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Research Article

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Total Phenolic, Flavonoid and Antioxidant Compounds of Guava Whey Juice Fortified by Moringa Olifera Aqueous Extract to Extend Shelf-life

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ABSTRACT

Background: Moringa is a tree which not a well-understood plant since it has not been entirely studied all over the world. Fruit Juice however, especially guava (Psidium guajava L) juice is very desirable for the consumer because of its fresh aroma and delicious flavor, but its shelf life is less than 6 days at 4±1°C. Therefore, the aim of the present study is to evaluate the chemical and biological properties of the Moringa oleifera leaves aqueous extract (M.O.A.E) as a natural preservative of fresh juices and to produce untraditional healthy products as well as prolong the shelf life. This study treated Fresh Guava Whey Juice (F.G.W.J) by 1.5 and 2.0% of (M.O.A.E), respectively. Results Proximate analysis ascertained that M.O.A.E is a vast source of vitamin C (156.26mg/100g), antioxidant activity (85.44%), and phenolic compounds (55.6 mg GAE/gm), respectively. Total phenolic compounds in F. G.W.J (control sample) reached to 50.5%, while the fortified beverage with 1.5 and 2.0% M.O.A.E, reached 64.8 and 76.3% respectively at zero time of storage. Both control and fortified samples of F. G.W.J with M.O.A.E (1.5 and 2.0%) showed a higher scavenging activity by 80.28 %, 93.39 and 98.39%, respectively after 2 months of storage. Results also indicated that M.O.A.E may be used as an antimicrobial agent with reasonable safety margins to inhibit bacterial growth in pharmaceutical and food applications. It appeared that using HPLC hydrolysis method is the most suitable process for the extraction of phenolic compounds from pure M.O.A.E and F.G.W.J fortified with M.O.A.E. Conclusion: It is concluded that it was applicable, successful and accessible to utilize moringa crop in producing many edible and preferable manufactured products. lastly, the fortification of F. G.W.J with 1.5 or 2% were the exact ratios for using M.O.A.E in such juice to prolong shelf life.

Key words: *Moringa Oleifera Leaves, Whey, Antioxidant Activity, Phenolic Compounds, Microbiological Analysis, Shelf Life.*

INTRODUCTION

Moringa oleifera lam (pterygosperma Gaertner) [1], are a rich source of nutrients and phytochemical components which allow these leaves to be utilized for producing important products such as Moringa tea, juice, and dairy products. These properties are attributed to the chemical components of Moringa Oleifera (M.O.) and can be used to help with a variety of health problems. Also, this medical tree has numerous economic applications and utilizations in human consumption [2,3]. The use of M.O. parts including roots, leaves, flowers, green pods, seeds, steams and petioles, have various medicinal, nutrient, bioactive, antioxidant, and antimicrobial properties [4]. Some of these components can be utilized in food industry as a natural preservative, that can be used as an alternative to synthetic preservatives in the future. Moreover, it can be recognized as a healthier product without synthetic additives [5,6]. Furthermore, M.O. is a perfect source of vitamin C, vitamin A, vitamin B, some minerals (specifically iron and calcium) and sulphur-containing amino acids (methionine and cysteine) [2, 4]. M.O.A.E. is rich in phytochemical such as phenols, flavonoids and alkaloids. These components have antimicrobial preservative effects [5, 6,7, 8, 9, 10]. The increase in the shelf life period in M.O.A.E. could be related to the presence of phenolic, hydrocarbon and alcoholic contain [11]. Dietary flavonoids possess antioxidant, anti-inflammatory, antiviral and antihistamine, properties. A

group of people prefer M.O. over Lipton tea [9]. The fresh leaves' extract contains flavonoids, saponins, tannins, terpenoids and glycosides [12]. The M.O. plant has been proven as a good antimicrobial agent [4]. The extract of the leaves has significant antimicrobial activity and effective in growth inspection of fungi. Moreover, M.O.A.E acts as an antioxidant. The major bioactive compounds of phenolics, for example quercetin and kaempferol are responsible for antioxidant activity [13]. Meanwhile, [6] detected from the dry M.O.L. extracts of methanol, ethanol and aqueous, that the aqueous extract values for phenolics and flavonoids were 24.67 and 14.32 (mg/g^{-1}), respectively. This indicated that aqueous is more suitable for extracting polar phenolic acid with antioxidant activity of 46.77 $mg.ml^{-1}$. This activity of leaves extract comes from phenolic compounds and flavonoids, and it could be implicated in human body protection against free radicals, which cause damage over time. These results established that leaves' extract could be a strong source of natural antioxidants with many human health benefits. Aqueous extract is the strongest extraction media able to dissolve most of the phenolic composites from samples, and it is more preferred to extract polar phenolic acid [6].

[3] conducted HPLC analysis to identify phenolic compounds in fresh and dried M.O.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), for fresh leaves. While 13.72, 6.97, 2.34, 33.80, 0.74, 27.14, 89.69, 1.27, 65.74 and 17.29 (mg/100g) for dried leaves, respectively. The results indicated rutin, caffeic acid and ferulic acid were the dominant phenolic components of M.O.L. extracts. This concentration of phenolic showed that M.O.E. has antioxidant activity. Furthermore, [6] determined phenolic compounds in the three M.O.L. extracts by high performance liquid chromatography (HPLC). It used ten phenolic compounds as standards (gallic acid, itaconic acid, protocathechuic acid, catechin, esculetin, catechol, tannic acid, ferulic acid, pyrogallol, and cinnamic acid). The results showed concentrations of the phenolic compounds expressed in (mg.100g-1) [6].

Studying M.O. trees which were planted locally in Saudi Arabia is crucial and more research on how to utilize this plant within industrial applications in Saudi Arabia and accordingly raising awareness of the effective uses of this crops besides encouraging the consumption of it, is needed [14].

No work has been done to investigate the aqueous extract of M.O. leaves for preserving fresh fruit juices in a refrigerator up to 2 months. Thus, the aim of this study is to evaluate the chemical, microbiological properties of (M.O.A.E) as a natural preservative of fresh juices and to produce untraditional healthy products and to prolong the shelf life.

MATERIALS AND METHODS

Materials

The fresh M.O. leaves (pterygosperma Gaertner), were purchased from Durat Al-Ezdihar Farm in jizan, Kingdom of Saudi Arabia (KSA). The fresh guava (Psidium guajava L), was purchased from the local market in Jeddah. The whey protein (liquid) was got from Halwani broth factory, Jeddah.

Chemicals

All chemicals used for juice analysis were purchased from from Al-shafei establishment Jeddah. Gallic acid and dionized water were obtained from King Fahd for medical research. The Packaging materials (glass bottles), air-tight glass jars, cheese cloth and muslin cloth were purchased from a local shop, Jeddah, Saudi Arabia.

Methods

Materials preparation

1. M. Oleifera Leaves (M.O.L):

The fresh M.O.L were harvested, washed to remove the dusts, blanched at 45°C for 30 secs and left to drain, then dried in air circulated oven at 50°C for 5h to avoid loss of active compounds. The dried leaves were ground to fine powder using a hand milling machine [14]. The powdered sample was then stored in an air-tight bottle for further use.

2. M. Oleifera Aqueous Extract (M.O.A.E):

The extraction process used was hot-water method (decoction) following the procedure of [15]. Fifty grams of powdered sample were soaked in 500 ml of distilled water and boiled for about 10 minutes. After boiling, the extract allowed to cool for a little time. Then it was double-filtered extract using cheese cloth, collected in a glass tray and allowed to cool. The filtrate was dried in air circulated oven at temperature of 70°C [10]. Then it was lyophilized at (-90 ˚C) and 5 pressure.

3. Processing Technology :

Fresh high quality Guava fruits were washed, peeled, cut to pieces and then pulped in a blender. Then filtered through a sterilized cheese cloth, the resultant juice was packed in sterilized bottles to mix immediately by different percentage of M.O.A.E and liquid whey protein [11]. The prepared materials were subjected to blend with M.O.A.E as follows:

A- Guava 20% + Whey beverages 80% (control sample)

B- Guava 20% +Whey beverages 80 % + M.O.A.E, 1.5%

C- Guava 20% +Whey beverages 80 % + M.O. A.E, 2.0%

The blends were Packed in sterilized glass bottles, packaging materials and stored in refrigerator at 4.0 ± 1 ° C till analysis.

4. Analytical Methods:

4.1. Physico-chemical analysis: Moisture content, crude protein, pH value and total soluble solids (T.S.S %) were determined according to the method described by [16]. Total titratable acidity and Vitamin C content were detected by the methods of [17].

4.2. Determination of antioxidant acivity (DPPH radical scavenging assay) detected by the methods of [18] using spectrophotometer, Model: PD-303UV, Apel. 0.5 g of sample with 15 ml of acid methanol by 1%HCL at room temperature for 1 hour was extracted. Then, the extract on 2500 RPM for 15 minutes was centrifuged, and the up layer was collected in beaker. The collected extract was adjusting its pH to reach (3). Then it was added to 50 ml methanol, and 0.05 or 1ml was taken (fresh sample) mixed with 5 ml of DPPH solution (0.025 g/L). The mixture and blank (1ml methanol mixed with 5 ml DPPH solution) were kept in the dark for 30 min at 23°C, after that the absorbance was read at 715 nm. For juice sample, 1ml of juice to 10 ml of distilled water was diluted. Then, 1 ml was taken mixed with 5 ml DPPH solution.

4.3. Determination of total phenolic compounds (TPC) were detected by the methods of [19] using spectrophotometer, Model: PD-303UV, Apel. In brief, 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. Ten ml of Na2CO3 (7%) solution was added to the mixture after 5 min. Then, 13 ml of deionized water was added and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE) per g of dried sample.

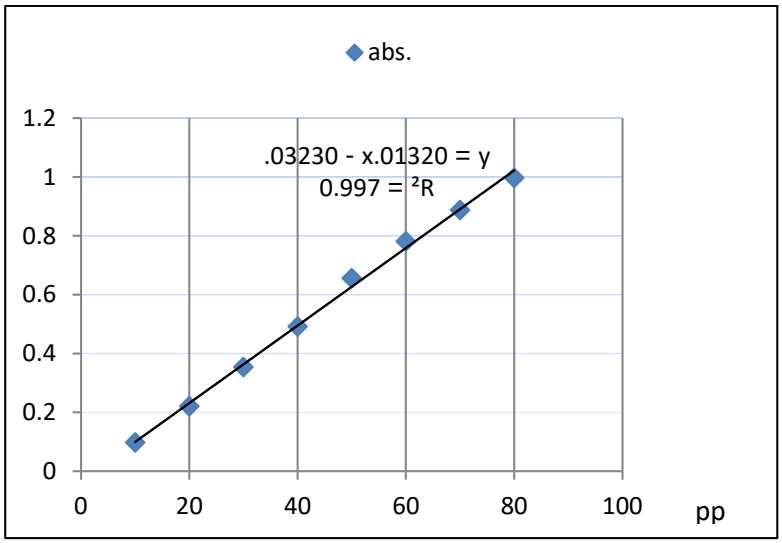

Figure 1. Standard Curve of Gallic acid

4.4. High Performance Liquid Chromatografy (HPLC) analysis of phenolic and flavonoid compounds: HPLC modification method analysis of phenolic and flavonoid compounds of F.G.W.J and M.O.A.E. were extracted by using HPLC -diode-array detection by the method of [20]. HPLC (Agilent 1260) analysis of phenolic and flavonoid compounds were performed on a reverse phase Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm), using a gradient program with two solvents system (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 transferred to a volumetric flask, then dissolved in methanol (10 mL). The serial dilutions were prepared in

methanol to get concentrations; 25, 50, 100, 150, 200, and 250 ng/µL. Each calibration level was analyzed in triplet. Antipyrine was used as internal standard at a final concentration of 100 ng/µL (Rt, 31.60 min). Each compound (Epicatechin, Antipyrine, Benzoic acid, Cinnamic acid, Chlorogenic acid, 4-O-Caffeoylquinic acid, Rutin) was injected separately applying scan mode DAD from 190 – 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.

4.4.1. Extraction process for juices by HPLC:

All juice samples (F.G.W.J) were prepared as follows: 6 mL of samples was sonicated for 60 min. Then, it was centrifuged at 5000 rpm/10 min, and filtered using Nylon membrane disks. Also, 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 used HCl method.

4.4.2. Extraction process for M.O.A.E powder by HPLC:

A weight of 550 mg of sample powder was transferred to a dry clean test tube, and mixed with 4 mL methanol. This mix was vortexed 30 secs, sonicated for 30 mins, centrifuged at 5000 RPM/10 min, filtered through 0.2 u Nylon syringe filter membrane and divided into two equal portions in two test tubes, each 2 ml., direct extraction was conducted before and after hydrolysis.

5. Microbiological analysis

The total bacterial account was determined using serial dilutions (10^{-3}) on plate count agar (PCA). The duplicate plates were incubated at 30 ˚C for 48 h. The enumeration of total yeasts and molds (YM) count with the same dilutions was also carried out on Rose Bengal Chloramphenicol agar (RBC) at 25 ◦C for 5 days. Results were expressed as "cfu (colony-forming units) /ml". The analysis was conducted using methods [21, 22, 23].

6. Sensory evaluation:

A sensory evaluation was conducted in laboratory of food and nutrition department, King Abdul Al-Aziz university, Jeddah. It was conducted in each juice on five attributes: taste, color, odor, texture and overall acceptability. The form of sensory evaluation test contained five point hedonic scales, 5-like extremely, 4-like moderately, 3- neither like nor dislike, 2- dislike moderately, 1- dislike extremely [24].

7. Statistical analysis:

All data obtained results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data were presented as mean± standard deviation (SD). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to [25]. All differences were considered significant provided that *P*-values were <0.05.

RESULTS AND DISCUSSION

The effect of Moringa Olivera extract mixed with guava whey protein were appeared clearly by their physicochemical composition analysis and microbiological tests. The evaluation was done in fresh juices (F.G.W.J) fortified with or without M.O.A.E. In order to identify the importance of the products, their physical, chemical composition and its relation to the shelf life were evaluated as shown in the following results.

Proximate composition :

The fresh leaves of Moringa Oleifera (M.O.L), boiled and extracted, (M.O.A.E) were analyzed for proximate analysis (Table 1). The results revealed that the M.O.L contained 69.85% moisture in the fresh leaves but it was decreased to 5.84% in M.O.A.E. These results were in agreement to [26] and [10]. In addition, fresh M.O.L. contained 7.77% protein which increased after extraction to 30.28% in M.O.A.E. Moreover, M.O.A.E were an excellent source of vitamin C (156.26±0.36mg/100g), antioxidant activity (85.44±0.17%) and phenolic compounds (55.60±0.04mg GAE/gm), respectively. The results of proximate analyses revealed that M.O.L was a vast source of nutrients for human around the world who lack in many nutritional eliminates such as protein, vitamins, phenolic and antioxidant activity. These results were nearly agreed with those reported by [26] and [6].

Table 1. Chemical analysis of fresh, boiled and extract of Moringa Olifera leaves

Chemical analysis%	M.O. samples		
	M.O. fresh leaves	M.O. fresh boiled leaves	M.O.A. E
Moisture	69.85 ± 0.24^{a}	-	5.84 ± 0.10^{b}
Protein	7.77 ± 0.10^{b}	-	30.28 ± 0.39^{a}
Vitamin C (Mg/100g)	139.46 ± 6.36^{b}	8.44 ± 0.64^{c}	156.26 ± 0.63^{a}
Antioxidant activity	68.65 ± 0.32^{c}	77.65 ± 0.21^{b}	85.44 ± 0.17^{a}
Total phenolic compounds (Mg GAE/g)	13.68 ± 0.04^{c}	38.76 ± 0.05^{b}	55.60 ± 0.04^{a}

Mean (N= 3±SD) with different litters in the same raw implies significant difference at $P \leq 0.05$ of chemical analysis of M.O. samples.

Physicochemical properties of juices : Physicochemical analyses were done at zero time, one month and at the end of storage period (60 days at 4.0 ± 1ºC). The evaluation treatments of different juices were showed in the following results

Total Soluble Solids (T.S. S%) : Results in Table (2) showed that F. G.W.J (control sample) contained 21.4% T.S.S. ; meanwhile, fortified F. G.W.J with M.O.A.E (1.5 and 2.0%) was 25% at zero time of storage. Storage of packed juices in glass bottles up to 60 days at 4 ± 1°C recorded a slight increase in T.S.S.% compared to zero time ones (table 2). This may be due to the absence of the microorganisms that cause the fruit juice to deteriorate as a result of sugar fermentation [27].

Total Titratable Acidity &pH value : Data in same Table (2) indicated similar changes in acidity and pH values according to treatments. Besides, a slightly noticeable increase was revealed out in titratable acidity and a decrease in pH value at zero time and throughout of storage period up to 2 months. These results matched with what was recorded by [28].

Table 2. Physicochemical properties of F.G.W.J. fortified with or without M.O.A.E during storage up to 2 months

Sample numbers	Zero Time			After One month			After two months		
	T.S.S	Acidity	pH	T.S.S	Acidity	pH	T.S.S	Acidity	pH
A	21.4 ±0.00	0.077 ±0.00	4.46 ± 0.10^{ab}	21.70 ±0.00	0.09 ±0.00	4.16 ± 0.02^{a}	21.8 ±0.00	0.096 ±0.00	3.97 ± 0.03^{b}
B	25.0 ±0.00	0.070 ±0.00	4.47 ± 0.10^{ab}	25.40 ±0.00	0.09 ±0.00	4.07 ± 0.10^{ab}	25.6 ±0.00	0.096 ±0.00	3.89 ± 0.04^{b}
C	25.0 ±0.00	0.077 ±0.00	4.70 ± 0.00^{a}	25.60 ±0.00	0.09 ±0.00	4.21 ± 0.10^{b}	26.0 ±0.00	0.128 ±0.00	4.04 ± 0.04^{a}

Mean (N= 3±SD) with different litters in the same raw implies significant difference at $P \leq 0.05$ of pH value of juices, A-- Guava 20% + Whey protein 80% (control sample), B- Guava 20% + whey protein 80 % + M.O.A.E, 1.5%, C- Guava 20% +Whey protein 80% + M.O.A.E, 2.0%.

Ascorbic acid content : In general, the results in Table (3) detected a decrease in ascorbic acid as a result of an increase of whey percentage and a decrease in fresh guava juice during preparation process. These results agree with authors [29, 30] who have investigated that, whey has poor in antioxidants contents, thus mixed whey with fruit juices showed decrease in vitamin C contents. In contrast, data ascertained that juices treated with M.O.A.E (2.0%) have a slightly high percentage of ascorbic acid content (7.29mg/100ml juice) when compared with control sample (A) (5.08 mg/100ml juice). However, it was decreased during storage up to 2 months by 17% and 9%, respectively for control sample A and C. Its might be attributed to the synergistic role of natural compound and vitamins were found in these juices [11].

Total phenolic content (TP): The M.O.A.E. might be regarded as a promising candidate as a natural plant extract being rich in phenolic compounds [6]. Table (3) concerning the considerable antioxidant capacity, presents the analytical data for total phenolic of the studied samples of fortified F.G.W.J with or without M.O.A.E. The total phenolic compounds of the extracts were expressed as Gallic Acid Equivalent in mg/ml. Considerable antioxidant capacity was also detected in three juices along storage period 1 and 2 months. TP in F. G.W.J. (control sample) reached to 50.5 mg GAE/ml, while the fortified juices with 1.5, 2.0 % M.O.A.E, were 64.8 and 76.3%, respectively at zero time of storage. After the storage period up to 2 months, it was reduced to 45.86, 60.07and 65.07, respectively.

Results ascertained that G.W.J fortified with M.O.A.E has positive effect on TP when compared to control sample [11].

Antioxidant activity: The differences in DPPH activity were observed among the different ratio of F.G.W.J. fortified with or without M.O.A.E Table 3). About 80.48 and 89.48% scavenging activity against free radicals from DPPH at zero time of storage were compared with control sample 70.55%. The M.O.A.E. showed a higher scavenging activity in the fortified juices with 1.5 and 2.0 %, during storage period up to 2 months. It was 80.28%, 90.39 and 98.39%, respectively, in control sample A, B and C after 2 months of storage (Table 3). This may be due to the higher amounts of polyphenols in M.O.A.E. These results agreed with [6, 31]. Consequently, F. G.W.J fortified with M.O.A.E. could be considered as a good source of antioxidant activity as a free radical scavenger and also prolonging the shelf-life of the product

Table 3. Ascorbic acid, phenolic compounds and Antioxidant Activity of F.G.W.J fortified with or without M.O.A.E. during storage period in glass bottles.

Sample numbers	Storage period of juices In months								
	Zero Time			After One month			After two months		
	Vitamin C mg/100 ml juice	Total phenols mgGAE/ml	Antioxidants activity%	Vitamin C mg/100 ml juice	Total phenols mgGAE/ml	Antioxidants activity%	Vitamin C mg/100 ml juice	Total phenols mgGAE/ml	Antioxidants activity%
A	5.08±0.00	50.50 ± 0.1^{c}	70.55 ± 2.13^{c}	4.17 ±0.00	46.33 ± 1.16^{c}	76.36 ± 1.10^{d}	.83 ±0.00	45.86 ± 0.08^{b}	80.28 ± 0.04^{c}
B	6.25±0.00	64.80 ± 0.1^{b}	80.48 ± 3.13^{b}	5.51 ±0.00	60.36 ± 0.23^{b}	90.33 ± 2.68^{b}	.17 ±0.00	60.07 ± 0.10^{a}	93.39 ± 0.04^{b}
C	7.29±0.00	76.31 ± 0.1^{a}	89.48 ± 3.13^{a}	6.70 ±0.00	72.67 ± 0.72^{a}	94.33 ± 2.68^{a}	.61 ±0.00	65.07 ± 0.10^{a}	98.39 ± 0.04^{a}

Mean (N= 3±SD) with different letters in the same raw implies significant differences at $P \leq 0.05$ of total phenols and antioxidant activity of juices, A-- Guava 20% + Whey protein 80% (control sample), B- Guava 20% +Whey protein 80 % + M.O.A.E, 1.5%, C- Guava 20% +Whey protein 80% + M.O. A.E, 2.0%.

Microbiological quality analysis of juices:

In this study, we focused initially on microbiological evaluation (total bacterial count and yeast & mold count). All evaluation tests were done 4 times (at zero time, after 15 days, after 30 days and at the end of storage period 60 days). The results showed that treatments B and C at zero time and during storage up to 2 months, did not detect total bacterial count and yeast and mold count, respectively, compared to control sample A ($21x10^3$and $32x10^3$). The results proved that the M.O.A.E contains good antimicrobial activity agents as presented by the composition of the secondary metabolites of the leaf extract. Also, it may be used as a natural antioxidant and antimicrobial agent with reasonable safety margins in pharmaceutical and food applications [6]. The M.O.A.E. contains hydrocarbon and alcoholic as well as phenolic, that might be considered as microbial inhibition and increased preservative activity (shelf-life) of fortified juices. In addition, it can be deduced that there is a relationship between the chemical components of M.O.A.E and the antimicrobial activity [11].

Sensory Evaluation Analysis:

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 F.G.W.J. fortified with or without M.O.A.E by two percentages (1.5, 2.0%), backed in glass bottles and stored up to 2 months, are shown in Table (4). After preparation of the juices, no major changes occurred in odor of all three juices. These samples were sensory evaluated for color, taste, odor, texture and acceptability. It could be clearly that nearly all samples products were almost palatable among different panelists. M.O.A.E (B and C) recorded a slight decrease in sensory parameters compared to control A, at zero time of storage. On the other hand, high scores of sensory attributes plus acceptability were given in samples B and C after storage in glass bottles up to 2 months. It could be indicated through the aforementioned obtained results that it was applicable, successful and available to utilize moringa crop in producing many palatable and preferable manufactured products. This result indicated that, the fortification of F.G.W.J with 1.5 or 2.0% was

the exact ratios for using Moringa leaves extract in such juices. These results are nearly accordance with those reported by [11].

Table 4. Sensory parameters of F.G.W.J fortified with or without M.O.A.E. during storage period in glass bottles

Sample numbers	Storage period of juices In months														
	Zero time					After one month					After two month				
	Taste	Odor	Color	Texture	Accitability	Taste	Odor	Color	Texture	Accitability	Taste	Odor	Color	Texture	Accitability
A	3.76 $\pm 1.17^{b}$	4.28 $\pm 1.17^{ab}$	4.60 $\pm 0.76^{a}$	3.0 $\pm 1.23^{b}$	3.80 $\pm 1.23^{c}$	2.59 $\pm 1.19^{c}$	3.67 $\pm 1.18^{b}$	3.67 $\pm 1.27^{b}$	3.63 $\pm 1.33^{b}$	3.28 $\pm 1.28^{bc}$	1.34 $\pm 1.23^{cd}$	1.50 $\pm 1.22^{b}$	3.37 $\pm 1.39^{bc}$	3.50 $\pm 1.30^{b}$	1.44 $\pm 1.31^{c}$
B	2.64 $\pm 1.12^{c}$	3.73 $\pm 1.10^{b}$	4 $\pm 1.10^{b}$	4.36 $\pm 0.92^{b}$	3.55 $\pm 0.69^{b}$	2.68 $\pm 1.04^{c}$	3.36 $\pm 1.22^{bc}$	3.86 $\pm 1.08^{b}$	3.91 $\pm 1.07^{b}$	3.23 $\pm 1.81^{bc}$	3.50 $\pm 1.29^{bc}$	3.31 $\pm 1.06^{cd}$	3.40 $\pm 1.29^{bc}$	3.68 $\pm 1.06^{b}$	3.50 $\pm 0.90^{b}$
C	2.64 $\pm 1.12^{c}$	3.73 $\pm 1.10^{b}$	4 $\pm 1.27^{b}$	4.36 $\pm 0.981^{b}$	3.55 $\pm 1.04^{b}$	2.42 $\pm 1.18^{cd}$	3.13 $\pm 1.45^{bc}$	3.75 $\pm 1.19^{b}$	4.13 $\pm 0.95^{ab}$	3.17 $\pm 1.05^{bc}$	3.66 $\pm 1.40^{bc}$	3.21 $\pm 1.08^{cd}$	3.43 $\pm 1.34^{bc}$	3.90 $\pm 1.03^{b}$	3.54 $\pm 1.02^{b}$
Avrage	3.28 $\pm 1.23^{bc}$	4.02 $\pm 1.15^{ab}$	4.32 $\pm 1.00^{ab}$	4.06 $\pm 1.09^{b}$	3.68 $\pm 1.07^{b}$	2.56 $\pm 1.13^{c}$	3.40 $\pm 1.29^{bc}$	3.75 $\pm 1.18^{b}$	3.88 $\pm 1.14^{b}$	3.16 $\pm 1.07^{bc}$	2.83 $\pm 0.30^{b}$	2.67 $\pm 1.02^{d}$	3.43 $\pm 0.03^{bc}$	3.69 $\pm 0.20^{b}$	2.82 $\pm 1.20^{bc}$

Mean (N= 3±SD) with different litters in the same raw implies significant difference at $P \leq 0.05$ of sensory properties of juices, A-- Guava 20% + Whey protein 80% (control sample), B-- Guava 20% +Whey protein 80 % + M.O.A.E, 1.5%, C- Guava 20% +Whey protein 80% + M.O. A.E, 2.0%.

Qualitative and quantitative Phenolic and flavonoid compounds of F.G.W.J fortified with or without M.O.A.E. and pure M.O.A.E which have been carried out by HPLC.

Four phenolic and three flavonoid compounds (according to Quercetin standard) were analyzed as standards then determined in four samples (Epicatechin (EP), Antipyrine (IS), benzoic acid (BE), cinnamic acid (CI), chlorogenic acid (CH), 4-O-Caffeoylquinic acid (CA), rutin (RU), quercetin (QU), (Figure-2). The concentrations of phenolic and flavonoid compounds expressed in **mg L^{-1}**of juices and **mg kg^{-1}** of M.O.A.E. Phenolic and flavonoid compounds in samples A, B, C and pure M.O.A.E. were examined before and after hydrolysis. The hydrolysis process means un-conjugated phenolics with sugars were determined (Figure 3).

Results in (Table 5 and figure 4) Indicated that control sample contained only three phenolics (EP, BE, and CI) with low concentration of (1.87, 1.19, 1.93 and unknown flavonoid compound with 3.71 mg L^{-1}). Meanwhile, sample (B) contained EP (14.21), BE (1.65), CI (1.73), CH (3.58), CA (1.54), RU (13.3), QU (13.19) and unknown flavonoid contained (37.71) mg L^{-1} after hydrolysi (figure 5 and table 5). Results also showed that phenolic, flavonoid compounds in Sample (C) had contained EP (15,45), BE (5.91), CI (3.45), CH (10.66), CA (2.80), RU (48.39), QU (43.28) while unknown flavonoid contained (126.08) mg L^{-1}after hydrolysis, respectively (figure 6 and table 5). However, the M.O.A.E showed eight compounds with the maximum concentration of phenolic and unknown flavonoid compounds (EP, BE, CI, CH, CA, RU, QU, unknown) (Figure7). The concentration of these compounds were 402.45,202.16,7.29,197.42,89.15,1404.3,185.6 and 5954.1 mg L^{-1}, respectively after hydrolysis.

Based on the results, the study indicated that the highest level of phenolic compounds (eight and seven) plus unknown flavonoid compounds were obtained by pure M.O.A.E and sample C [(Guava 20% +Whey protein 80 % + M.O.A.E., 2.0%)], respectively. In comparison with the control sample which contained only three phenolic and unknown flavonoid compounds with low concentration of 1.87,1.19,1.93 and 3.71 (mg L^{-1}), it seems that hydrolysis is the most suitable process for extraction of phenolic compounds (un-conjugated with sugars) and the major constituent of flavanols exist as conjugated form with sugar from M.O.A.E and sample C which contained Guava 20% +Whey beverages 80 % + M.O.A.E, 2.0 % (figures 7 and 6).

Conclusively, results indicated that Epicatechin (EP), rutin (RU), quercetin (QU), benzoic acid (BE) and chlorogenic acid (CH), were predominant phenolic compounds in F.G.W.J fortified with M.O.A.E. Whereas, the others are dominant phenolic constituents. This attributes to the increase ratio of M.O.A.E. in F.G.W.J (Table 5). Numerous investigations of qualitative composition of M.O plant extracts revealed the presence of high concentrations of

phenolic compounds obtained using polar solvents [32, 33]. Methanol and water extract of M.O. were the strongest extraction media that were able to dissolve most of the phenolic compounds from samples. Methanol is able to extract semi-polar phenolics while water is more favored to polar phenolic acid. Therefore, M.O. might be considered as a promising candidate as a natural plant rich in phenolic compounds [6].

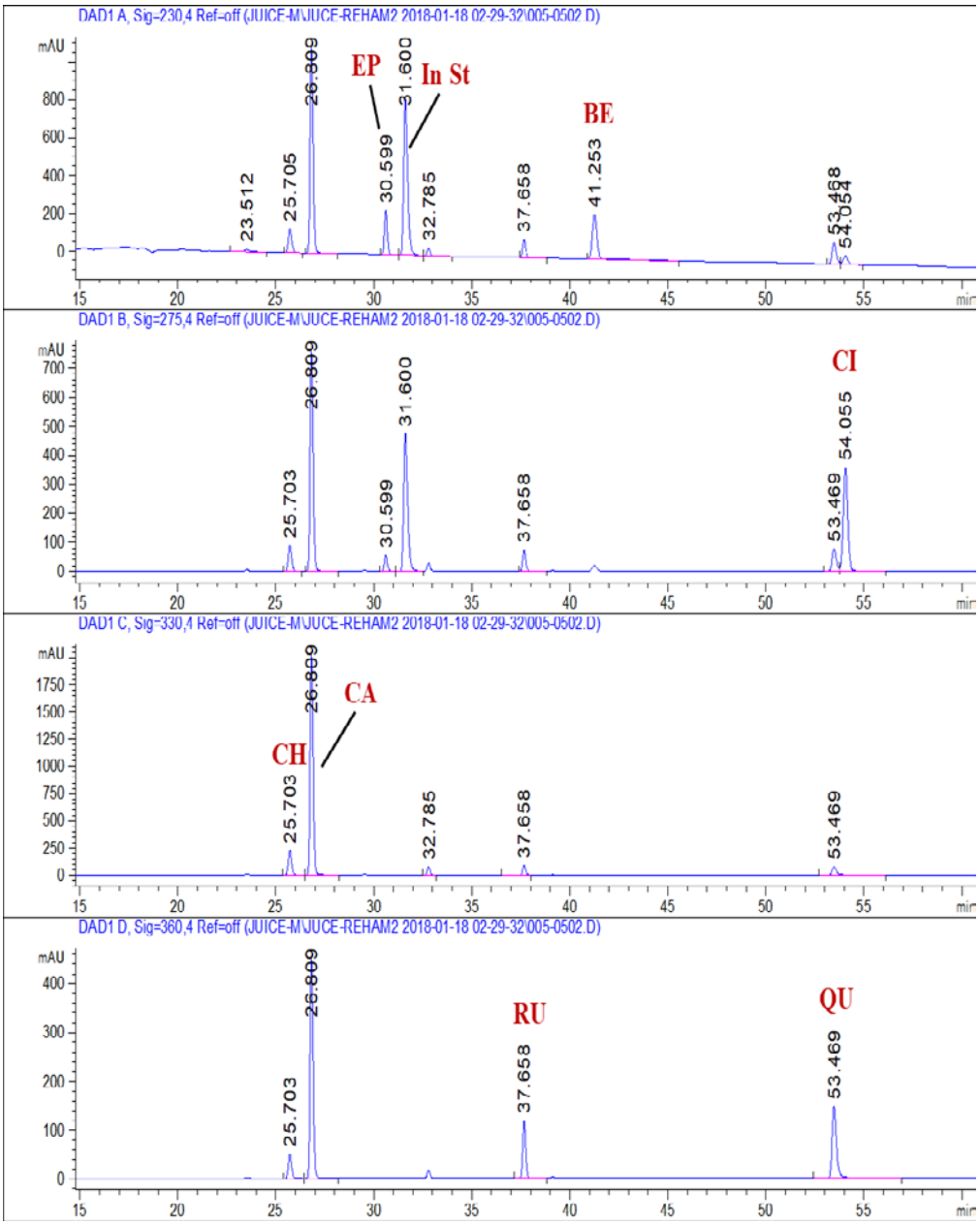

Figure 2. Representative chromatogram of calibration solution containing 200 ng/µL of each standard phenolic compounds measured at; 230 nm (EP, epicatechin 30.59 min; In St, antipyrine 31.60 min; BE, benzoic acid 41.25 min), 275 nm (CI, cinnamic acid 54.05 min), 330 nm (CH, chlorogenic acid 25.70 min;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

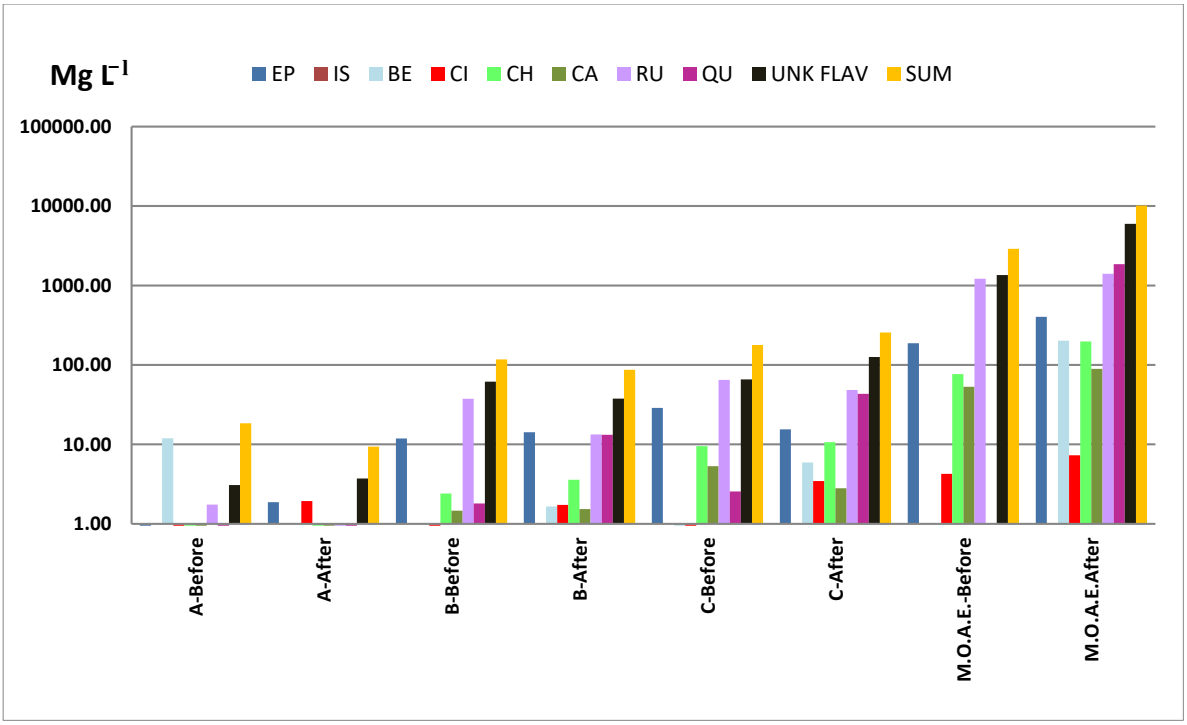

Figure 3. Histogram of phenolic compounds recovered from analyzed samples after and before acidic hydrolysis followed by SPE C18. The components were EP, epicatechin; In St, antipyrine; BE, benzoic acid; CI, cinnamic acid; CH, chlorogenic acid; CA, 4-O-Caffeoylquinic acid; RU, rutin; QU, and quercetin.

Table 5. Analysis of phenolic and flavonoids compounds in GWJ with or without M.O.A.E before and after hydrolysis by using high performance liquid chromatography (HPLC)

Phenolic and unknown flavonoid conpounds↓	Samples *							
	A	A	B	B	C	C	M.O.A.E-	M.O.A.E
	Hydrolysis							
	before	after	before	after	before	after	before	after
	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg kg^{-1}	mg kg^{-1}
EP	0.52	1.87	11.87	14.21	28.77	15.45	187.14	402.45
IS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BE	0.00	1.19	0.00	1.65	0.54	5.91	0.00	202.16
CI	0.86	1.93	0.80	1.73	0.79	3.45	4.25	7.29
CH	0.11	0.25	2.41	3.58	9.49	10.66	76.49	197.42
CA	0.06	0.14	1.46	1.54	5.32	2.80	53.20	89.15
RU	1.74	0.85	37.50	13.33	64.85	48.39	1214.99	1404.26
QU	0.12	0.61	1.80	13.19	2.56	43.28	0.00	1850.56
UNK FLAV	3.08	3.71	61.48	37.71	65.84	126.08	1351.24	5954.10
SUM	18.44	9.37	117.33	86.94	178.17	256.01	2887.30	10107.40

* A- Guava 20% + Whey beverages 80% (control sample), B- Guava 20% +Whey beverages 80 % + M.O.A.E, 1.5%, C- Guava 20% +Whey beverages 80 % + M.O. A.E, 2.0%.

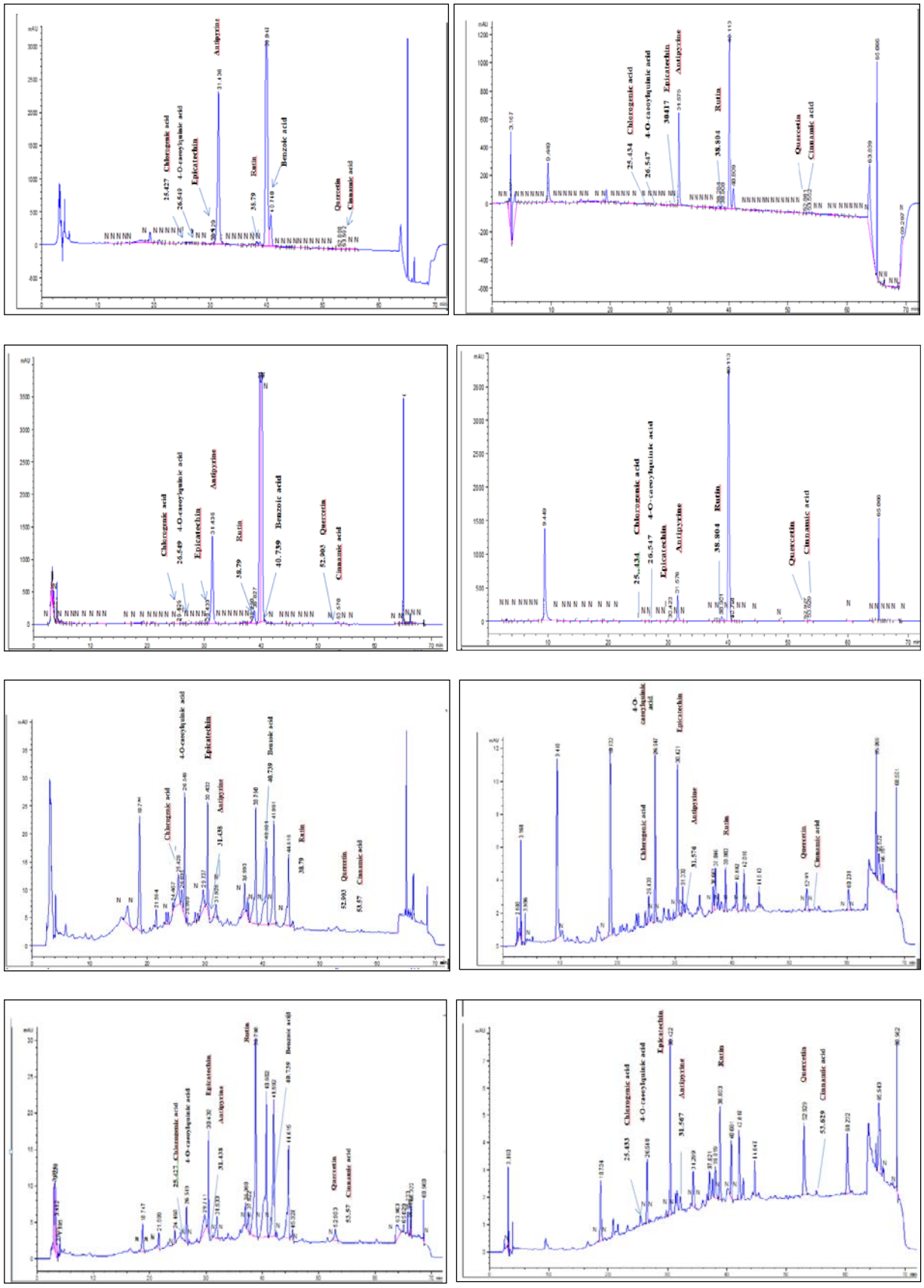

Figure 4: HPLC phenolic compounds of controle sample juice (A) before and after hydrolysis, at 230, 275, 330 and 360 nm (N = unkown flavonoied compounds at 360 and above)

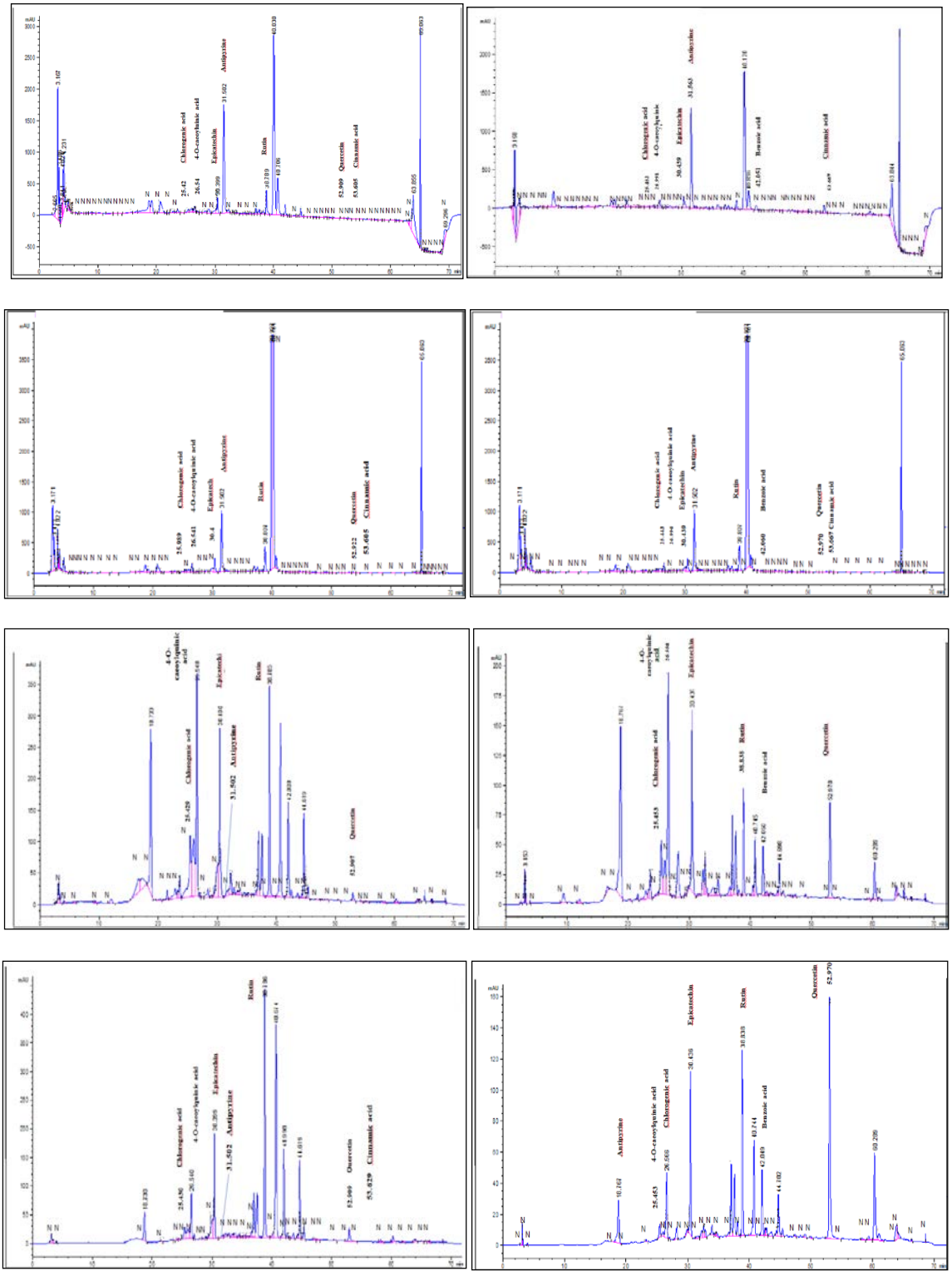

Figure 5: HPLC phenolic compounds of sample juice (B) before and after hydrolysis, at 230, 275, 330 and 360 nm (N = unkown flavonoied compounds at 360 and above)

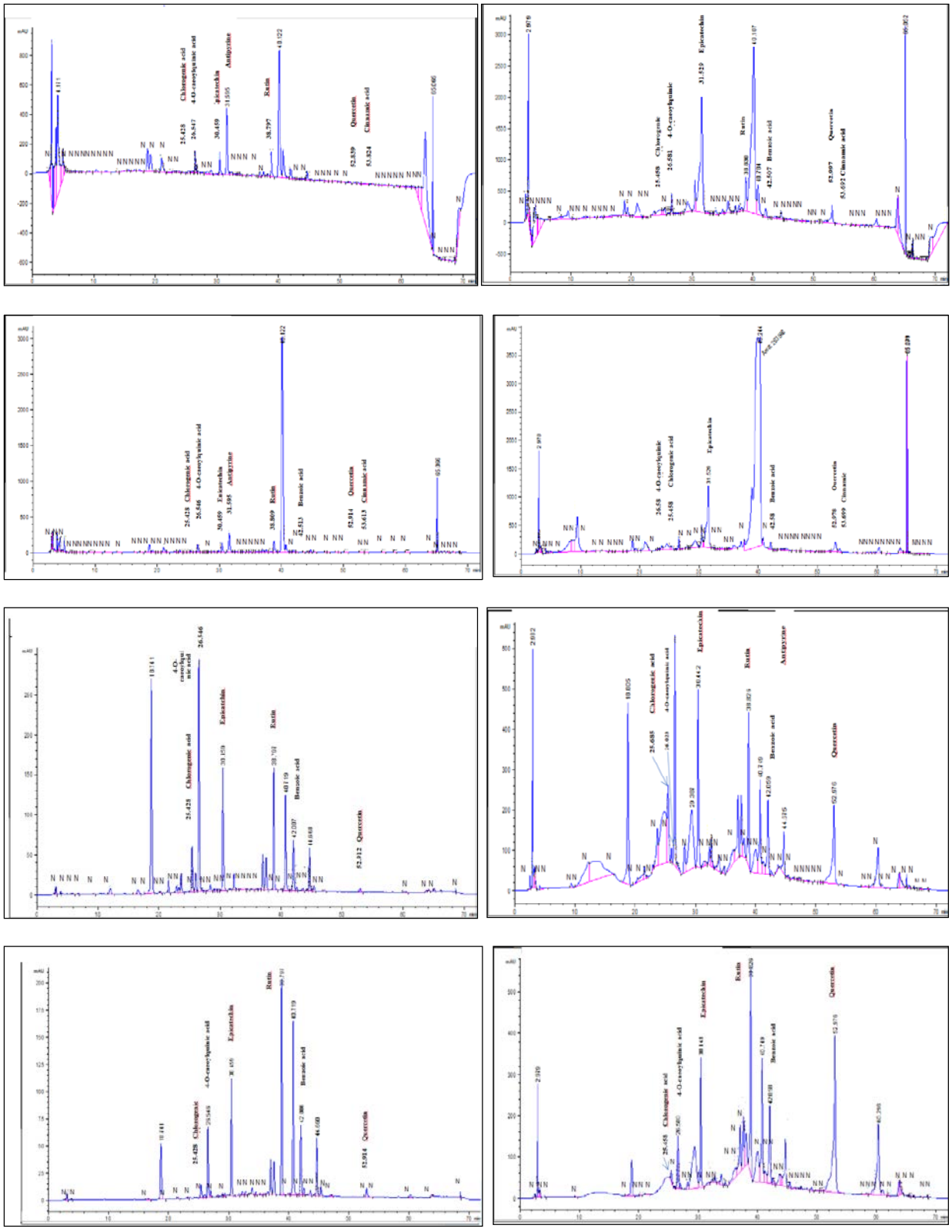

Figure 6: HPLC phenolic compounds of sample juice (C)before and after hydrolysis, at 230, 275, 330 and 360 nm (N = unkown flavonoied compounds at 360 and above)

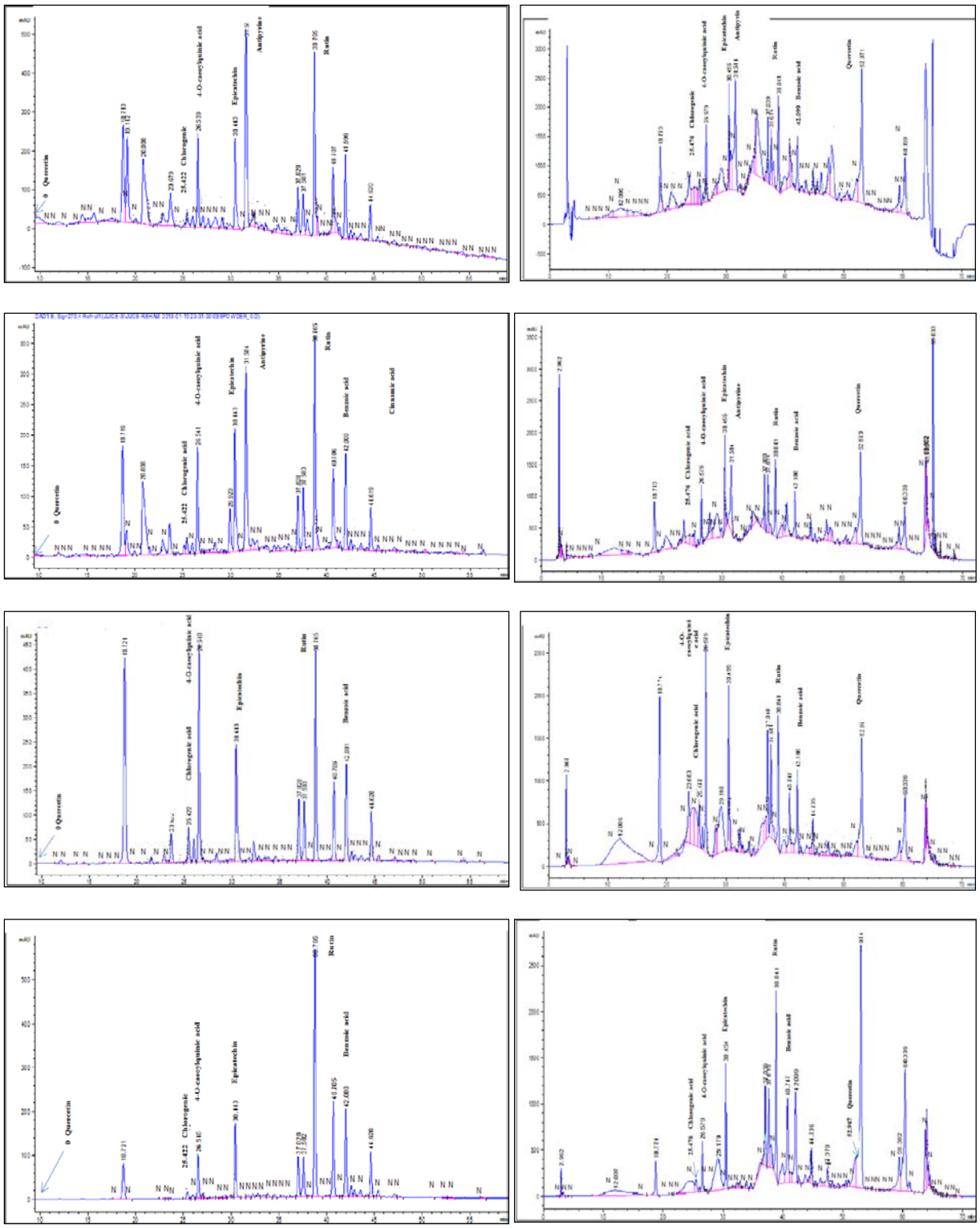

Figure 7. HPLC phenolic compounds of M.O.A.E powder before and after hydrolysis, at 230, 275, 330 and 360 nm (N = unkown flavonoied compounds at 360 and above)

CONCLUSION

M.O.L is an excellent source of nutrition for the humans around the world who lack in many nutritional eliminates such as protein, vitamins, phenolics and antioxidant activity.
The M.O.A.E. might be stared as a promising candidate of 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, F.G.W.J fortified

with M.O.A.E. could be considered as a significant source of antioxidant, free radical scavenger and shelf-life prolonger. It was applicable, successful and accessible to utilize moringa crop when producing many pleasant and preferable manufactured products. This result indicated that, the fortification of F.G.W.J with 1.5 or 2.0% of M.O.A.E. was the exact ratios for using Moringa Olivera leaves' extract in such juices.

Finally, results indicate that Epicatechin (EP), rutin (RU), quercetin (QU), benzoic acid (BE) and chlorogenic acid (CH), were predominant phenolic compounds in F.G.W.J fortified with M.O.A.E. whereas, the others are dominant phenolic constituents. However, further researches are needed to test the sanitizing and preservative effects of M.O.A.E on foods.

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