in the hot-air dryer at 70°C. After drying, all samples were wrapped in aluminum foil and analyzed for moisture and lycopene content. The average moisture content of the samples was determined gravimetrically by drying in a conventional oven at 105 °C for 24h (AOAC 1990). Tomato seeds were dried in the same laboratory conditions at 60°C for 24h.

Nutritional composition

Proximate composition

The samples were analyzed for proximate composition (moisture, protein, fats, and ash) using the AOAC procedures (AOAC 1990). The crude protein content ($\text{N} \times 6.25$) of the samples was estimated by the Kjehldal Method; the total lipids were extracted by the method of Folch et al. (1957) and by Soxhlet apparatus and determined gravimetrically; the ash content was determined by incineration at 600 ± 15 °C; the total sugars were determined using the Dubois Method (Dubois et al. 1956). The energetic value was determined as done in tomatoes by Pinela et al. (2012), multiplying the amount of each nutrient for the correspondent caloric content, according to the European document for food, n°1169/2011 (2011).

Fatty acids profile

Fatty acid methyl esters (FAMES) were prepared by acid-catalysed transmethylation using tricosanic acid (C 23:0) as internal standard (Lepage & Roy 1984) and analyzed as previously reported (Messina et al 2013).

Antioxidants compositions

3.3.1. Extraction and quantification of lycopene

Extraction and quantitative determination of lycopene were conducted according to Fish et al. (2002). A mixture of solvent hexane: acetone and ethanol 95% (50:25:25; v/v/v) was added to a known weight of tomato peels and seeds. The assay was carried on triplicate.

Tubes containing tomato samples and the mixture of solvents were laid on their sides in a container that contained ice and then homogenated with ultraturrax for 5 minutes. Finally, they were transferred on magnetic stirrer. After 30 minutes, a volume of deionised water was added to each tube and the samples stirred for another 10 minutes. Stirring was stopped and tubes were left at room temperature for 5 min to allow for phase separation. The absorbance of the hexane (upper) layer was measured at 503 nm versus a blank of hexane solvent. The lycopene content of tissue was then estimated by Equation (1):

$$\text{Lycopene (mg/kg tissue)} = (A_{503} \times 31.2)/g \text{ tissue}$$

The supernatant was then dried and weight to estimate the recovery of lycopene. The dried residual was frozen at -20 °C for further analysis.

Carotenoids

Carotenoids content was determined by the method described by Nagata and Yamashita (1992). The sample (1g) was vigorously shaken with 10 ml of acetone/hexane (4:6; v/v) for 5 min, and then filtered. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Content of β -carotene was calculated according to the Equation (2):

$$\beta - \text{carotene (mg/100ml)} = 0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$$

The results were expressed as mg of β -carotene/ g of sample.

The filtrate was then dried and weight to estimate the recovery of carotenoids. The dried residual was frozen at -20 °C for further analysis.

Total phenolics content

Crude phenolic extract of peels and seeds of tomato was prepared. In brief, sample (1g) was extract with 20 ml aqueous ethanol (80%) for 5 min with ultraturax then shaken with magnetic stirrer at room temperature for 1h. The extracts were then filtrated and evaporated in polyvap. The dried residual material was crude phenolic extracts which was frozen at -20°C for further analysis. Total phenolics in the tomato pomace were analyzed using Folin-Ciocalteu's assay as described by Oki et al. (2002). Gallic acid was used as standard for calibration (5- 500 mg/ml) and results were expressed as mg of gallic acid equivalents (GAE) per g of the extract.

Tocopherols content

Tocopherols content was determined as described by Ryyananen et al. (2002) with brief modifications. Tomato pomace (peels and seeds) (1g) was accurately weighed into a 30 ml Pyrex glass tube with a Teflon screw cap. Ascorbic acid (0.1g), ethanol (5ml) and water (2 ml) were added. After mixing the tube with the vortex mixture, 2ml of KOH (60%) was added. The tube was capped and transferred to a boiling bath for 25 min. The tubes were cooled in an ice-water bath. Then the suspension was extracted twice with 10ml of n-hexane and distilled water (9:1; v/v). Tubes were shaken with the vortex mixture for 1 min then centrifuged for 2 min. Finally, after separation of the phases, the organic layer were collected and evaporated to dryness. The total amount of extractable material was gravimetrically determined and the extraction yield was calculated as mass of extract/mass of dry raw material, expressed as percentage. Extracts were conserved at -20 °C for further analysis.

Evaluation of antioxidant activity

3.4.1 DPPH Free Radical Scavenging assay

Hydrogen atom- or electron-donation ability of the corresponding extracts was measured from the bleaching of the purple-coloured ethanol solution of DPPH. This spectrophotometric assay uses stable 2,2'-diphenylpicrylhydrazyl (DPPH) radical as reagent. One hundred of various concentrations of extracts or standards were added to 500 µl of ethanol solution of DPPH (0,2 mM) and 400 µl of Buffer Tris-HCl (100 mM, pH 7.4). After 30 min of incubation period at room temperature in darkness, the absorbance was read against the blank at 517 nm. Inhibition of DPPH free radical in percent (I%) was calculated in Equation (3):

$$I\% = 1 - (A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. The values of inhibition were calculated for the various concentrations of the extracts.

Reducing power

Reducing power was determined as described by Yen and Chen (1995). Different concentrations of the extracts (0.04- 2.5 mg/ml) and different concentrations of Gallic acid (0.002-0.062 mg/ml) were mixed with phosphate buffer (0.2 M – pH 6.6), potassium ferricyanide [$K_3 Fe(CN)_6$] (1%), trichloroacetic acid (1%) and ferric chloride $FeCl_3$ (0.01%). The mixture was vigorously shaken and then incubated at 50 °C for 30 min. Subsequently we add a volume of TCA and the solution was centrifuged at 3000 rpm for 10 min. Finally the supernatant solution was mixed with distilled water and $FeCl_3$ (1:1:2; v/v/v) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

For each sample two extracts were obtained and all the assays were carried out in duplicate. The results are expressed as mean values and standard deviation (SD).

The statistical differences were evaluated for each parameter by the analysis of variance (ANOVA). Before analysis, the degree of heterogeneity was assessed by the Cochran's test (Underwood 1997). Data were processed by Statistica (version 8.0, Statsoft, Inc., Oklahoma).

Table S1: Nutritional composition and energetic value of tomato peels (P) and tomato seeds (S)^a.

	P	S
Moisture (g/100g fw)	3.25 ± 0.35	4.66 ± 0.83
Ash (g/100g dw)	20.60 ± 1.89	17.67 ± 1.89
Proteins (g/100g dw)	9.30 ± 0.24	30.23 ± 0.25
Lipids (g/100g dw)	5.65 ± 0.49	26.53 ± 0.38
Total sugars (Monosaccharids) (g/100g dw)	16.14± 0.01	1.32 ± 0.00
Carbohydrates (g/100g dw)	45.06 ± 0.01	19.59 ± 0.02
Energy (Kcal/100g dw)	268.29± 0.67	438.05± 0.84

^aData are mean values of duplicate determination ± standard deviation, carried on a total of 5 pooled samples.

Table S2– Fatty acids content (Area %) of tomato seeds oil.

Fatty acids	Mean ± S.D.
14:0	2.94 ± 0.4
16:0	18.2 ± 0.7
16: 1ω-7	7.28 ± 0.4
18:0	5.05 ± 0.6
18:1ω-9	33.18 ± 0.53

18:2 ω -6	34.36 $\pm$ 1.02
Total Saturated	25.18 $\pm$ 1.34
Total Unsaturated	74.82 $\pm$ 0.8