Chemical and Biological Studies on Some Bioactive Components Separated During Olive Oil Extraction

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5. Summary

The olive tree (*Olea europaea L.*) is known as the oldest cultivated tree in the world and is an evergreen tree belongs to Oleaceae family. Olive is considered one of the important fruit crops in Egypt with a high economic value.

Olive oil is one of the very few oils that can be consumed in its natural form, thus preserving all its natural constituents. The composition of olive oil can give valuable information in understanding their functional, qualityand nutritional properties. The main type of fatty acid found in all kinds of olive oil is MUFAs.The most common processes for produce olive oil are used: traditional pressing mills (mainly employed by small producers), the three-phase system, and the two-phase system.

The extraction of olive oil results in the production of huge amount of wastes, accounting to solid waste (olive pomace, OP), olive leaves (OL) and liquid waste (olive mill wastewaters, OMWW) from olive fruits. Such substantial amounts of wastes may have harmful effects on the environment.

Our investigation aims to study the effect of extraction methods on the amount and the quality of produced oil. In addition to that try to produce useful material from wastes which produced during the production of olive oil and study the effect of bioactive components separated from these wastes on health and growth of rats.

The results show that:

5.1. Olive oil extraction processing and physiochemical properties:

5.1.1. Olive oil and their by-product yields as affected by different extraction methods used:

The content of olive oil of produced from CP3P method was higher than that produced from PP method. Where the extracted oil was 13.35±0.15 and 11.75±0.25 for CP3P and PP methods respectively.

The percentage of OMWW as shown 54.45±0.15 and 53.25±0.45 for CP3P and PP methods respectively. The high percent of CP3P method may be due to the large amount of water used during production in a comparison with PP method.

5.2. Physiochemical olive oil properties:

5.2.1. The flow rate of olive oil:

The flow rate of olive oil was 4.709±0.78s and 4.688±.035s for CP3P and PP methods respectively.

5.2.2.Acid value of olive oils:

The acid value and acidity (percent as oleic acid) were (0.56±0.005, 0.283±0.002) and (0.60±0.005, 0.302±0.005) for CP3P and PP methods respectively.

5.2.3. Refractive index of olive oils:

The average outcomes of the refractive index have been obtained by refractometer, the RI values for PP and CP3P were given the same value (1.470±0.00) at 25 °C.

5.2.4. Saponification value of olive oil samples:

Thesaponification value of olive oil produced from CP3P method had higher saponificationvalue (197±2.86mg KOH g^{-1})than that of PP method (191.47±12.79 mg KOH g^{-1}).

5.2.5. Peroxide value of olive oil samples:

As results of peroxide values showed no significant differences between different production methods.

5.2.6.Insoluble impurities values of olive oil:

The Insoluble impurities are diverse with the extraction methods of olive oil, for CP3Pmethod shown the high value of insoluble impurities with value of 2.239±0.477, while for the PP method was 1.402±.064.

5.3. The wastes and their physiochemical properties:

5.3.1. Water content of olives:

The results of water content were confirmed with a previous report that the water content of olive varies from 47.55 to 52.89%.

5.3.2. Electrical conductivity (EC) ofolive mill wastewater:

The electric conductivity of CP3P method was 3.12 mS/cm, meanwhile the EC of PP method was 3.56 mS/cm.

5.3.3. Total suspended solids (TSS) of olive mill wastewater:

The TSS for CP3P method was higher than the TSS of PP method, where it was 0.41 and 0.277 for CP3P and PP methods respectively.

5.3.4. The pH of olive mill wastewater:

The pH of OMWW from CP3Pmethodwas 5.620. On the other hand, the pH of the PP method was 5.22.

5.3.5. The total solids inolive mill wastewater:

The total solids of OMWW produced from CP3P and PP methods were found to be 3.04 and 4.29%, respectively.

5.3.6. The color of olive mill wastewater:

The color of OMWW observed from CP3P method was reddish-brown, meanwhile the color of OMWW observed for PP method was black.

5.4. Chemical composition of leaves and pomace:

Pomace produced by PP method has a higher percent of moisture content, ash and residual oilthan the extraction with CP3P method. Meanwhile, it has low content in nitrogen free extract.

Olive leaves according to the results, it could be considered as a rich source of minerals.

5.5. Extracts yield of Bioactive compounds from wastes:

The results show that the extracts yield values of OPCM, OPPM, and OLM of methanolic extract were 7.22, 5.38, and 24.57 % respectively for conventional method. Whilethe extracts yield values of OPCM, OPPM, and OLM of methanolic extract were 10.02, 8.64, and 38.98% respectively

5.6. Effect of olive oil extraction methods on bioactive components of olive pomace extracts:

Extract from olive pomace produced from PP extraction method contains a high percent of Squalene, stearic acid, phytol, undecanal, Campesterol and β- Sitosterol therefore the olive oil produced bythe PP extraction method may have low stability against oxidative rancidity in comparison with the olive oil which produced by the CP3P extraction method.

5.7. Bioactive components of olive leaves extract:

The olive leaves extract contains Hydroquinone (8.41), β- Sitosterol (7.51), Squalene (4.11), Tyrosol (1.73), and Hydroxytyrosl (1.6) which have antioxidants activity. The results showed that olive leaves extract is a rich source of natural antioxidants and may increase the stability of edible oil against oxidative rancidity.

5.8. Determination of total phenolic compounds of by-products extracts:

The olive leaves extract was the highest percent of total polyphenols in comparison with olive pomace produced from different extraction methods (101.52±0.18 mg GAE/g DW). Meanwhile, there is no marked differences between the two extraction methods where the total polyphenol content of olive pomace from CP3P method (**OPC**) and olive pomace from PP method(**OPP**) extracts were 96.78±0.23 and 96.89±0.21 respectively.

5.9. Evaluation of antioxidant activity ($DPPH^{\bullet}$) radical -scavenging activity:

The outcomes of scavenging properties of the OPPM, OPCM, and OLM extracts proved that the increasing the concentrations of all tested extracts cause increasing inhibition ratio. Once low concentration 31.25µg/mL was used, the scavenging activity was ranged from 26.72% for OLM extract to 35.49% for OPPM extract. While, when the concentration was increased to 125 µg/ml the Scavenging activity was increased to 76.18%, 38.25%, and 71.25% for OLM, OPCM, and OPPM respectively. The extract that showed relatively high scavenging activity was (OLM), which contained the highest amount of total phenolic compounds.

5.10. ($ABTS^{\bullet+}$) radical -scavenging activity:

The inhibition of scavenging $ABTS^{+}$effect of OLM, OPPM and OPCM extracts on $ABTS^{+}$,exhibited that the radicalswereincreased with increasing the concentration.

5.11. Oxidative Stability of soybean oil as affected by the addition of olive leaves extract:

5.11.1. Peroxide value under acceleration storage:

The significant differences ($p<0.05$) werenoticedbetween the PVs of the control and the samples contain 5, 10, 50, 100, and 200 ppm of OLE and 200 ppm BHT. The PV values of control were the higher thanall treatments. Nevertheless, the samples contain 5, 10, 50, and 100 ppm from olive leaves extract showed higher PVs compared to the samples containing 200 ppm of olive leave extract and BHT. The samples containing 200 ppm of olive leaves extract and BHT nearly have the same antioxidant activity.

5.11.2. Thiobarbituric acid under acceleration storage:

The TBA value for soybean oil samples treated with olive leaves extract, BHT and control. The TBA values for all treatments and control increase with increasing the incubation period until 24 hours. The TBA values in control are higher than all treatments until 24 hours. The TBA values for the control and treatments fluctuated between decreasing and increasing until 72 hours. After 72 hours the TBA value of treatments is lower than control that is mean olive leaves extract and BHT have inhibited the oxidative rancidity.

5.12. Influence of different extraction methods on fatty acid composition of olive oils:

The fatty acid composition affected by the extraction method. The results indicated that themain fatty acid composition of olive oil extracted by CP3P and PP extraction methodswas oleic acid (74.11-79.19%), palmitic (13.9-9.56%), linoleic (6.94-8.30%), stearic (1.56-2.36%), and palmitoleic (0.75-1.66%), respectively. The olive oil extracted by CP3P is lower in oleic content (74.11%) than the olive oil extracted by PP (79.19%).

The olive oil produced from both extraction methods is rich in linoleic (ω-6), where it was 6.94% and 8.30% for CP3P and PP extraction methods respectively. The linoleic fatty acids (belonging to the omega-6) and α-linolenic fatty acids (belonging to the omega-3) are considered essential.

The ω-6 / ω3 ratio of the PP extraction method was relatively higher than that reported by the joint Food and Agriculture Organization/World Health Organization committee, whereas the CP3P extraction method showed comparable ratio with that reported by WHO/ joint FAO (16.60 and 9.50, respectively).

The results also indicated that MUSFAs for oil produced by CP3P and PP extraction methods were 74.11 and 79.19 respectively.

5.13. Biological investigation of olive leaves extracts:

All of the existing animals in this investigation take an oral dose of olive leave extract. The male rats had been fed onvariancediets depending upon the kinds of oils were used in this investigation. The male rats were separatedinto 5 groups. The selection of dietary fat was relied on varied in fatty acid composition of these oils and fats.

The rats have beenpartition into 5 groups by the experimental meals for:

Group (1):the rats were fed on basal diet with (Control (corn oil 7%)).

Group (2): rats were fed on basal diet with (Corn oil with OLE 100 mg/day/kg).

Group (3): rats were fed on basal diet with (Extra virgin olive oil 7%).

Group (4): rats were fed on basal diet with (Tail fat7%).

Group (5): rats were fed on basal diet with (Tail fat with OLE 100mg/day/kg)

5.13.1.Clinical symptoms:

The rats in third group which fed on olive oil showed a high appetite for the diet, a normal moving in their plastic cage, no diarrhea and no increase in their temperature. All other groups except the third group showed a relatively stable in rat weight.

5.13.2.Growth of rats and organs weight:

The results refers that the group 2 (corn oil with a dosage of OLE) the highest body weight gain (18.59%±6.82), while the rats in group 3, those fed on diet mixed with olive oil (7%) showed a decrease in body weight gain in comparison with control group (-5.78%±0.65). These differences in weight gains could be accounted for by the difference in fatty acid composition.

4.13.3. The observation of organs weight:

The results showed there is not significant differences of weight of all organs for all groups, that is mean OLE has no side effect on these organs within used concentration.

5.13.4. Change in serum lipid profile:

Atherosclerosis is the single largest cause of death and disability in the Western world and in the next two decades will be the leading cause of death worldwide. Total plasma cholesterol (TPC), high densitylipoprotein (HDL), low density lipoprotein (LDL) and triglycerides (TG) are used as indicator for atherosclerosis.

5.13.4.1. Blood triglycerides level:

The triglycerides for rats in group 4 which fed on tail fat have the highest serum TG 122.00±7.48 mg/dl in comparison with all other treatments, on the other hand the rats in group 5 which fed on tail fat with OLE have TG 99.67±6.55 mg/dl. These results indicated that OLE reduce the triglycerides to that value of group 1 (control) which fed on corn oil only.

5.13.4.2. Blood total cholesterol level:

The results showed that the rats in all groups at the end of the experiments have total cholesterol ranging from 99.67 to 100.67 mg/dl except group 4 which fed on tail fat (122.00 ±7.48mg/dl). The highest value of serum TC for rats in group 4 which fed on tail fat may be due to the high percent of total saturated fatty acid. We noticed that the olive leaves extract which contains a high percent of natural antioxidants prevent the harmful effect of tail fat.

5.13.4.3. Blood HDL blood level:

The results of Blood HDL analysis showed no significant differences within all groups at the end of the experiment.

5.13.5.4. Blood LDL blood level:

The results showed that the rats in all groups at the end of the experiments have LDL ranging from 29.40 to 35.67 mg/dl except group 4 which fed on tail fat (40.2mg/dl). The highest value of serum LDL for rats in group 4 which fed on tail fat may be due to the high percent of total saturated fatty acid.

5.13.5.5. Blood VLDL blood level:

The results of VLDL showed that no marked differences in VLDL at the end of experiment between groups 1, 2, 3 and 5. Meanwhile, the rats in group 4 which fed on tail fat have the highest VLDL value (22.8 mg/dl). We observed that the group 4 which fed on tail fat has the highest value in TG and VLDL.

5.13.5.6. Total lipidsin serum blood:

The results mentioned the group 4 which fed on tail fat has the highest value of total lipids. Meanwhile the group 3 which fed on olive oil has the lowest value of total lipids. The results of total lipids showed that the olive leaves extract and olive oil reduced the total lipids.

5.13.6.Change in kidney function:

The results were reported thatthe blood urea nitrogen (BUN) and creatinine (Creat) in the serum blood of rats which feeding of diets having different types of oils and fats with dosage with OLE. The group 2 and 5 showed lower BUN, where it was(23.53±3.29 and 22.65±2.62) respectively, in comparison with the zero time. At the end of the experiment, the group 1 (control) which fed on corn oil showed the highest BUN between all treatments (26.00±1.63).

5.13.7.Change inliver function:

The results of analysis of alanine transaminase (ALT) levels in the group 2 showed the lowest in ALT (33.00±2.16) which fed on corn oil with OLE in comparison with the group 1 (control) which fed on corn oil. The addition of OLE to tail fat decrease ALT from 49.00±7.12 to 41.00±1.63 this may be due to the natural antioxidants which found in OLE. At the same time addition of OLE to corn oil decrease ALT from 50.00±5.72 to 33.00±2.16.

The results of the analysis of aspartate transaminase (AST) levels were showed the same trend for ALT.

5.14. Histopathological examination of heart:

Microscopic investigation of the heart of Albino male rats were taken place in all experimental groups at the end of experiment period (60 days) to determine the extent of tissue deteriorationcaused by corn oil 7% (control), corn oil with OLE dosage, Olive oil 7%, tail fat 7% (positive), and tail fat 7% with OLE dosage (negative).

5.14.1. Histopathological observations of the liver under light microscopy:

Microscopically, heart of rat from group 1 revealed the normal histological structure of cardiac myocytes. Meanwhile, heart of rats from group 2 showed slight edema between the cardiac myocytes. Associated with few inflammatory cells infiltration. Furthermore, the sections from group 3 revealed no histopathological alterations. Moreover, heart of rats from group 4 showed sections revealed focal necrosis of cardiac myocytes associated with inflammatory cells infiltration. Otherwise, heart of rats from group 5 revealed no histopathological alterations.