

# The Shelf life Stability of Mixed Fruit and Vegetable Juice with *Moringa Oleifera* Leaves Extract

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## Abstract

**Background:** *Moringa oleifera* leaves (M.O.L.) have medical and nutritional properties, which make these leaves appropriate for using to make various food products such as juices. Fruits and vegetables commonly consumed as a beverage and juice. Mostly, to have higher quality juice with fresh taste, the standard is often fresh juice not-processed, however, their shelf life is less than 6 or 7 days depends on the fruit and vegetable types. Thus, they have been preserved by several methods such as thermal process. Therefore, the aim of this study was to evaluate the treated fruit and vegetable juices mixed with fresh M.O.L extract (F.M.O.L.E.) to produce untraditional healthy products as well as prolonging the shelf life with negligible loss of bioactive components. **Methods:** in this study each fruit and vegetable were boiled in water, blended, filtrated and then mixed as follows: Treatment 1, F.M.O.L.E. 40% with Pineapple 38%+Carrot 20 % +Ginger 2%. Treatment 2, mixed F.M.O.L.E. 50% with Pineapple 38% + Carrot 10 % + Ginger 2%. Treatment 3, F.M.O.L.E. 60% with Pineapple 28% +Carrot 10% + Ginger 2%. Microbiological, Sensorial and physicochemical analysis were conducted on juices (packed in glass bottles) during storage at 4±1°C. **Results:** Chemical analysis ascertained that F.M.O.L. is an exceptional source of ascorbic acid (139.46 mg/100g), Meanwhile, F.M.O.L.E. is a respectable source of phenolic compounds (38.76 mg GAE/mg) and antioxidant activity (77.65%), respectively. Results of sensory evaluation indicated that treatment (1) and (2) were acceptable between diverse panelists. Physicochemical analysis indicated that both treatment (3) and (2) have the lowest content of total acidity and highest contents of pH value, ascorbic acid (6.25 mg/100ml), total phenolic (73.21 and 66.41 mg GAE/ml) and antioxidant activity (76.33 and 72.10%), respectively at zero time and after one month of storage time. For now, these parameters were affected by storage time, the pH value, ascorbic acid, phenolic components and antioxidant activity were decreased during storage time of all treatments. Moreover, the color analysis of L\*, a\* and b\* value displayed that all treatments were light (L\*value) plus, tend to yellowness (+b\* value) more than color redness (+a\* value) at zero time. All values increased after one

month of storage time. Microbiological analysis showed that there is a growth of yeasts after one month of storage time in all treatments. Although, treatment (2) was not exceeding the maximum count permitted of the Gulf Standard. Therefore, the cooking process of fruit and vegetables to produce juices, mixed with F.M.O.L.E by 50% treatment (2) preserve the juice during storage time up to one month compared to other treatments. From HPLC analysis of treatment (2), it appeared that Epicatechin (EP), chlorogenic acid (CH), 4-O-Caffeoylquinic acid (CA), rutin (RU), quercetin (QU) were predominant phenolic components in treatment (2). Whereas, the others are dominant phenolic constituents, benzoic acid (BE) and cinnamic acid (CI). **Conclusion:** from all the aforementioned obtained results of sensory evaluation and other analysis emerged a successful and appropriate ways to produce fruit and vegetable juices mixed with 40% or 50% of F.M.O.L.E.

**Keywords:** Fruit and vegetable juice, shelf life, *Moringa Oleifera* extract, *Moringa oleifera* leaves.

## Introduction

*Moringa oleifera* Lamarck leaves (*pterygosperma* Gaertner) (Makkar and Becker, 1996), are rich source of nutrients and phytochemical components, which allow these leaves to be utilized to produce important products such as *Moringa* tea, juice, and dairy products. The use of M.O. leaves (M.O.L.) part have various medicinal, bioactive, antioxidant, and antimicrobial properties (Gopalakrishnan et al., 2016). The leaves have been used to cure 24 medicinal conditions, six of which include: asthma, flu, bone setting, impotence, heartburn and syphilis in Ugandan rural societies (Kasolo et al., 2010). In addition, it is useful for people with cardiovascular disease as it helps reducing cholesterol (Farooq et al., 2012; Adeyemi et al., 2014). It was found that fresh leaves have a protein content equivalent to 2 times that of milk, ascorbic acid content to 7 times that of oranges, vitamin A content to 4 times that of carrots, calcium content 4 times that of milk, potassium content 3 times that of bananas, and an iron content 3 times that of spinach (Farooq et al., 2012; Belay and Sisay, 2014). Moreover, the leaves contain high percentages of all the essential amino acids. Therefore, they can serve as a high-quality source of protein for people who do not get protein from meat sources (Mishra et al., 2012). In addition, it was proven that M.O. plant is superior as antimicrobial agent (Gopalakrishnan

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et al., 2016). The fresh M.O.L. extract (F.M.O.L.E.) has antimicrobial effects because of bioactive compounds (phytochemicals) such as alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids (Vinoth et al., 2012; Amabye and Tadesse, 2016). (El-massry et al., 2013) conducted HPLC analysis to identify phenolic compounds in F.M.O.L.E. Results showed Gallic acid (14.22 mg/100g), Chlorogenic acid (8.62 mg/100g), Ellagic acid (4.78 mg/100g), Ferulic (36.79 mg/100g), kaempferol (1.80 mg/100g), quercetin (28.56 mg/100g), rutin (97.68 mg/100g), syringic acid (2.66 mg/100g) caffeic acid (68.25 mg/100g), and catechin (18.16 mg/100g). The results indicated rutin, caffeic and ferulic acid were the dominant phenolic components in M.O.L. extracts. This concentration of phenolic showed that M.O.L. extract has antioxidant activity. The main bioactive components are responsible for antioxidant activity such as kaempferol and quercetin (Farooq et al., 2012). These results revealed that M.O.L. extract could be a strong source of natural antioxidants with many human health benefits. Aqueous extract is the strongest extraction medium able to dissolve maximum of the phenolic composites from the samples, and it is more preferred to extract polar phenolic acid (El-Sohaimy et al., 2015). Besides, M.O.L.E. can be utilized in food industry as a natural preservative, that can be used as an alternate to synthetic preservatives in the future (Bukar et al., 2010; El-Sohaimy et al., 2015). Studying M.O. trees which were planted locally in Saudi Arabia is critical, and more research on the way of its use in industrial applications in Saudi Arabia are needed. Furthermore, raising awareness of the effective uses of this unutilized crop and encouraging the consumption of it are needed. Fruits and vegetables are commonly consumed as juices, which are used as an ingredient or flavoring agent in foods industry (Ibrahim, 2016), but their shelf life is short. Consequently, there are various techniques to increase their shelf life such as pasteurization, nonthermal physical techniques, freezing, chilling, water activity, modified atmosphere packaging, and the addition of natural antimicrobials. The most common method is thermal processing, which has negative effect due to loss of original flavor and taste compounds occur in juices (Aneja et al., 2014). Nevertheless, it is still the most effective method to guarantee microbial safety and enzyme deactivation (Rawson et al., 2011). In the most studies on the effects of heat treatment on the total phenolic content, the results are contradicting. Generally, in developing countries the most common applied method in processing unit operations is heat. Cooking process (boiling) of vegetables lead to loss of antioxidant compounds in water, so it is bouncing to consume the water used for boiling to obtain the optimum benefits of bioactive components present in vegetables (Chipurura et al., 2010). Furthermore, it was referred that cooked moringa leaves (boiled) provide more bio-available iron and antioxidant content (Yang et al., 2006). The combination of the preservation methods with natural antimicrobial compounds in the preservation of juices with improved microbiological safety and acceptable organoleptic properties might be a new trend (Pandey and Negi, 2018). Therefore, the aim of the current study is to evaluate the treated fruit and vegetable juices mixed with fresh M.O.L extract (F.M.O.L.E.) to produce untraditional healthy products as well as prolong the shelf life with negligible loss of bioactive components.

## Materials and Methods:

### Materials

The fresh *Moringa oleifera* leaves (*Pterygosperma* Gaertner) were purchased from Durat Al-Ezdihar Farm in jizan, Kingdom of Saudi Arabia (KSA). The fresh fruits and vegetables varieties namely, pineapple (*Ananas comosus*), carrot (*Daucus carota* L.), ginger (*Zingiber officinale*) were purchased from the local market in Jeddah city. All were purchased during the years of 2016-2017. The cheese cloth was purchased from a local shop for fabrics (Al-Dahab street, Al-balad), Jeddah, Saudia Arabia.

### Chemicals for chemical analysis:

Ascorbic acid, meta phosphoric acid, 2,6 di-chloro phenol indophenol, and Folin-Ciocalteu's phenol reagent were purchased from Scientific Supply House, Jeddah. Sodium carbonate was obtained from Al-Shafei establishment. The chemicals used in crude protein analysis were obtained from Halwani factory, Jeddah. Standard Gallic acid (GAE), and deionized water was obtained from King Fahd for Medical Research Center. Sodium hydroxide (NaOH) and Methanol were obtained from laboratory of food and nutrition department. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich company, Jeddah.

### Chemicals for HPLC methods:

Nylon Syringe membrane disks, 0.20 u, 33 mm were purchased from Milli-Q (Poole, U.K.). 186007197C - Clear Glass 12 x 32 mm Screw Neck Total Recovery Vial, with Cap and PTFE Septum, 1 mL Volume, 100/pkg were purchased from Waters (milford, U.K.). Chromabond® Solid Phase Extraction column, C18 ec, 3 mL/500 mg (Macherey -Nagel GMBH, Duren, Germany). All standards components (Epicatechin, Antipyrine, Benzoic acid, Cinnamic acid, Chlorogenic acid, 4-O-Caffeoylquinic acid, Rutin) were purchased from Sigma Aldrich, purity > 99.8 % (Poole, U.K.). Sodium sulfate anhydrous (Na<sub>2</sub>SO<sub>4</sub>) and Methanol (MeOH) were purchased from Sigma Aldrich.

Packaging materials: The glass bottles and air-tight glass jars were purchased from Bin Shihoun Commercial Center, Al-balad, Jeddah.

### Methods:

#### Material's preparation:

1. Preparation of Fresh *Moringa oleifera* Leaves (F.M.O.L.) plus other vegetables and fruits:

The fresh M.O.L (7 kg) were harvested, separated from stalks, sorted (to remove the yellow and brown leaves), which would have changed the color of the final product. The sorted leaves, washed

to remove the dusts (Badejo et al., 2014). Other materials including carrot, ginger, and pineapple were washed, peeled, cut in slices and weighted (100 g each one).

#### 2. Preparation of pineapple, carrot, ginger, fresh *Moringa Oleifera* Leaves extract (F.M.L.E.):

One hundred grams (100 g) of cleaned *Moringa oleifera* leaves, sliced carrot (*Daucus carota*) and sliced ginger (*Zingiber officinale*) were slurred in 200 ml, 600 ml and 200 ml of treated water (Boiled at 100°C and cooled; boiled ginger and carrot for 30 minutes and moringa for 15 minutes), respectively, using a commercial laboratory blender (food and nutrition laboratory blender, Jeddah, KAU) at low speed of 19,000 rpm for 5 mins. The slurries were filtered using sterilized cheese cloth (Out et al., 2013), with some modifications. Cleaned peeled, and sliced pineapples were treated with water (Boiled at 100°C and cooled; boiled pineapple for 15 minutes). It was pulped using commercial laboratory blender (Food and nutrition laboratory blender, Jeddah, KAU) at low speed of 18,000 rpm for 5 mins and filtered using a sterilized cheese cloth.

Filtrates of Moringa, ginger, carrot and pineapple slurries were centrifuged (Hermile labortechnik GmbH, low speed model Z200A – 8x14ml bottles) to obtain a clear extract. All filtrated materials were packed in sterilized bottles, to mix immediately by different percentage.

#### 3. Experimental processing technology:

The prepared filtrates were subjected to blend together with F.M.O.L. as follows:

1. F.M.L.E. extract 40% + Pineapple juice 38%+Carrot extract 20 % +Ginger extract 2%.

2. F.M.L.E. extract 50% + Pineapple juice 38%+ Carrot extract 10 % + Ginger extract 2%.

3. F.M.L.E. extract 60% + Pineapple juice 28% +Carrot extract 10 % + Ginger extract 2%.

The blends were Packed in sterilized glass bottles, packaging materials and stored in refrigerator at  $4.0 \pm 1$  °C. Treatments conducted Microbiological, sensorial and physicochemical analysis at zero time and after one month of storage time.

#### 4. Analytical Methods:

##### 4.1. Physico-chemical analysis:

Determination of Moisture content, crude protein, pH value and total soluble solids (T.S.S %) were conducted according to the method defined by (AOAC, 2007). Total titratable acidity and

ascorbic acid content were detected by the methods of (Ranganna, 1977). Color hunter (L, A, B values) was determined by using Chroma Meter CR-400, Konica Minolta, SN: 888212539.

##### 4.2 Determination of antioxidant activity

The antioxidant activity (DPPH radical scavenging assay) was determined by the methods of (Su and Silva, 2006) using spectrophotometer (PD-303UV, Apel). It was performed using 0.5 g of sample with 15 ml of acid methanol by 1% HCL (at room temperature) for 1 hour. Then the extract was centrifuged at 2500 rpm for 15 minutes, the upper phase collected in a beaker, and the pH adjusted to 3. After that, it was added to 50 ml methanol, and 0.05 or 1ml of fresh sample was mixed with 5 ml of DPPH solution (0.025 g/L). The mixture and blank (1 ml methanol mixed with 5 ml DPPH solution) were kept in the dark for 30 min at 23°C, and the absorbance was read at 715 nm. For the juice sample, 1 ml of juice was diluted with 10 ml of distilled water; then, 1 ml of that mixed with 5 ml of DPPH solution.

##### 4.3 Determination of total phenolic compounds (TPC)

TPC was detected by the methods of (Saeed et al., 2012) using spectrophotometer (PD-303UV, Apel). One ml of sample (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. Then, 10 ml of Na<sub>2</sub>CO<sub>3</sub> (7%) solution was added. After 5 min, 13 ml of deionized water was added and mixed thoroughly. Then kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The TPC was specified from extrapolation of calibration curve, which was made by preparing gallic acid solution. The estimation of TPC was carried out in triplicate. The TPC was expressed as mg of Gallic Acid Equivalents (GAE) per g of dried sample or per ml of juice.

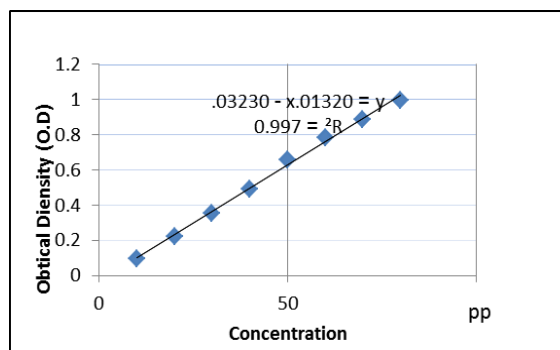


Figure 1: Standard Curve of Gallic acid

##### 4.4. High Performance Liquid Chromatography (HPLC) analysis of phenolic and flavonoid compounds:

HPLC modification method analysis of phenolic and flavonoid compounds of juice (Treatment 2). were extracted by using HPLC -diode-array detection by the method of (Mullen et al., 2007). HPLC (Agilent 1260) analysis of phenolic and flavonoid components were done on a reverse phase Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), using a gradient program with two solvent

systems (A: 0.2% formic acid in acetonitrile: water (1:1) B: 0.2% formic acid in water) at a constant solvent flow rate, 0.9 mL/min. Calibration solution: Ten mg of quercetin was added to a volumetric flask, then dissolved in 10 mL methanol, and serial dilutions were prepared.

#### 4.4.1. Extraction process for juices by HPLC:

Methanol was used to get concentrations 25, 50, 100, 150, 200, and 250 ng/ $\mu$ L. Each calibration level was analyzed in triplet. Antipyrine was used as internal standard at a final concentration of 100 ng/ $\mu$ L (Rt, 31.60 min). Each composite (Epicatechin, Antipyrine, Benzoic acid, Cinnamic acid, Chlorogenic acid, 4-O-Caffeoylquinic acid, and Rutin) was injected separately applying scan mode DAD from 190 to 400 nm. The UV-VIS scan of each compound was saved and matched with the detected compounds in each sample. Unknown flavones were characterized by two or three maxima in UV and visible range. Also, the concentration of unknown flavonols were calculated as quercetin. All juice samples (F.G.W.J) were prepared as follows: 6 mL of samples was sonicated for 60 min, centrifuged at 5000 rpm/10 min, and filtered using Nylon membrane disks. Then, 5.5 mL clear solution was gotten. 2 mL of clear solution was extracted two times using SPE C18 ec 500 mg/3-mL columns. Then, juices were extracted by solid phase extraction before and after hydrolysis of sugars by using HCl method.

#### 4.5 Microbiological analysis:

The total bacteria was counted using serial dilutions ( $10^{-3}$ ) on plate count agar (PCA). The duplicate plates were incubated at 30 °C for 48 h. The account of total yeasts and molds (YM) was done with the same dilutions on Rose Bengal Chloramphenicol agar (RBC) at 25 °C for 5 days. Results were expressed as "colony-forming units (CFU)/ml". The analysis was conducted using methods of (Gulf standards and Saudi standards, 1994).

#### 4.6 Sensory evaluation:

Sensory evaluation was conducted in laboratory of food and nutrition department, girls' section, King Abdul Al-aziz university, Jeddah. 69 students were chosen randomly, the first stage included 37 students and second stage included 32 students. They seated in stalls with proper lighting and asked to judge each juice on five characteristics: taste, color, odor, texture and overall acceptability. The form of the sensory evaluation test was contained five-point hedonic scale, 5-like extremely, 4-like moderately, 3- neither like nor dislike, 2-dislike moderately, 1-dislike extremely (Spangler and Mook, 1978).

#### 4.7 Statistical analysis:

All obtained data were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data were displayed as mean $\pm$

standard deviation (SD). Analysis of Variance (ANOVA) test was used for defining the significances among different groups according to (Armitage, 1987). All differences with p-values  $\leq$  0.05 were considered significant.

## Results and Discussion

The effect of cooking process and *Moringa oleifera* extract mixed with fruit and vegetable juice (pineapple, carrot and ginger) were showed clearly by their physicochemical composition analysis and microbiological tests. The evaluation was done on processed juices mixed with F.M.O.L extract. Thus, their physicochemical composition and its relation to the shelf life were evaluated as shown in the following results.

#### *The chemical composition of F.M.O.L:*

The fresh *Moringa oleifera* leaves (F.M.O.L) and fresh *Moringa oleifera* leaves extract (F.M.O.L.E) were analyzed for proximate analysis (Table 4.1). The results revealed that the F.M.O.L contained 69.85% moisture, 7.77% crude protein, and 139.46 mg/100g ascorbic acid. These results were nearly the same as the results of (Okafor and Ogbobe, 2015). The total phenolic compounds in F.M.O.L. were 13.6 mg GAE/g. This value is considered higher when compared with those reported by (Khandelwal et al., 2014). Also, the antioxidant activity was low (68.65 %). These didn't agree with those reported by (El-massry et al., 2013). Sometimes, the differences in composition induced due to the damage during enzymatic processes which continued for long periods from collection to marketing of leaves. Furthermore, factors such as source of raw materials, collection and extraction method have far reaching effect on the proximate and phytochemical components of medicinal plants (Adeyemi et al., 2014). Meanwhile, the F.M.L.E. has the lowest value of ascorbic acid (8.44 mg/100g). This might be due to the fact that ascorbic acid breaks down when fresh leaves are heated (boiled) at 100°C for 15 min to prepare the extract, also when exposed to air and light during preparation (Chipurura et al., 2010; Otu et al., 2013). But high amounts of total phenolic and antioxidant activities were observed (38.76 mg GAE/g and 77.65 %, respectively). Although, the extract was prepared by boiling fresh leaves, which has a negative effect on vitamin C, But the results were not expected. This agreed with (Yang et al., 2006) who found that the boiling process of M.O. leaves increased the availability of iron and antioxidant content. Moreover, this boiling process can maximize utilization of the required nutrients epically from the M.O. leaves (Nweze and Nwafor, 2014; Gopalakrishnan et al., 2016). The results of chemical analysis revealed that fresh M.O.L. is an excellent source of ascorbic acid. Meanwhile, F.M.O.L.E. is a respectable source of phenolic components (38.76 mg GAE/mg) and antioxidant activity (77.65%). These results were nearly agreed with those reported by (Okafor and Ogbobe, 2015) and (Subudhi and Bhoi, 2014).

Table 1- The chemical analysis of fresh *Moringa oleifera* leaves and its juice

Chemical analysis (%)	M.O.L. samples	
	F.M.O.L.	F.M.O.L.E.
Moisture	69.85 ± 0.24	-
Protein	7.77 ± 0.10	-
Ascorbic acid (mg/100g or 100ml)	139.46 ± 6.36 <sup>a</sup>	8.44 ± 0.64 <sup>b</sup>
Total phenolic (mg GAE/g or ml)	13.68 ± 0.04 <sup>b</sup>	38.76 ± 0.05 <sup>a</sup>
Antioxidant activity	68.65 ± 0.32 <sup>b</sup>	77.65 ± 0.21 <sup>a</sup>

Means ± standard deviation (N= 3±SD); For each sample within a column, means not sharing the same alphabetical letter are significantly different at  $p \leq 0.05$

The sensory evaluation of treated fruit and vegetable juices mixed with F.M.O.L.E:

Organoleptic evaluation could be considered as one of the most important aspects in juice blend technique since it reflects the consumer/preference. Data concerning sensory evaluation of fruit and vegetable juices mixed with F.M.O.L.E. by three percentages

(40, 50 and 60 %), packed in glass bottles and stored up to one month, are shown in Table (2). These juices were sensory evaluated for taste, odor, color, texture and overall acceptability. The observed taste, odor and overall acceptability of treatment (1) that have been mixed with 40% F.M.O.L.E. had the highest scores at zero time. On the other hand, treatment (2) mixed with 50% F.M.O.L.E. and (3) mixed with 60% F.M.O.L.E. had non-significant differences between all parameters at zero time of storage. Meanwhile, after one month of storage treatment (2), followed by treatment (1) take highest scores in taste, odor and overall acceptability compared to treatment (3). After preparation of the juices and during storage, no major changes occurred in scores of color and texture between all three juices. Treatment (1) and (3) had a slight decrease in sensory parameter scores compared to treatment (2) after one month of storage. It could be indicated through the aforementioned obtained results that it was fruitful and appropriate to produce treated fruit and vegetable juices mixed with 40% and 50% of F.M.O.L.E. These results are nearly accordance with those reported by (Out et al., 2013).

Table 2- The sensory evaluation of treated fruit and vegetable juices mixed with F.M.O.L.E. during storage at  $4 \pm 1^\circ\text{C}$  up to one month

Treatments	Storage period of juices in months									
	Zero time					After one month				
	Taste	Odor	Color	Texture	overall acceptability	Taste	Odor	Color	Texture	overall acceptability
1	3.36 ± 1.40 <sup>bc</sup>	3.55 ± 1.01 <sup>b</sup>	4.09 ± 0.87 <sup>b</sup>	4.27 ± 1.08 <sup>b</sup>	3.77 ± 1.15 <sup>b</sup>	3.09 ± 1.51 <sup>c</sup>	3.44 ± 1.34 <sup>bc</sup>	4.03 ± 1.06 <sup>b</sup>	4.00 ± 1.08 <sup>b</sup>	3.38 ± 1.24 <sup>c</sup>
2	3.04 ± 1.29 <sup>c</sup>	3.30 ± 1.35 <sup>c</sup>	3.70 ± 1.17 <sup>b</sup>	3.56 ± 1.34 <sup>b</sup>	3.44 ± 1.09 <sup>bc</sup>	2.75 ± 1.46 <sup>c</sup>	3.59 ± 1.37 <sup>b</sup>	4.03 ± 0.10 <sup>b</sup>	4.16 ± 1.11 <sup>b</sup>	3.47 ± 1.14 <sup>bc</sup>
3	3.30 ± 1.20 <sup>c</sup>	3.33 ± 1.21 <sup>c</sup>	4.00 ± 0.88 <sup>b</sup>	3.89 ± 1.12 <sup>b</sup>	3.48 ± 1.12 <sup>bc</sup>	2.66 ± 1.58 <sup>cd</sup>	3.28 ± 1.37 <sup>c</sup>	3.91 ± 1.15 <sup>b</sup>	4.03 ± 1.12 <sup>b</sup>	3.31 ± 1.15 <sup>c</sup>

Means ± standard deviation (N= 3±SD); For each treatment within a column, means not sharing the same alphabetical letter are significantly different at ( $p \leq 0.05$ )

1- F.M.O.L.E. 40% + Pineapple 38%+Carrot 20 % +Ginger 2%

2- F.M.O.L.E. 50% + Pineapple 38%+ Carrot 10 % + Ginger 2%

3- F.M.O.L.E. 60% + Pineapple 28% +Carrot 10 % + Ginger 2%

The physicochemical analysis of treated fruit and vegetable juices mixed with F.M.O.L.E:

Physicochemical properties of juices:

Physicochemical analyses were done at zero time and after one month of storage period, meanwhile it was done after two months just for treatment (2) (60 days at  $4.0 \pm 1^\circ\text{C}$ ) (because it did not spoil after one month of storage). The evaluation of treatments of different juices showed the following results:

Total Soluble Solids (T.S. S%):

Results in Table (3) showed that treatments 1, 2, and 3 contained 12.2, 12.2, and 14% T.S.S, respectively at zero time of storage. A slight increase in T.S.S.% was recorded in packed juices in glass bottles stored up to 60 days at  $4 \pm 1^\circ\text{C}$  compared to zero-time ones (Table 3). Meanwhile, treatment (2) contained 16.8% T.S.S after two months of storage. This may be due to the absence of the microorganisms, which cause the sugar fermentation of the juice (El-kady et al., 2015). Meanwhile, Ameh et al. (2015) reported the increase in T.S.S.% of juice due to the high concentration of solids, typically sugars, vitamins and minerals etc., which are dissolved

in the juice. Also, during processing treatment of juices, the increase of T.S.S% may be due to conversion of insoluble polysaccharides to soluble polysaccharides and insoluble pectin to soluble pectin. It could be concluded that treatment 3 has highest total soluble solids.

Total Titratable Acidity & pH value:

Data in same Table (3) indicated similar changes in acidity and pH values according to treatments. Besides, a slightly noticeable increase was revealed out in titratable acidity and slight decrease in pH value during storage period up to one months, as well as in treatment (2) after two months of storage. This result matched with what was recorded by (Molinar and Silva, 1996). It could be concluded that both treatments (3) and (2) have lowest content of total acidity, and highest contents of pH value than treatment (1).

Ascorbic acid content:

In general, the results in Table (3) detected increase in ascorbic acid as a result of an increase of F.M.O.L.E percentage at zero time. Data ascertained that juices with F.M.O.L.E (50 and 60%) have a slightly high percentage of ascorbic acid content (6.25

mg/100ml juice) when compared with the treatment that had 40% of F.M.O.L.E (5.21 mg/100ml juice). It might be attributed to the natural compound and vitamins that were found in F.M.O.L.E. These results agree with the previous studies (Yang et al., 2006) that revealed boiling M.O.L. enhances antioxidant contents. However, it was decreased during storage up to one months by 20.3%, 19.8%, and 19.8%, respectively for treatments 1, 2 and 3. Meanwhile, in treatment (2), it was decreased during storage up to two months by 31.5%. This result matched with what was recorded by (Otu et al., 2013). It could be concluded that both treatments (3) and (2) have highest contents of ascorbic acid at zero time and during storage up to one month compared to treatment (1).

*Total phenolic content (TP):*

The F.M.O.L.E. might be regarded as a natural plant extract being rich in phenolic components (Pari et al., 2007; Vinoth et al., 2012; Subudhi and Bhoi, 2014; Amabye and Tadesse, 2016). Table (3) presents the analytical data for total phenolic compounds of the studied treatments of fruit and vegetable juices mixed with F.M.O.L.E. Total phenolic in treatment (1) reached 58.61 mg GAE/ml, while treatment (2) and (3) to 66.41 and 73.21 %, respectively at zero time of storage. After the storage period up to one months, it was reduced to 53.46, 59.32 and 68.39 mg GAE/ml, respectively. Results ascertained that increased percentage of juices with M.O.L.E. has positive effect on TP when compared to treatment (1). However, it was decreased during storage up to one months by 8.9%, 10.7%, and 6.6%, respectively for treatments 1, 2 and 3. Meanwhile, in treatment 2, it was decreased during storage up to two months by 62.5%. This result matched with what was recorded by (Castro-López et al., 2016), that decrease of phenolic content during storage may be due to the decrease of polyphenolic compounds that prevent microbial growth. Some others mentioned that the difference in total phenolic contents

during storage may be due to several factors such as extraction conditions, and the yield and bioactivities of the treatments. It could be concluded that both treatments (3) and (2) have highest contents of total phenolic compounds at zero time and during storage up to one month.

*Antioxidant activity:*

The differences in DPPH activity were observed among the different percentages of treated fruit and vegetables juices mixed with F.M.O.L.E. (Table 3). About 69.68, 72.10 and 76.33% scavenging activity against free radicals (DPPH) were observed at zero time of storage of treatments 1, 2 and 3, respectively. The higher percentage of M.O.L.E. in the juices showed a higher scavenging activity with 70.81 and 71.59 % respectively for treatments 2 and 3 after one month of storage period, when compared to 68.52% in treatment 1. This may be due to the higher amounts of phenolics in M.O.L.E. These results agreed with (Pari et al., 2007; Vinoth et al., 2012; Subudhi and Bhoi, 2014; Amabye et al., 2016). Consequently, treated fruits and vegetable juices mixed with F.M.O.L.E. could be considered as a respectable source of antioxidant activity as a free radical scavenger and also prolonging the shelf-life of the product

However, it was decreased during storage up to one months by 1.6%, 1.9% and 6.2%, respectively for treatments 1, 2 and 3. Meanwhile, in treatment (2), it was decreased during storage up to two months by 2.5%. This result matched with what was recorded by (Klimczak et al., 2007; Owczarek et al., 2004); this decrease may be connected to the decrease in phenolic components and ascorbic acid contents which were observed in all treatments during storage up to one month. It could be concluded that both treatments 3 and 2 have highest contents of antioxidant activity at zero time and during storage up to one month.

Table 3- The physicochemical analysis of treated fruit and vegetable juices mixed with F.M.O.L.E. during storage at 4±1 °C up to one month

Contents%	Zero time			After one month		
	1	2	3	1	2	3
T.S.S	12.2 ± 0.00 <sup>b</sup>	12.2 ± 0.00 <sup>b</sup>	14 ± 0.00 <sup>a</sup>	13 ± 0.00 <sup>b</sup>	13 ± 0.00 <sup>b</sup>	14.2 ± 0.00 <sup>a</sup>
Acidity	0.028 ± 0.00 <sup>a</sup>	0.018 ± 0.00 <sup>b</sup>	0.018 ± 0.00 <sup>b</sup>	0.060 ± 0.00 <sup>a</sup>	0.030 ± 0.00 <sup>b</sup>	0.030 ± 0.00 <sup>b</sup>
pH value	4.26 ± 0.01 <sup>b</sup>	4.48 ± 0.01 <sup>a</sup>	4.43 ± 0.04 <sup>a</sup>	4.11 ± 0.01 <sup>b</sup>	4.19 ± 0.01 <sup>a</sup>	4.17 ± 0.01 <sup>a</sup>
Ascorbic acid (Mg/100ml)	5.21 ± 0.00 <sup>b</sup>	6.25 ± 0.00 <sup>a</sup>	6.25 ± 0.00 <sup>a</sup>	4.15 ± 0.00 <sup>b</sup>	5.01 ± 0.00 <sup>a</sup>	5.01 ± 0.00 <sup>a</sup>
Total phenolic (MgGAE/ml)	58.61 ± 0.83 <sup>c</sup>	66.41 ± 0.42 <sup>b</sup>	73.21 ± 0.63 <sup>a</sup>	53.46 ± 0.95 <sup>c</sup>	59.32 ± 0.99 <sup>b</sup>	68.39 ± 0.14 <sup>a</sup>

Means ± standard deviation (N= 3±SD): For each treatment within a column, means not sharing the same alphabetical letter are significantly different at (p ≤ 0.05) ND = Not Detected.

- 1- F.M.O.L.E 40% + Pineapple 38%+ Carrot 20 % +Ginger 2%
- 3- F.M.O.L.E 60% + Pineapple 28% +Carrot 10 % + Ginger 2%

- 2- F.M.O.L.E 50% + Pineapple 38%+ Carrot 10 % + Ginger 2%

*The color values of L\*, a\*, and b\* of treated fruit and vegetable juices mixed with F.M.O.L.E:*

Color analysis of juices: color analysis was done at zero time and after one month of storage period, meanwhile it was done after two months (60 days at 4.0 ± 1°C) just for treatment (2) (because it isn't

spoiled after one month of storage). The evaluation treatments of different juices revealed the following results:

Color L\*: The L\* parameter indicates of lightness which ranges from 0 (black) to 100 (white). The color lightness (L\*) value of all treatments was high at zero time of storage. After one month of storage time was highest in treatment (3). The color lightness (L\*)

value was increased in treatments 1, 2, and 3 by 16.8, 20.2, and 36.4 %, respectively, during storage up to one month compared to zero time. Meanwhile, it was increased by 30.0% in treatment 2 during storage up to two months compared to zero time. Color a\*: The a\* parameter indicates red color at positive value (+a\*) or green at negative value (-a\*). The color redness (+a\*) positive value of treatment (3) was highest. The color redness (+a\*) positive value was increased in treatments 1, 2, and 3 by 720.3, 498.6, and 278.4 %, respectively, during storage up to one month compared to zero time. Meanwhile, it was increased by 130.4% in treatment (2) during storage up to two months compared to zero time. Color b\*: The b\* parameter indicates the degree of yellow at

positive value (+b\*) or blue at negative value (-b\*). The color yellowness (+b\*) positive value of treatment (3) was highest at zero time and after one month of storage time. The color yellowness (+b\*) positive value was increased in treatments 1, 2, and 3 by 220.8, 269.3, and 259.7 %, respectively, during storage up to one month compared to zero time. Meanwhile, it was increased by 60.4% in treatment (2) during storage up to two months compared to zero time. These variations in color properties (L\*, a\* and b\*) values of juices may be due to the precipitation, leading to instability of the juice during storage (Adiamo et al., 2017).

Table 4- The color of L\*, a\*, and b\* values of Fruit and vegetable juices mixed with F.M.O.L.E. during storage at 4±1°C up to one months

Contents%	After processing (at zero time)			After one month		
	1	2	3	1	2	3
L*	20.45 ± 0.30 <sup>a</sup>	20.56 ± 0.28 <sup>a</sup>	20.93 ± 0.32 <sup>a</sup>	23.88 ± 0.27 <sup>b</sup>	24.72 ± 0.33 <sup>b</sup>	28.54 ± 1.09 <sup>a</sup>
a*	0.69 ± 0.07 <sup>b</sup>	0.69 ± 0.08 <sup>b</sup>	1.11 ± 0.14 <sup>a</sup>	5.66 ± 0.09 <sup>a</sup>	4.13 ± 0.08 <sup>b</sup>	4.20 ± 0.06 <sup>b</sup>
b*	2.31 ± 0.13 <sup>b</sup>	2.25 ± 0.14 <sup>b</sup>	3.08 ± 0.16 <sup>a</sup>	7.41 ± 0.11 <sup>c</sup>	8.31 ± 0.30 <sup>b</sup>	11.08 ± 0.66 <sup>a</sup>

Means ± standard deviation (N= 3±SD): For each treatment within a column, means not sharing the same alphabetical letter are significantly different at (p ≤ 0.05) ND = Not Detected.

1- F.M.O.L.E. 40% + Pineapple 38%+Carrot 20 % +Ginger 2%  
3- F.M.O.L.E. 60% + Pineapple 28% +Carrot 10 % + Ginger 2%

2 F.M.O.L.E. 50% + Pineapple 38%+ Carrot 10 % + Ginger 2%

#### The microbiological analysis of treated fruit and vegetable mixed with F.M.O.L.E:

Evaluation tests were done two times (at zero time, and at the end of storage period of 60 days). At zero time of all treatments, we did not detect bacteria, yeasts and molds, but there was growth of yeasts ( $1 \times 10^{-3}$ ) in treatment (1), which did not exceed the gulf standards limits (Maximum Count permitted for yeasts,  $1 \times 10^{-3}$ ). After one month of storage there was growth of yeasts in treatment (1) and (3) ( $50 \times 10^{-3}$  and  $15 \times 10^{-3}$ , respectively), which exceeded the gulf standards limits (Maximum Count permitted for yeasts,  $1 \times 10^{-3}$ ). Meanwhile, there was growth of bacterial count

in treatment (1) and (2) ( $1 \times 10^{-3}$  and  $1 \times 10^{-3}$ , respectively), which did not exceed the gulf standards limits (Maximum Count permitted for bacterial,  $1.0 \times 10^{-4}$ ). The results proved that cooking process (boiling at 100°C) and mixed processed juices with the F.M.O.L.E 50%, extend the shelf life of treatment (2). Furthermore, F.M.O.L.E. contains respectable antimicrobial agents, and might be used as a natural antioxidant and antimicrobial agent in pharmaceutical and food applications (El-Sohaimy et al., 2015). The F.M.L.E. contains hydrocarbon and alcoholic as well as phenolic, that might be considered as microbial inhibitor and increase preservative activity (shelf-life) of encouraged juices.

Table 5- The microbiological analysis of treated fruit and vegetable juices mixed with F.M.O.L.E. during storage at 4±1°C up to one month

Microorganism	Storage period (months)					
	Zero time			After one month		
	1	2	3	1	2	3
Total Bacterial	ND	ND	ND	$1 \times 10^{-3}$	$2 \times 10^{-3}$	ND
Molds	ND	ND	ND	ND	ND	ND
Yeasts	$1 \times 10^{-3}$	ND	ND	$50 \times 10^{-3}$	$1 \times 10^{-3}$	$15 \times 10^{-3}$

ND = Not Detected.

1- F.M.O.L.E 40% + Pineapple 38%+Carrot 20 % +Ginger 2%  
3- F.M.O.L.E 60% + Pineapple 28% +Carrot 10 % + Ginger 2%

2- F.M.O.L.E 50% + Pineapple 38%+ Carrot 10 % + Ginger 2%

Table 6- The recommended microbiological standards for any fruit juice; all numbers are as per ml of juice consumed

Parameter	Total viable count	Yeasts and Mold
Maximum Count Anticipated	$5.0 \times 10^3$	100
Maximum Count permitted	$1.0 \times 10^4$	$1.0 \times 10^3$

Source: Gulf Standards (2000).

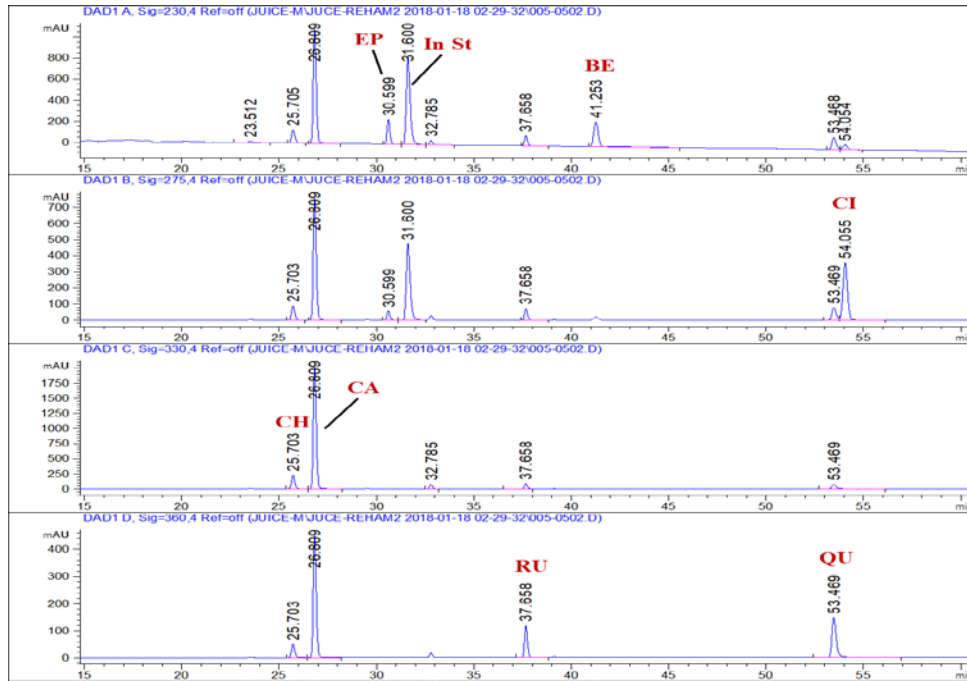
#### Qualitative and quantitative Phenolic and flavonoid compounds of treatment (2) which have been carried out by HPLC, after two months of storage:

Four phenolic and three flavonoid compounds (according to Quercetin standard) were analyzed as standards then determined in treatment 2 (F.M.O.L 50% + Pineapple 38%+ Carrot 10 % +

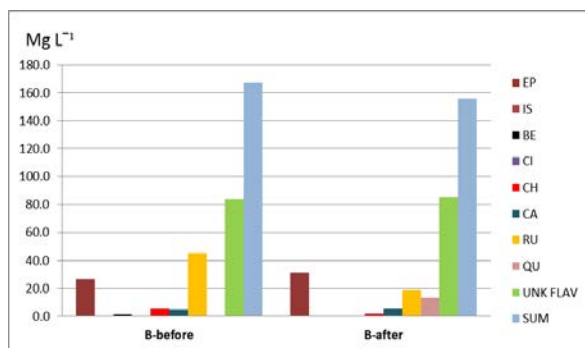
Ginger 2%): (Epicatechin (EP), Antipyrine (IS), benzoic acid (BE), cinnamic acid (CI), chlorogenic acid (CH), 4-O-Caffeoylquinic acid (CA), rutin (RU), and quercetin (QU) (Figure 2). The concentrations of phenolic and flavonoid components expressed in  $\text{mg L}^{-1}$  of treatment (2). Phenolic and flavonoid compounds in treatment (2) was examined before and after hydrolysis. The hydrolysis process means determined un-conjugated phenolics with sugars (Figure 3). Results (Table 7 and figure 4) indicated that

treatment (2) contained only three phenolics, EP, BE, and CH, with concentration of 26.6, 1.1, and 5.2, as well as two flavonoids, CA and RU, with concentrations of 4.8 and 45.1 mg L<sup>-1</sup>, respectively, and also unknown flavonoid compound (84 mg L<sup>-1</sup>) before hydrolysis process. Meanwhile, it contained EP (31.3), CH (2.0), CA (5.2), RU (18.8), QU (13.2) and unknown flavonoid contained (85.2 mg L<sup>-1</sup>) after hydrolysis (figure 4 and table 7). It could indicate that the highest level of phenolic plus unknown flavonoid compounds were obtained before and after hydrolysis process, and it seems that both hydrolysis or without hydrolysis process are suitable for extraction of phenolic compounds. Conclusively, results showed that Epicatechin (EP), chlorogenic acid (CH), 4-O-Caffeoylquinic acid (CA), rutin (RU), and quercetin (QU) were

predominant phenolic components in treatment (2). Whereas, the others are dominant phenolic constituents. This may attribute to the increase in the percentage of F.M.O.L.E. (50%) in processed juices (Table 5). Many studies of qualitative composition of M.O plant extracts reported that high concentrations of phenolic components were obtained using polar solvents (Čanadanović-Brunet et al., 2008; Stanković, 2010). Methanol and water extracts of M.O. were the strongest extraction media able to dissolve most of the phenolic components from samples. Methanol is able to extract semi-polar phenolics whilst water is more preferred to polar phenolic acid. Therefore, M.O. could be considered as a natural plant rich in phenolic components (El-Sohaimy et al., 2015).



**Figure 2.** Representative chromatogram of calibration solution containing 200 ng/μL of each standard phenolic compound measured at 230 nm (EP, epicatechin 30.59 min; In St, antipyrine 31.60 min; BE, benzoic acid 41.25 min), 275 nm (CI, cinnamic acid 54.05 min), 330 nm (CH, chlorogenic acid 25.70 min; CA, 4-O-Caffeoylquinic acid 26.80 min), and 360 nm (RU, rutin 37.65 min; QU, quercetin 53.47 min)



**Figure 3.** Histogram of phenolic compounds recovered from analyzed treatment (2) after and before acidic hydrolysis, followed by SPE C18. The components were EP, epicatechin; In St, antipyrine; BE, benzoic acid; CI, cinnamic acid; CH, chlorogenic acid; CA, 4-O-Caffeoylquinic acid; RU, rutin; QU, quercetin.

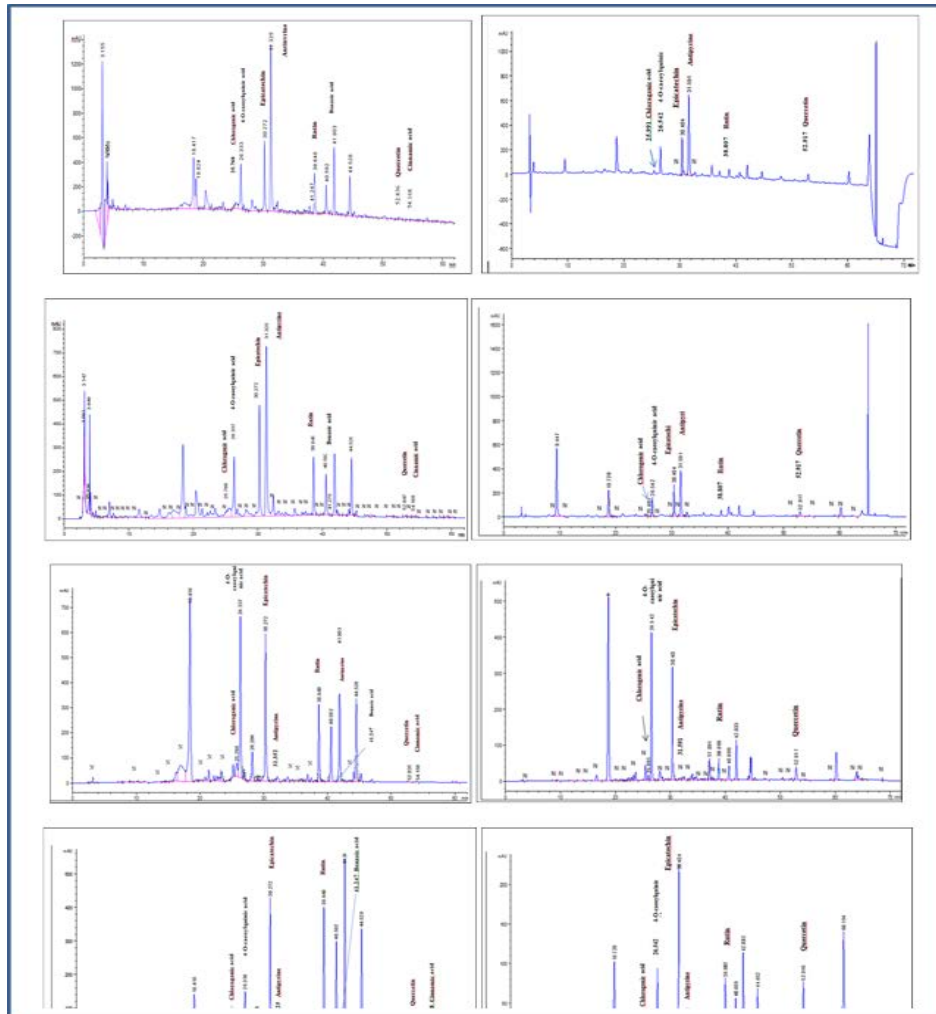
**Table 7- Analysis of phenolic and flavonoids components in treatment (2) before and after hydrolysis by using high performance liquid chromatography (HPLC)**

Phenolic and unknown flavonoid compounds ↓	Treatments	
	2	2
	before Hydrolysis mg L <sup>-1</sup>	after Hydrolysis mg L <sup>-1</sup>
EP	26.6	31.3
IS	0.0	0.0
BE	1.1	0.0
CI	0.3	0.0
CH	5.2	2.0
CA	4.8	5.2
RU	45.1	18.8
QU	0.4	13.2
UNK FLAV	84.0	85.2
SUM	167.4	155.7

EP, epicatechin; In St, antipyrine; CH, chlorogenic acid; CA, 4-O-Caffeoylquinic acid; BE, benzoic acid; CI, cinnamic acid; RU, rutin; QU, quercetin

2- F.M.O.L.E50% + Pineapple 38%+ Carrot 10 % + Ginger 2%





**Figure 4:** HPLC phenolic compounds of treatment (2) before and after hydrolysis, at 230, 275, 330 and 360 nm (N = unknown flavonoid compounds at 360 and above).

**Conclusion:**

*Moringa oleifera* leaves are excellent sources of nutrients for the humans around the world that have deficiency in many nutritional eliminates such as protein, vitamins, phenolics and antioxidant activity. The F.M.O.L.E. might be stated as a natural plant extract, rich in phenolics and hydrocarbon. It might be considered as a microbial inhibitor and as a natural preservative in juices. Consequently, treated fruit and vegetables juices mixed with F.M.O.L.E. can be considered as sources of natural antioxidant, and shelf-life prolonger. It was applicable, successful and attainable to utilize *Moringa* crop when producing many preferable manufactured products. This result indicated that, the treated fruit and vegetables juices mixed with 40 and 50% of F.M.O.L.E. was the exact percentages, by using Fresh *Moringa oleifera* leaves' extract in such juices. Finally, results indicated that Epicatechin (EP), chlorogenic acid (CH), 4-O-Caffeoylquinic acid (CA), rutin (RU), and quercetin (QU) were predominant phenolic compounds in Pineapple juice 38%+ Carrot juice 10 % + Ginger 2% mixed with 50% of F.M.O.L.E. While, the others are dominant phenolic components. However,

further researches are needed to test the sanitizing and preservative effects of F.M.O.L.E on foods, and when accompanied with other preservation process.

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