ORIGINAL ARTICLE

TOMATO POMACE VALORIZATION BY OIL AND BIOACTIVE COMPOUNDS EXTRACTION: CASE OF SOUSS-MASSA REGION

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| Received May 01, 2020 | | Accepted May 22, 2021 | | Published May 27, 2021 | | ID Article | Abbassi-Ref3-ajira100529 |

ABSTRACT

Background: Tomato processing industry are dedicated to the preparation of tomato concentrates and their derivatives. Tomato processing byproduct, also known as tomato pomace (peel and seeds) represents around 5–30% of the main product. If these wastes remain unused, they not only add to the disposal problem, but also aggravate environmental pollution. **Methods**: In this research, physical-chemical characteristics of peel and seeds extracts of tomatoes cultivated in the region of Souss-Massa, are studied to evaluate the possibility of production and exploitation of oil and bioactive compounds that constitute these extracts obtained by Soxhlet extraction using polar and non-polar solvents. **Results**: The seed oil extracted has a dark yellowish color and obtained with a yield of 14.53%. Its measured characteristics revealed that this oil is perfectly beneficial nutritionally to human health due to its richness in unsaturated fatty acids and has quality parameters close to those indicated in the Codex Alimentarius of fats and oils. The content of total phenolics in the extracts was determined spectrometrically according to the Folin−Ciocalteu procedure and calculated as gallic acid equivalents (GAE). Among extracts obtained, remarkable high antioxidant activity and high total phenolic content (GAE > 20 mg/g) were found in peel extracts $(22.75 - 32mg\ GAE/100g\ d.w.)$ compared to seeds extracts. The antiradical activity of peel and seeds extracts was evaluated by the DPPH test. The overall results showed that the methanol peel extract has strong antioxidant capacity with EC50 reached $(88.73\mu g/ml)$. **Conclusion**: To fully exploit these important sources of natural antioxidants, further characterization of the phenolic composition is necessary. But overall, these results remain useful for developing standards on these materials.

Keywords: *Valorization; Tomato pomace; residues; extraction; Soxhlet.*

1. INTRODUCTION

Tomatoes (Solanum lycopersicum L., family Solanaceae) are the second most cultivate and consumed vegetable crop, next to potatoes, with a global annual production reaching 100 million tons [1]. Morocco is the 15th world producer of tomatoes with more than 1.4 MT according to the FAO.

Fresh tomato fruits and tomato-based commercial products occupy an important place in the human diet thanks to its richness in antioxidants. Epidemiological studies have shown that regular consumption of fruits and vegetables, including tomatoes, plays a key role in preventing cancer and cardiovascular problems [2, 3, 4 , 5]. Additionally, its high consumption provides > 85% of the total dietary intake of lycopene [6].

The tomato processing industry leads to the production of an immense quantity of by-products (5–30% of the main product). Primarily used as livestock feed or disposed of in a landfill. The tomato pomace is nearly 33% seed, 27% skin, and 40% pulp, whereas the dried form contains approximately 44% seed and 56% skin and pulp [7]. This biomass is at the root of several economic and environmental problems [8].

So, the value of recovering these residues is thus linked both to the protection of the environment, to the reduction of the loss of raw material resources and to transform these residues into bioproducts with high value-added in the food and pharmaceutical fields, in order to make them an economic and scientific gain rather than a loss. These residues are currently of great interest since they have an excellent source of natural antioxidants including polyphenolic compounds and carotenoids [9, 10, 11].

Many recent studies and reviews have focused on the valorization of waste or by-products generated in the food manufacturing to produce biofuels, agro-industrial, biodegradable plastics, bioactive and nutraceuticals [12, 13, 14] [15, 16].

Similarly, the tomato waste is rich in bioproducts with high value-added in the food, pharmaceutical and energy fields. The tomato pomace presents an important source of commercially vital carotenoids, lycopene, astaxanthin, tocopherols, terpenes, and sterols which have diverse applications in the feed, pharmaceuticals, and cosmetic industries [17, 18 - 10- 1]. Monika Knoblich et al., (1999) showed that the peel byproduct contained 100.8 g protein, 256.4 g ash and 299.4 g acid detergent fiber kg−1 [4]. The seeds byproduct contained 202.3 g protein, 51.8 g ash and 537.9 g acid detergent fiber kg−1 [11]. Firestone et al. [12] found that the composition of tomato seed oil in fatty

acids essentially similar to that of low-linolenic acid soybean oil. The highest concentration of total phenolics content is mainly concentrated in the epidermis because of the intensity of the light radiation received by the cells of the epicarp [13]. Toor and Savage [14], reported that the total phenolics content (expressed in mg gallic acid / 100 g d.w.) of tomato skin and seeds was 29.1 and 22.0, respectively [15]. In this research, physical-chemical characteristics of peel and seeds extracts of tomatoes cultivated in the region of Souss-Massa, are studied to evaluate the possibility of production and exploitation of oil and bioactive compounds that constitute these extracts obtained by Soxhlet extraction using polar and non-polar solvents.

2. MATERIELS AND METHODES

2.1. Waste preparation

Tomatoes (38Kg) were taken from a farm in the Sous Massa area. They were washed by hand with water to remove all dirt. Then, were put in hot water (67°C) a few minutes (2 to 3min) then the peel removes easily. The tomatoes were emptied of their contents and cut in half with a knife, the seeds were filtered and soaked in water at room temperature overnight to remove the gelatin that surrounds them, then washed and filtered with a strainer.

The peel was dried into oven at 50°C for 48 hours. The seeds have been in air-dried for 3 days. The dried residues were ground in the blender. We obtained 81g of seed powder and 42g of peel which represents respectively 0.21% and 0.34% of the weight of the fresh fruit. The powders obtained were extracted by Soxhlet extraction using various organic solvents and several physico-chemical analyzes.

2.2. Extraction procedure

2.2.1. Tomato Seeds Oil Extraction

The oil was extracted from the tomato seeds powder using a Soxhlet device. 30g of powder are weighed into a cartridge which is introduced into the Soxhlet. The extraction is carried out with n-hexane (300ml) brought to reflux for 3 to 4 hours. The solvent is then evaporated in a rotary evaporator and the crude oil collected is stored in a flask.

2.2.2. Extraction of phenolic compounds from tomato seeds

After extracting the oily fraction of the seeds powder with n-hexane, a quantity of 30g of cake (the defatted powder) is extracted with 200ml of solvent selected using Soxhlet for 4 hours. The solvent is then evaporated in a rotary evaporator to obtain crude extracts. Three solvents of different polarity (ethyl acetate, ethanol and methanol) were used.

2.2.3. Extraction of phenolic compounds from tomato peel

30 g of peel powder was extracted with 200ml of ethyl acetate by the Soxhlet method for 5 hours of interaction between the plant material and the solvent. Another trial was carried out under the same conditions using the same amount of powder in 200ml of methanol. The crude extracts were recovered after evaporation of the solvent.

- **Spectrometric determination of total phenolics content (Folin-Ciocalteu test).**

Many studies have discussed the use of the Folin-Ciocalteu reagent to determine total polyphenols [19, 20]. Polyphenols present in these extracts react with Folin-Ciocalteu reagent which is composed of a mixture of specific redox reagents : phosphotungstic acid (H3PW12O40) and phosphomolibdic acid (H3PMO12O40) of yellow color, is reduced during the oxidation of polyphenols in alkaline solution to form a blue complex constituted by oxides of tungsten (W8O23) and molybdenum (MO8O23) that can be quantified by visible-light spectrophotometry [21]. The maximum absorption of the blue pigments depends on the alkaline solution (pH usually controlled with sodium carbonate) and the level of oxidized phenolic compounds [20]. Four concentrations of each crude extract were prepared by the dilution method in 1.5ml Eppendorf tubes using methanol. 5µL of each concentration are taken in a test tube by adding 1.7mL of distilled water and 300µL of Folin-Ciocalteu reagent diluted 10 times, then 0.5mL of NaCO3 (20%) is added to the solution after 3 minutes. The mixture was incubated at room temperature for 1h and the absorbance read at 760nm.The results obtained are expressed in milligram equivalent of gallic acid per 100g of dry matter from an equation of the linear regression deduced from the calibration curve.

The antiradical activity of various antioxidants is determined using the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH*). In its radical form, DPPH* has an absorption band at 515nm which disappears upon reduction by an antiradical compound [22, 23]. The antiradical activity of crude extracts which were prepared from tomatoes seeds and skin was measured by DPPH. Under the effect of antioxidants, the DPPH is reduced by passing from its violet color to the yellow color. 2 mg of each crude extract was taken in a test tube and dissolved in 10 ml of methanol. Five concentrations such as 12.5µg/mL, 25µg/mL, 50µg/mL, 100µg/mL and 200µg/mL were prepared by dilution. A solution of DPPH is prepared by adding 100ml of methanol to 3.3mg of DPPH powder with magnetic stirring. A volume of 1.5ml of each dilution (different concentrations) was taken in a test tube and 2.5ml of DPPH solution added. The tubes were placed in the dark at room temperature for 90 minutes. A measure of absorbance was

made at 517nm. Under the same conditions an ascorbic acid control was carried out. The antioxidant activity of the crude extracts was calculated using the following equation (1):

$$\%Inhibition = \frac{\text{Astandard} - \text{Aextract}}{\text{Astandard}} \times 100 \quad \textbf{(1)}$$

With:

- $\mathbf{A_{standard}}$: Standard Absorbance (Methanolized DPPH)
- $\mathbf{A_{extract}}$: Absorbance for each dilution of crude extract

EC50 is the concentration of test sample required to scavenge 50% of the DPPH free radical, it is determined either graphically or calculated by the linear regression equation of the plotted graphs, based on the inhibition percentages as a function of different concentrations of fractions tested.

3. RESULTS AND DISCUSSION

Physico-chemical characteristics of the powders

Low water content explains a high content of dry matter. The analysis performed to determine the water content of seeds and peels reveals that the average value of the water content of seeds is 8.73% or 91.27% dry matter, and the peel has a water content of 6.5% or 93.5% dry matter. The ash content is the total amount of minerals in a sample. The average ash value found for the seeds is 8.62%, while for dried tomato peels we obtained 3.5%.This result agrees with that described by previous researcher [24], which is 2 to 9%, whereas for dried tomato peels is 3.5% this value close to that reported by Navarro-González et al., (2011) which is 3.1% [25].

Physical-chemical characteristics of tomato seeds oil

The oil extracted from the tomato seeds powder has a dark yellowish color and obtained with a yield varied with heating; reached 14.53% after 3 hours. The average yield is 13.6%, this value is in the range found in Al-Wandawi et al., (1985) which is between 14 and 21% [26].

The density of the extracted oil is (0.892-0.910), it is less dense than sunflower oil (0.920-0.925) and near to olive oil density (0.910-0.916).

The acid number of the oil studied is 2.6 mg KOH/g fat body. This value meets the standard set by the Codex Alimentarius for fats and oils that are 4 mg KOH/g fat body.

The saponification number (SN) is 175mg KOH/g fat body. It is lower than the SN of olive and sunflower oils which are respectively (185-196) and (188-193). These results show that tomato seeds oil contains fatty acids with carbon chains longer than those of the fatty acids present in these two types of oil.

Spectrometric determination of total phenolics content:

After extrapolation of the results of the optical density on the calibration curve, the different total phenolics content of tomato residues calculated and expressed in gallic acid equivalent are shown in Table 1.

Table 1: Total Phenolic Content of tomato seeds and peels.

Raw extracts		Total phenolics (mg GAE/100g d.w.)
Seeds	Ethanol	18.74
	Methanol	7.4
Peels	Methanol	32
	ethyl acetate	22.75

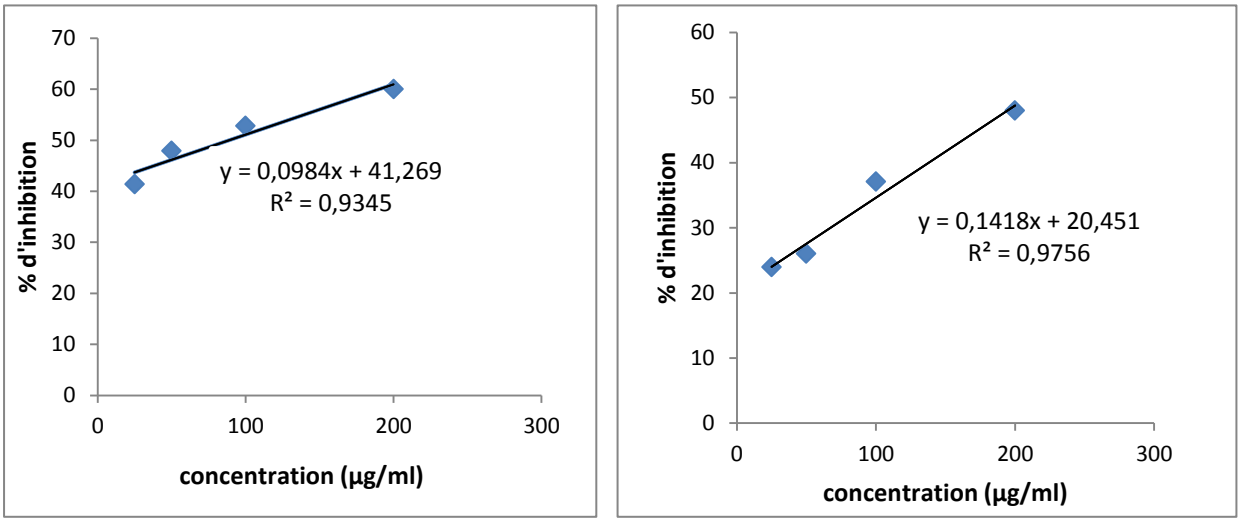
The total phenolic content was higher in peel extracts (22.75-32 mg GAE/100g d.w.). As for seeds, the significant total phenolics content was found in the EtOH crude extract (18.74 mg GAE/100g DM).

These results bring attention to the richness of peel in phenolic compounds compared to the seeds. And that may be due to the nature of the phenolic compounds contained in these residues and by the assay method used, the assay by the Folin-Ciocalteu reagent is not very specific.

The results obtained confirm the difference in the content of total phenolic compounds between seeds and peel. The EC50 values and antiradical power (ARP) of peel extracts compared to a standard antioxidant (ascorbic acid) are shown in Table 2 and Figure 1.

Table 2: Antioxidant activity, EC50 and antiradical power (ARP) of peel extracts compared to a standard antioxidant (ascorbic acid).

Concentration of extracts (µg/mL)	% Inhibition		Reference antioxidant
	MeOH	EtOAc	
12,5	38.88	23.16	*Ascorbic acid*
25	41.34	23.92	
50	47.87	26	
100	52.77	37.07	
200	60	47.98	
EC50 (µg/mL)	88.73	208.38	14.24
ARP	0.011	0.0047	0.07



(a) (b)

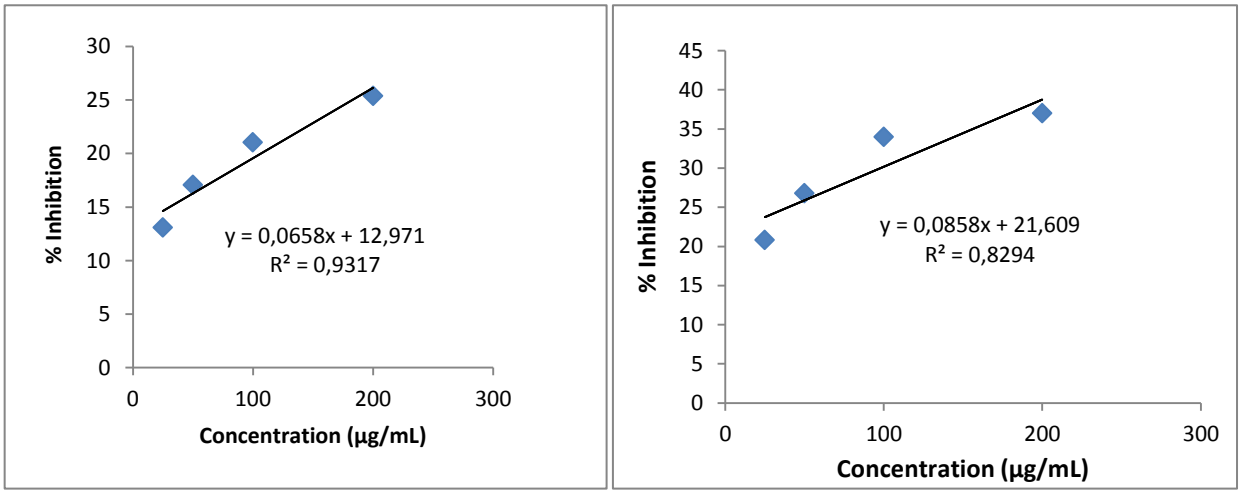
Figure 1: The variation curves of the DPPH inhibition as a function of the concentration of the peel extracts: (a) extract of MeOH and (b) extract of EtOAc.

The methanol peel extract had a good antioxidant capacity with EC50 achieved (88.73µg/mL), this extract was able to reduce the stable free radical DPPH which reflected by the change of the color from purple to yellow. This antioxidant activity is mainly due to the presence of phenolic compounds (32 mg GAE/100g d.w.).

Seeds extracts also render the free radical (2.2-diphenyl-1-picrylhydrazyl) stable with very little activity. The results obtained are illustrated in Figure 2 and Table 3. This result show that the reducing power of the seeds extract is lower than the peels extract. However, its EC50 values are higher (331-562.75µg/mL), which can be explained by the low content of phenolic compounds (7.4-18.74mg GAE/100g d.w.).

Table 3: Antioxidant activity, EC50 and antiradical power (ARP) of seeds extracts compared to a standard antioxidant (ascorbic acid)

Concentration of extracts (µg/mL)	% Inhibition		Reference Antioxidant
	EtOH	MeOH	
12.5	19.6	12.3	Ascorbic Acid
25	20.8	13.09	
50	26.8	17.06	
100	34	21.03	
200	37	25.4	
EC50 (µg/mL)	331	562.75	14.24
ARP	0.003	0.0017	0.07



(a (b

Figure 2: Percentage inhibition of DPPH depending on the concentration of seeds extract: (a) MeOH extract, (b) EtOH extract.

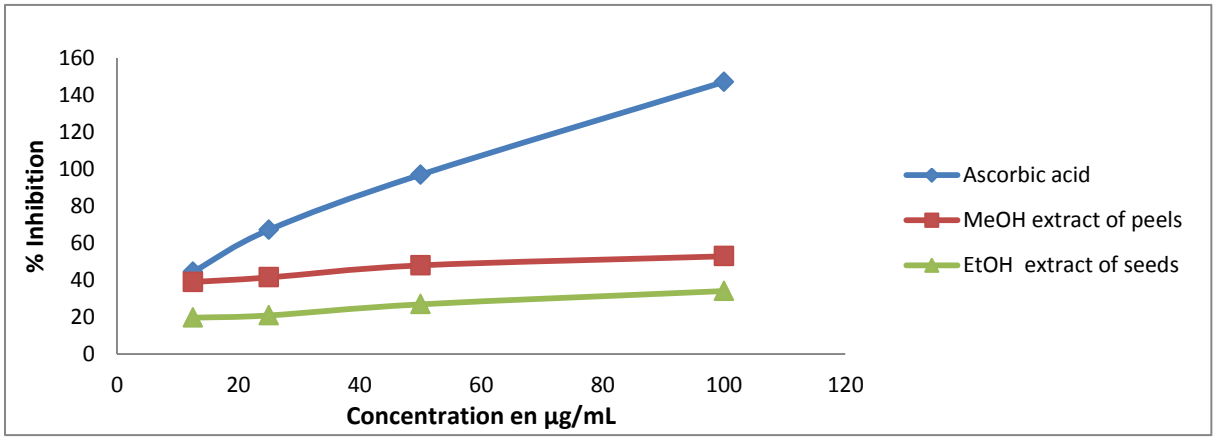


Figure 3: The comparison of percentage inhibition of peel MeOH extract and seeds EtOH extract with ascorbic acid.

Figure 3 shows the comparison of the results of percentage inhibition of the radical DPPH according to concentration of the compounds tested. The percentage inhibition of DPPH increases with the concentration for the three samples: ascorbic acid and the two extracts studied. The percentage of inhibition of ascorbic acid remains higher than that of the methanolic extract of peel which is greater than that of the extract ethanolic of seeds.

4. Conclusion

The results of the present research suggest that the tomato seeds oil can be nutritionally beneficial to human health, by its high content of unsaturated fatty acids and various other elements. The oil obtained has quality parameters close to those indicated by the Codex Alimentarius for fats and oils. The content of total phenolics in the extracts was determined spectrometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalents (GAE). Among extracts obtained, remarkable high antioxidant activity and high total phenolic content (GAE > 20mg/g) were found in peel extracts (22.75-32mg GAE/100g d.w.) compared to seeds extracts.

The antiradical activity of peel and seeds extracts was evaluated by the DPPH test. The overall results showed that the methanol peel extract has strong antioxidant capacity with EC50 reached (88.73µg/mL).

in conclusion, tomato wastes are valuable as a source of comestible oil, polyphenols and bioactive compounds. To fully exploit these important sources of natural antioxidants, further characterization of the phenolic composition is necessary. But overall, these results remain useful for developing standards on these materials.

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Cite this article: Najat Abbassi, Said Ait Talhajt, Salah Fadel, and Mohamed Ahra. TOMATO POMACE VALORIZATION BY OIL AND BIOACTIVE COMPOUNDS EXTRACTION: CASE OF SOUSS-MASSA REGION. 2021*; 12(5): 211-216.*