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ORIGINAL ARTICLE

Study of Carbohydrate, Antioxidant, Nutrient and Genomic DNA Quantification of Olive Treated with Alkaline and Acidic Solvent: An Innovation

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ABSTRACT
The experiment was conducted to investigate biochemical content, nutritional and DNA characterization of olive fruit treated with alkaline (Sodium chloride anhydrous) and acidic solvent (5% acetic acid, vinegar). The treatments were used as control (no water), water control, 10% sodium chloride anhydrous (NaOH), Vinegar (5% acetic acid), Vinegar + NaOH and Vinegar + NaOH + Hot water treatment. Our results showed that inverted sugar and glucose content were higher in the Vinegar and NaOH treated olive than in other treatments. Fructose content was the highest in Vinegar + NaOH treated fruit. Nutrient contents NO3 K, Ca and Na were found higher in the treated fruit than the control fruit. Moreover, maximum K content was observed in the case of all treatments compared to the other nutrient content. The highest acidic (lower pH) condition (sour) was found in treated fruit. DNA yield was found higher in water control than acid and alkaline treated olives. DNA band was wider in the olive treated water control compared to the NaOH, vinegar, Vinegar + NaOH and Vinegar + NaOH + Hot water treatment. Finally, results suggest that vinegar + NaOH treated olive fruit was the best for fresh olive homemade processing after harvesting for edible purpose.
Keywords: olive, vinegar, sugars, nutrient content and DNA band

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INTRODUCTION

The olive (*Olea europaea*) contains a significant role as fruit crops especially in the Middle East and African countries. It is of major sources of fruit industries in the Mediterranean region as the source of olive oil [5, 2]. Saudi Arabia produced 20% of olive in the world demand [4, 8]. Olive fruit and olive oil have been used as the food scene for the healthiest alternative to other edible oils. Olive keeps an important role as nutritional and medicinal fruit for all over the world. Some studies have been suggested that olive oil assisted to reduce the levels of low density lipoprotein [6]. The study has been observed that female had got satisfactory defend against ovarian cancer who had taken greater olive oil [18]. Studies showed that olive or olive oil might keep a potential role in decreasing trends or threat of different kinds of cancer, especially colon, breast, ovarian and prostate cancers. It has been stated that 1,031 having ovarian cancer as well as 2,411 lacking of cancer out of approximately 3,500 Italian women [15]. Consuming the maximum quantity of olive oil had the minimum of ovarian cancer and decreased 30% of certain disease [15]. Khandaker *et al*., [13] reported that wax apple fruit contained phenol and anthocyanin content which could protect the cancer and chronic disease and cardiovascular disease [10, 11]. Nowadays it is found in literatures that fresh olive juice helps to reduce cancer other than olive oil. Postharvest quality of olive keeps a significant role to use it as food products. It is difficult to use as edible food of directly harvested olive fruit due to the bitterness like acidic condition. It would be easier to eat while alkaline or acidic medium can change its bitterness characteristics due to the changing of biochemical and nutritional value and genomic quality. There is no literature found that postharvest olive

can be used as edible fruit as nutritional and medicinal value. This is why, the current study has been designed and attempted to investigate the effect of (alkaline, NaOH) and acidic solvent (vinegar) on the biochemical content like pH, sugar, glucose, antioxidant, flavonoid and nutrient (Na, K , Ca, NO3) as well as DNA quantification of fresh olive fruit.

MATERIALS AND METHODS

Materials

Matured olive fruit was harvested from olive garden, Hail and Al-qasim region, Saudi Arabia.

Methods

Five kilogram olive fruit were collected randomly from the garden. Twenty olives were employed for the each treatment. Total of 120 olives were used for the experiment. Randomized Complete block design was designed. The treatments were (Fig. 1). The treatments were used as control (no water used), water control (olive was soaked into the water), 10% sodium chloride anhydrous (NaOH), Vinegar (5% acetic acid), Vinegar + NaOH and Vinegar + NaOH + Hot water treatment (Olive was heated for 2 hours by oven at 100 ^{0}C with water, vinegar and NaOH). The samples were soaked for 5 days. Afterwards, samples were taken out from the solution and put it in the freezer.

Juice preparation or extraction

The samples were ground with motor and pestle and filtered the extract and finally extracted olive juice was separated and stored in the freezer.

Data analysis

Biochemical (glucose, inverted sugar and fructose) content was determined. Finally DNA isolation and quantification were done by gel electrophoresis.

Glucose content test:

Glucose was checked by using glucose refractometer. Three drops of olive juice sample were placed on the disc of the meter and data were observed and documented.

Inverted sugar investigation

Inverted sugar was investigated by using inverted sugar refractometer. Three drops of olive juice sample were placed on the disc of the meter and data were observed and recorded.

Fructose content investigation

Fructose was tested by using fructose refractometer. Three drops of olive juice sample were placed on the disc of the meter and data were investigated and analyzed.

Total antioxidant investigation

1mM Trolox Standard Solution was used. Water was poured to each well to make the volume to 100 µL. Samples were directly added to the wells. For small molecule TAC, samples were diluted at 1:1ratio with Protein Mask. 20 µL of sample was used into wells. Distilled water was put in making the volume of 100 µL. 100 µL of Cu2+ Working Solution was added to all standard and sample wells and mixed properly using a horizontal shaker and the reaction was incubated for 90minutes at room temperature. The plate was protected from light at the time of incubation and finally made the measurement of the absorbance at 570 nm (A570).

TSS and pH test

Total soluble solid (%brix) was determined by Refractometer. pH was determined by pH meter.

Flavonoid investigation

Total flavonoid content (FC) was investigated with aluminum chloride colorimetric assay, using catechin as a standard.

Total Phenol investigation

Total phenolic content was evaluated by using the Folin-Ciocalteu assay [19]. Folin-Ciocalteau (FC) colorimetry is consisted of a chemical reduction of reagent, tungsten mixture and molybdenum oxides. 1ml of juice, gallic acid calibration standards, folin- Ciocalteau (FC) reagent were stored in the dark and separated until reagent has appeared as green, Sodium carbonate solution (100-ml) was utilized in the volumetric flask. Spectrophotometer was placed to 765 nm, having 1-cm, 2-ml plastic or glass cuvettes. 1ml of extract was poured to 25 ml of volumetric flax, having 9 ml of distilled water. 1 ml of Folin – Ciacalteu's phenol reagent was poured to the mixture. The solution was diluted with distilled water and mixed well. Finally, the solution was incubated at room temperature. Absorbance was determined against reagent blank at 750 nm using an UV-Vis Spectrophotometer.

Nutrient content investigation

Nutrient content (N, as NO3, K and Ca) was investigated using Horiba NO3, K and Ca meters (USA). 1 drop of juice sample were placed on the disc sensor of the meter using small dropper and data were observed and listed.

DNA isolation

5ml CTAB was heated (1210µl mercaptoethanol was added to each 5ml CTAB) in a centrifuge tube (blue-topped of 50ml) at 60-65^{o}C. Fruit skin was separated and wrapped with aluminium foil and stored in freeze having liquid nitrogen. Sample (1.0 g tissue/5ml CTAB) was stored for 2 days at –20 0C liquid Nitrogen. Fruit tissue was crumbled in cold pestle of liquid nitrogen. Ground fruit samples were added 0.5 spatula of PVPP powder using one spatula of fine sand. Powder was scraped into dry tube and poured heated buffer and mixed smoothly. CTAB volume was adjusted to get a slurry-assembled consistency then incubated for 60 min at 60 o C. The same volume of chloroform/iso-amyl alcohol (24:1) was poured and mixed well 2for 3min, then transferred to the centrifuge tubes. The rotation was 5,000rpm in spin. Supernatant was taken out by using wide-bore paste to clean tube and repeated chloroform extraction. DNA was precipitated having 0.66 vol. of cold isopropanol and kept overnight. DNA was spooled out for 2min at 10,000rpm. DNA sample was transferred to the 5ml buffer for 20min for washing then dried briefly. 1µl 10mg/ml of RNAse enzyme was added to each 1ml T.E./DNA mixture and stored for 60min at 37 o C. It was diluted in TE, then added 0.3vol 3M sodium acetate. Spooled DNA was removed, dried and stored in freeze until required.

DNA Quantification and characterization

DNA weight was measured by electric balance using eppendrop tubes.

Materials

Electrophoresis, micropipette, Gel tray and comb, 3loading dye, ethidium bromide, agarose, 1X TBE buffer, 1.5 ml eppendorf tubes

Method of DNA characterization

A 0.8% agarose gel was prepared using 99.2% 1x TAE and 0.1µl of Ethidium bromide (10mg/ml)/ 10ml solution. Load samples was undiluted and in a 1 in 10 dilution with 3µl loading buffer. Incubated for 2 hours at 38 0C then loaded loading dye (31 ul) into each sample. Micropipet was adjusted to 11 ul and load the samples in lanes 2-6. In lane 1, DNA standard added the (1 ug of DNA) standard (Lambda/HindIII digestion [10 ul sample]) plus 1 ul of loading dye. It was run at 100 volts for 1.5 hour. The gels were stained for 5 minutes in ethidium bromide and de-stain having water for 2 min. DNA fragments were migrated rapidly in the gel matrix based on size.

Statistical Analysis

Data were analyzed statistically by DMRT. Standard error and DMRT were employed.

RESULTS

Inverted sugar content was higher in NaOH and Vinegar than in control, water control and other treated fruit (Table 1). However, glucose content was the highest in the NaOH +Vinegar treated fruit (Table 1). Besides, fructose content was higher in NaOH +Vinegar and NaOH +Vinegar +hot water treated olive than in control, water control, NaOH and Vinegar alone treated olive (Table 1). The highest TSS content was found in the NaOH +Vinegar treated fruit. Moreover, the highest pH was found in the NaOH +Vinegar +hot water treated olive (Table 2). In Table 3, it has been exhibited that nutrient contents like NO3-, K+ and Ca++ were higher in the fruit treated with NaOH, vinegar, NaOH+Vinegar and NaOH +Vinegar+hot than in control (no water) and water control fruit. However, the highest K+ and Ca++ content were found in NaOH+Vinegar treated fruit as well as NO3- was the highest in the NaOH +Vinegar+hot water treated fruit. In addition, Na+ content was higher in NaOH NaOH +Vinegar and NaOH +Vinegar +hot water than in control, water control, and Vinegar alone treated olive (Table 3). Flavonoid content was higher in NaOH alone, vinegar alone and NaOH +Vinegar than in control, water control and NaOH +Vinegar+hot water (Table 4). Total antioxidant was found to be higher. However, the highest flavonoid content was found in NaOH treated fruit. Total antioxidant content was higher in NaOH, vinegar, NaOH +Vinegar and NaOH +Vinegar+hot water treated fruit than in control, water control shown in Table 4. However, the highest total antioxidant was found in the NaOH +Vinegar treated fruit. DNA yield was higher in the fruit treated with water control, vinegar + NaOH and vinegar + NaOH+ boiling olive compared to the fruit treated with NaOH and vinegar (Table 5). Fig.1 shows the fruit structure after the treatment application. DNA band/probe was wider in the fruit treated with water control, vinegar + NaOH and vinegar + NaOH+ boiling olive than in the fruit treated with NaOH and vinegar (Fig. 2).

Table 1: Glucose, fructose and inverted sugar measurement at different treatment. Means followed by the common letters are not significantly different at the 5%level by Duncan's Multiple Range test (DMRT). Mean ± SE (n= 10).

Treatment	Glucose Content (%)	Fructose Content (%)	Inverted Sugar Content (%)
Control (No water)	5.03± 0.1a	2.1± 0 a	6.6±0.2a
Water	11.5±0 b	3.3±0.1 a	9.3±0.1a
NaOH	13.3± 0.2bc	12.3± 0.2b	17.5±0.5c
Vinegar (V	13.7± 0.1bc	13.3±0.1b	17±0.4bc
Vinegar + NaOH	14.9±0 c	15.4±0.2c	14.8±0.3b
NaOH + Hot water+V	12.4±0.1b	15.1±0.1c	13.3±0.2b

Table 2. TSS and pH determination at different treatments. Means followed by the common letters are not significantly different at the 5%level by Duncan's Multiple Range test (DMRT). Mean ± SE (n= 10).

Treatment	TSS /Brix (%)	pH
Control (No water)	4.0 ±0.05a	2.5±0 a
Water	4.2±0.02a	4.0 ±0.01a
NaOH	5.0±0.01a	4.5±0.01a
Vinegar (V)	4.8±0.03a	5.5±0.02a
Vinegar + NaOH	9.2±0.05b	7.6±0.03ab
NaCl + Hot water+V	8.1±0.04b	8.0±0.05b

Table 3. Nutrient content determination at different treatments.Means followed by the common letters are not significantly different at the 5%level by Duncan's Multiple Range test (DMRT). Mean ± SE (n= 10).

Varieties	K+ (PPM)	Ca++ (PPM)	NO3- (PPM)	Na+ (PPM)
Control (No water)	903±6.1a	37±0.2a	485±1.0a	18±0.01a
Water	949±5.1a	38±0.2a	550±1.5a	18±0.03a
NaOH	1127±4.2a	111.3±0.7b	637±2.4 a	420±0.9c
Vinegar (V)	2308±5.3b	113.4±0.6b	807±2.6b	80±0.5bc
Vinegar + NaOH	3600±4.6c	120.4±0.5b	904±1.9b	430±0.7
NaOH + Hot water+V	2200±3.9b	117.0±0.6b	957±1.8b	443±0.6c

Table 4. Flavonoid and antioxidant determination at different treatments. Means followed by the common letters are not significantly different at the 5%level by Duncan's Multiple Range test (DMRT). Mean ± SE (n= 10).

Varieties	Flavonoid (mg/100g)	Total antioxidant (mg/100g)
Control (No water)	1.25±0.001a	350±0.8b
Water	1.25±0.002a	350±0.7b
NaOH	36.05±0.05c	755±0.6c
Vinegar (V)	10.40±0.03b	418±0.3a
Vinegar + NaOH	16.60±0.07b	985±0.5d
NaOH + Hot water+V	3.70±0.004a	574±0.9c

Table 5. DNA yield of olive at different treatment. Mean ± SE (n= 10).

Treatments	DNA yield (ng/ul)
Control (No water)	89±0.6
Water	89±0.6
V+NaOH	88±2.7
Vinger	68±1.9
NaOH	30±8.1
V+NaOH+ boiling	88±2.3

DISCUSSION

From the above mentioned results it has been seen that NaCl anhydrous (NaOH), vinegar and hot water (Heat treatment), water control treatment showed better performance in the case of biochemical content, carbohydrate, nutrient, flavonid, antioxidant and phenol content as well as DNA characterization . It may be due to the water condition (soaking), NaCl (NaOH), vinegar an dhot water treatment (heat). Hossain *et al.,* [9] reported that pH, Soluble solid content (SSC), biochemical and nutritional value were affected by postharvest preservation techniques like light, heat, temperature, hot water etc. of elephant apple fruit [7]. Cinday [3] stated that NaCl and KCl reduced the bitterness of fruit and increased saltiness. Jungbunzlauer. [12] described that the bitterness of stevia and erythritol fruit in light soft drinks has been reduced by sodium chloride. Anderson [1] stated that the brussel sprouts were soaked in cold water for 10 to 15 minutes and put a medium or large pot of water on to boil. Once the water was boiled and added the salt to the water. Then it has become cool down and added the balsamic vinegar or freshly squeezed citrus and resulted the reducing the bitterness. Olive fruit and olive oil are a good source of vitamin E and other necessary phytonutrient components including polyphenol and flavonoid which shows a significant anti-inflammatory properties and delay aging. In addition to that it assists in the restoration of body tissues. A defensive mechanism against cancer, liver disorders, atherosclerosis and inflammations has been exhibited by using olive oil [17, 20] reported that potassium and phenol content in olive (local variety) were affected by water use and other physical factors. She described that potassium content varied 4.76 -6.55g/1kg and phenol content was varied from 1-12.4mg/kg at different treatments in fresh fruit after harvest. Moreover, K+ and NO3-, content was higher in the case of all treatments compared to the other nutrient content (Table 2). This might be due to the effects of water, hot water, NaOH and vinegar treatment. DNA band/probe was wider in alkaline and acidic mixture treatments than in acid and alkaline alone treatment. DNA might denature by the given acid (5% acetic acid) and alkaline ((NaOH) treatments. This is why, band was not shown there. This might be the effects acid and alkaline solution. The effect may be responsible for the strength of cell membranes, leads protein folding and the introduction of membrane proteins [16]. It was reported that estimated 1320–30% of entire genes in the genomes convert membrane proteins [14]. Therefore, it can be concluded that our results show10% sodium chloride anhydrous (NaOH), Vinegar (5% acetic acid), Vinegar + NaOH and Vinegar + NaOH + Hot water treatment are better solution than control and water control to reduce bitterness of fresh olive and increase biochemical and nutritional value. Finally it seems that Vinegar + NaOH is the best treatment.

Fig. 1. Photograph shows the olive with different solvent. 1: NaOH + Hot water+V, 2: Vinegar + NaOH, : 3: Vinegar (V), 4: NaOH, 5: water 6: Control (no water).

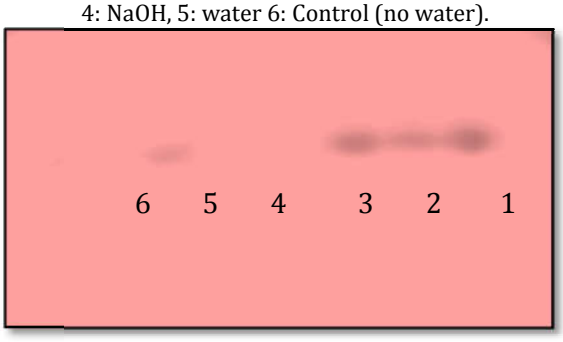

Fig. 2. DNA band characterization in different treatments. 1. DNA ladder (standard), 2. Water, 3. V+NaOH+boiling, 4. NaOH 5.Vanger, 6. V+NaOH,

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