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Chromatography Profile of Sugars and Minerals Detected in Ajwa and Tahini in Comprising with Their Mixture

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Abstract

There are different eating habits spread in the world that depend on social or personal habits. Consumption of two or more food types is a popular social behavior and a traditional recipe from the Arabian culture. Ajwa is one of the most important date types which consume every day in Gulf countries especially in Saudi Arabia. Tahini is a prevalent type of oily paste sauce used in middle east that prepared from sesame seeds mechanically hulled and pulverized. Ajwa and tahini are traditionally consumed together in many communities. The aim of current study was to explore the influence of mixing tahini with Ajwa date on the sugar and mineral profiles. Mineral and sugar values in samples under investigation were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Gas Chromatography- MS (GC-MS) respectively. Certified Reference Standards for minerals and sugars were applied for calibration and concentration detection purposes. Mixture condition showed different variation of minerals and sugar in relation to pure samples. Most mineral levels were decreased in the mixture. Potassium, magnesium, calcium, manganese, and copper content varied significantly among Ajwa date and tahini mixture. Similarly, combining ajwa with tahini significantly reduce the overall sugar content of ajwa by more than half. In conclusion, these techniques enabled us to analyze sugar and mineral concentrations pattern of Ajwa and Tahini components, which undergo a nutritional significant change of sugar and mineral concentrations after co-consuming of ajwa and tahini. Our study demonstrated that combining ajwa and tahini resulted in a considerable shift in sugar level observed in ajwa, as well as minerals level in both pure jwa and tahini samples.

Keywords: Ajwa, Tahini, Gas Chromatography- Mass Spectrometry, Inductively Coupled Plasma-Mass Spectrometry

Full-length article **Corresponding Author*, e-mail: research_inst@yahoo.com

1. Introduction

These Phoenix dactylifera L is one of the most important and famous natural resource comprising of vitamin, minerals, fibers, sugars, phenols, and flavonoids as well (Baliga *et al.*, 2011;Hassan *et al.*, 2017; Imsya and Malik, 2023). Ajwa date is a unique type of dates recognized as a holy fruit which have been recommended in sunnah and hadith because of their health benefits (Ahmed *et al.*, 2022; Hassan *et al.*, 2017). Its ancient tree grown specifically in Al Medina district of Saudi Arabia (Mallhi *et al.*, 2014). Previous studies clarified the role of dates in the medical field and the influence of ajwa on people health conditions. For a decade, all date types have been used as an excellent healthy food in middle east, north Africa and in Gulf countries (Al-Farsi *et al.*, 2008b; Al-Khalili *et al.*, 2023). Ajwa date consumes daily in Saudi society as essential sweet, snack, and in normal diet. The ability of ajwa to decrease some illness incidents specially inflammation, oxidation stress, diabetic, and cardiac diseases has been reported previously (Vayalil, 2011; Najafi, 2011; Al-Khalili *et al.*, 2023). Moreover, the consumption of ajwa with other type of foods or drinks is traditional habits among several communities. With regards to tahini, it's prepared from sesame seeds with different manufacture methods. Tahini contains essential natural compounds include proteins, minerals, phenols, and polyunsaturated fats. The medical role of tahini was reported previously which derived from sesame seeds containing sesaminol glucosides, sesamolin and sesamin (Pathak et al., 2014). The implication of sesame seeds in decreasing cancer cases, inflammatory incident and lowering blood pressure was published (Sumaina and Laban. 2021). In contrast, allergic reaction could be developed for some individuals resulting from tahini and sesame seeds intake. Tahini is consumed as sauce, butter or as a type of desserts or type of salads. Additionally, tahini served as a dipping for date fruits and ajwa in several countries (Kilci and Çetin. 2023). Although tahini has numerous health benefits, it should be consumed in moderation intake, especially for those watching their calorie or fat intake. Additionally, people with any allergy type should avoid tahini as well.

Different eating habits and lifestyles playing a major role in people health condition. For instance: the health anticipated benefits of each food can be affected when two or more types mixed and consume together such as dates with tahini, and mixing yogurt with fruits (Fernandez and Marette, 2017) The components of different foods may interact with each other when two or more types consumed together. This may lead to alter the nature or the quantities of the contents of each food type that could affect their beneficial outcome. The existing study aimed to evaluate the impact of mixing ajwa and tahini on their sugar and mineral quantities using two different type of chromatogram techniques.

2. Methodology

2.1. First: The assessment of the minerals

- ***Sample Preparation***

Tahini and Ajwa dates were purchased from regular Saudi market. Ten Ajwa flesh were weighed; then mean was assessed. Samples prepared from a mixture of three Ajwa flesh which weighed (22 g) and crushed provide a homogeneous Ajwa paste. The weight of Tahini used to dip three Ajwa weighed 5 g. Negative control contained 22 g of Ajwa paste with 30 ml of hot Milli-Qwater mixed properly using glass rod and vortex (Corning® LSE™ vortex mixers). While Tahini sample composed 22 g Ajwa paste added to 5 g of Tahini mixed with 25 ml of hot Milli-Qwater (hot water was added in calculated amounts to level out the material quantity of the three test samples).

- ***Standard Preparation***

Certified Reference Standards (TCT Inorganic Ventures) for each Macro element under investigation were diluted with 5% nitric acid solution (HNO3, 65% Supr apur®) to calibration levels matching the concentrations of elements after the dilution of the digested samples. Macro elements (Na, K, Ca, P, Cu, Fe, Mn, Mg, and Zn) were determined at certain mass to charge ratio optimized with the minimum interference using Standard Mode and kinetic energy discrimination mode (KED) as mode detectors.

- ***Instrumentation and reagents***

The samples were analyzed using a Nexion 350D Inductively Coupled Plasma Mass Spectrometer (ICP-Mass) (Perkin Elmer, USA) with Synergistix Software (v 1.0). In a Teflon vessel (model xp-1500 plus, CEM Corporation, Matthews, USA) a mixture of 2.5 ml of nitric acid (HNO3, 65% Supr apur®) with 1 ml of hydrogenperoxide (H2O2, 30% Suprapur®) were added to 0.5 ml of homogeneous Ajwa paste and then mixed with 0.5 ml of tahini sample. Samples placed into hotblock (35-Well 50ml HotBlock™ digestor) at 150ºC until the digestion was achieved. Digested samples were cooled down and diluted with 10 ml Milli-Qwater (18.2 MΩ), and then filtered with 0.45 µm syringe filters. Before analysis, samples were diluted with 5% HNO3 in a ratio1:5 and 1:10, and reagent blanks were prepared similarly to the samples.

2.2. Second: The assessment of the Sugars

Similar control and test sample conditions were applied for sugar extractions purposes. The samples were vortex (Corning® LSE™ vortex mixers) and sonicated (Q2000 Sonicator®) for at least 1hour at 60°C. To remove any insoluble materials, 10 ml of 96% ethanol added then samples were centrifuged at 3000-5000rpm for 15 - 20 min. Final solution (supernatant) was evaporated to dryness under nitrogen stream to obtain the extraction.

- ***Standard Preparation***

Certified Reference standards of sugars are prepared from Analytical Grade Fructose (Sigma CAS Number: 57-48-7), Glucose (Sigma CAS Number: 50-99-7), Sucrose (Sigma CAS Number: 57-50-1), Lactose (Sigma CAS Number: 64044-51.5), Maltose (Sigma CAS Number: 6363-53-7), Sorbitol (Sigma CAS Number: 50-70-4), and Galactose (Sigma CAS Number: 59-23-4). Mixed sugar standards for calibration were prepared separately by dissolving 10 mg of each reference standard sugar with 800 µl dried Pyridine (MERCK Index-No 613-002-00-7).

- ***Oximation and Silylation***

The analysis of carbohydrates by GC-MS requiring oximation process for all sample under investigation. Sample oximation procedure: 800 µl of each sugar sample and sugar standard solutions were added to 50 g of methyl hydroxylamine hydrochloride, incubated on heat plate for 60 min at 85°C. Each sample was react with 200 µl of Silylation reagent BSTFA (N,O-bis(trimethylsilyl) trifluoroacetamide) incubated on heat plate for 40 min at 80°C, centrifuged at 25°C for 10 min at 8000× g, and then injected into GC-MS.

- ***Instrumentation & Measurement***

The oximated sample (1µl) was injected in an Agilent 7890B GC-FID System with Open Lab CDS Chem Station system (rev C.01 06). The GC-FID Parameters were optimized as follow: Gas chromatography column: ZB (30m x 0.25mm x 0.25µm). Carrier Gas: Helium Column Oven Temp: 80ºC, Injection Mode: Split mode, Detector: Flame ionization detector.

3. Results

3.1. Calibration

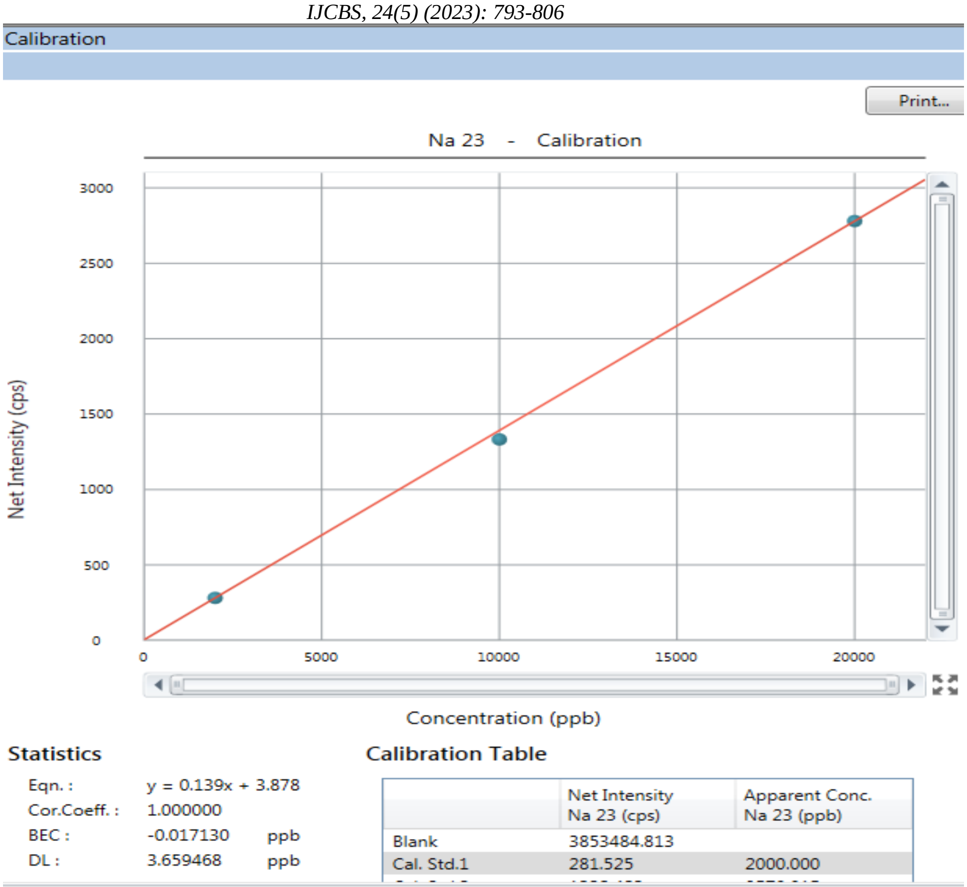
The quantification of minerals in the present work was assessed using standard graphs that achieved by Certified reference material TCT Inorganic Ventures solution analysis. Calibration curves of mineral elements shown in Figure 1 which validated using regression analysis. The intercepts were almost zero indicating to minimum matrix interference. Spearson's correlation factors (r^2) were ≥0.9998 for samples investigated herein and all points were on straight lines (Figure 1). The results indicated linearity within the concentrations used and therefore the regression equations used to quantify the minerals in all samples. Data illustrated in Figure 2 confirm the target minerals stability through the analysis period.

3.2. Mineral contents

Our results provide the nutritional comparison aspects between of sugar and minerals for Ajwa dates, tahini separately and in their combination. Minerals and sugar were corelate numerous physiological functions of the human body. Ajwa flesh enriched with minerals, and sugar, tahini also enriched with minerals but has no sugar. The quantification of minerals under investigation is shown in (Table 1). Pure Ajwa and tahini samples exhibited high mineral amounts than in the mixture for most elements. With regards to Ajwa date, the most abundance minerals were potassium and magnesium (8722 & 856.4 mg/kg correspondingly). While calcium and phosphorous reveal second profusion element concentrations (638.7 & 647.4 mg/kg respectively). Similar contents of sodium (24.2 mg/kg) and zinc (22.7mg/kg) were observed in ajwa sample. Phosphorous, potassium, magnesium, and calcium concentrations in pure tahini sample were abundant with the presented order. Obvious reduction of mineral levels in the mixture (ajwa / tahini) condition comparison to pure samples illustrated in Figure 3.

3.3. Sugar contents

Standard sugars (glucose, galactose, sucrose, lactose, and fructose) were analyzed under the same gas chromatogram operating conditions. Sharp and distinguished peaks for standards analysis presented in Figures 4A.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Na 23 - Calibration
Net Intensity (cps)
3000
2500
2000
1500
1000
500
0
0
5000
10000
15000
20000
Concentration (ppb)
Statistics
Eqn. : y = 0.139x + 3.878
Cor.Coeff. : 1.000000
BEC : -0.017130 ppb
DL : 3.659468 ppb
Calibration Table
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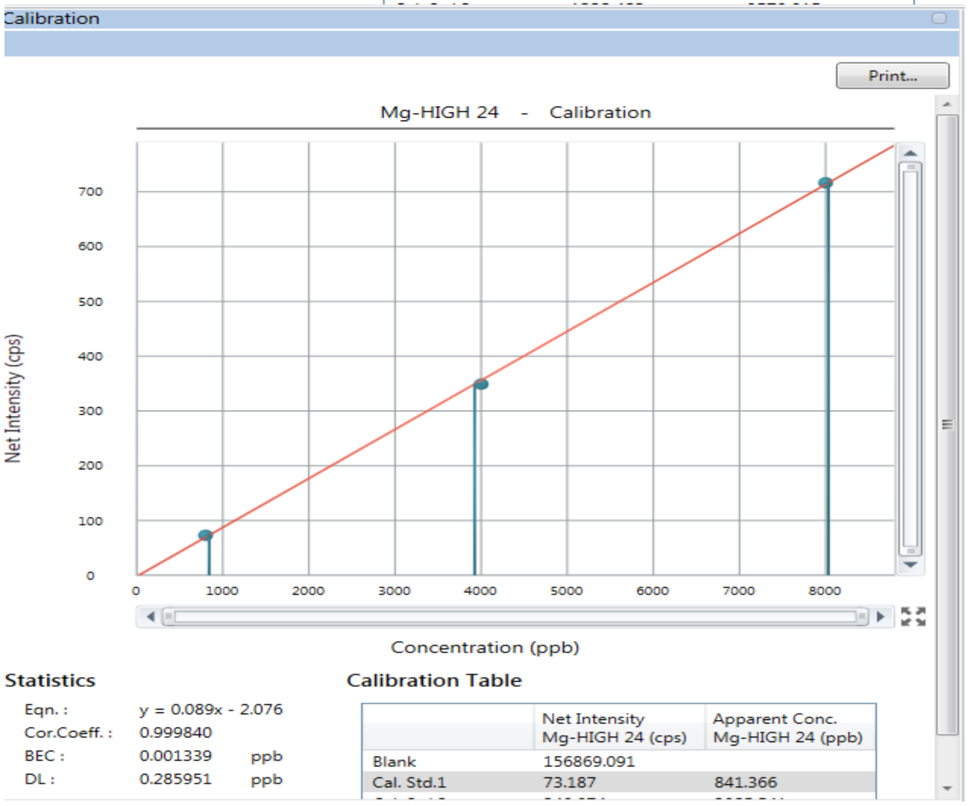
Apparent Conc. Na 23 (ppb)
Blank 3853484.813
Cal. Std.1 281.525 2000.000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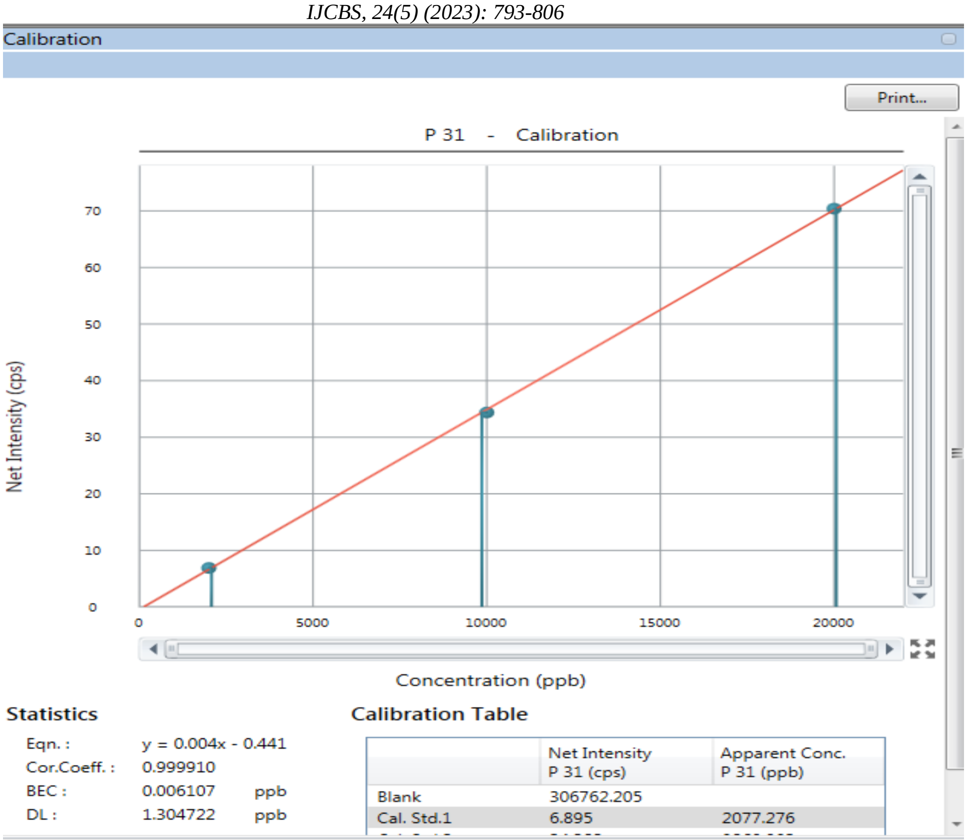
Mg-HIGH 24 - Calibration
Net Intensity (cps)
700
600
500
400
300
200
100
0
0
1000
2000
3000
4000
5000
6000
7000
8000
Concentration (ppb)
Statistics
Eqn. : y = 0.089x - 2.076
Cor.Coeff. : 0.999840
BEC : 0.001339 ppb
DL : 0.285951 ppb
Calibration Table
Net Intensity Mg-HIGH 24 (cps)
Apparent Conc. Mg-HIGH 24 (ppb)
Blank 156869.091
Cal. Std.1 73.187 841.366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P 31 - Calibration
Net Intensity (cps)
70
60
50
40
30
20
10
0
0
5000
10000
15000
20000
Concentration (ppb)
Statistics
Eqn. : y = 0.004x - 0.441
Cor.Coeff. : 0.999910
BEC : 0.006107 ppb
DL : 1.304722 ppb
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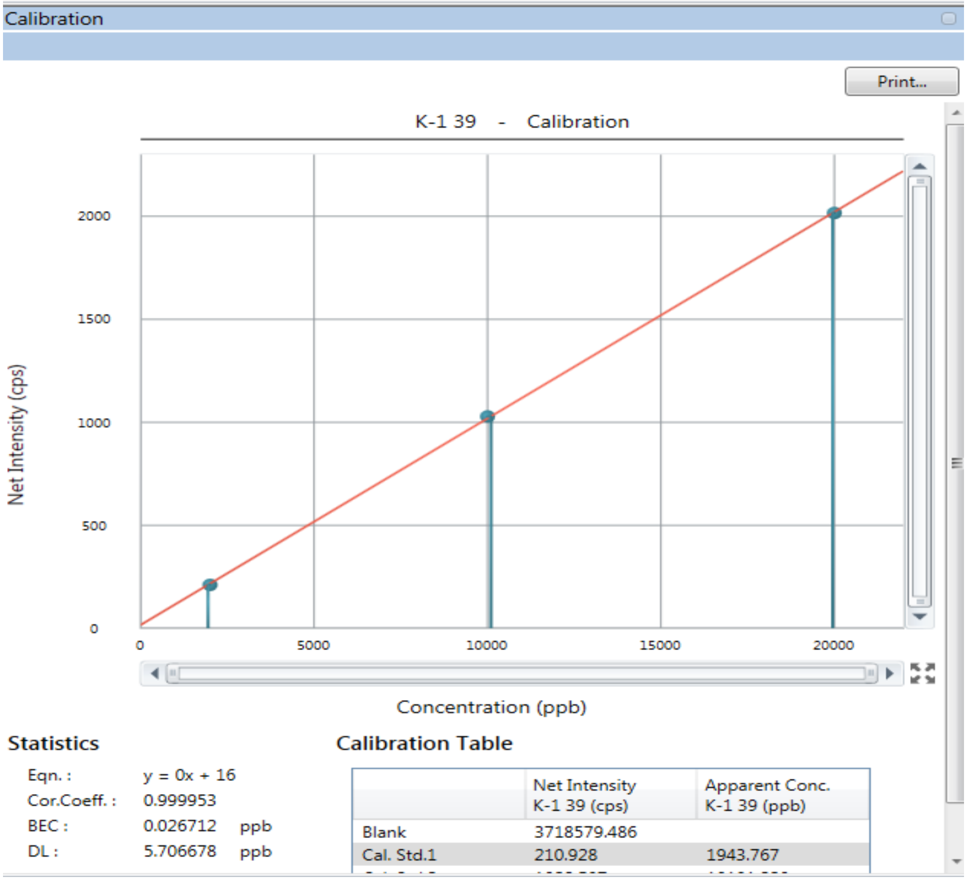
Apparent Conc. P 31 (ppb)
Blank 306762.205
Cal. Std.1 6.895 2077.276


Calibration
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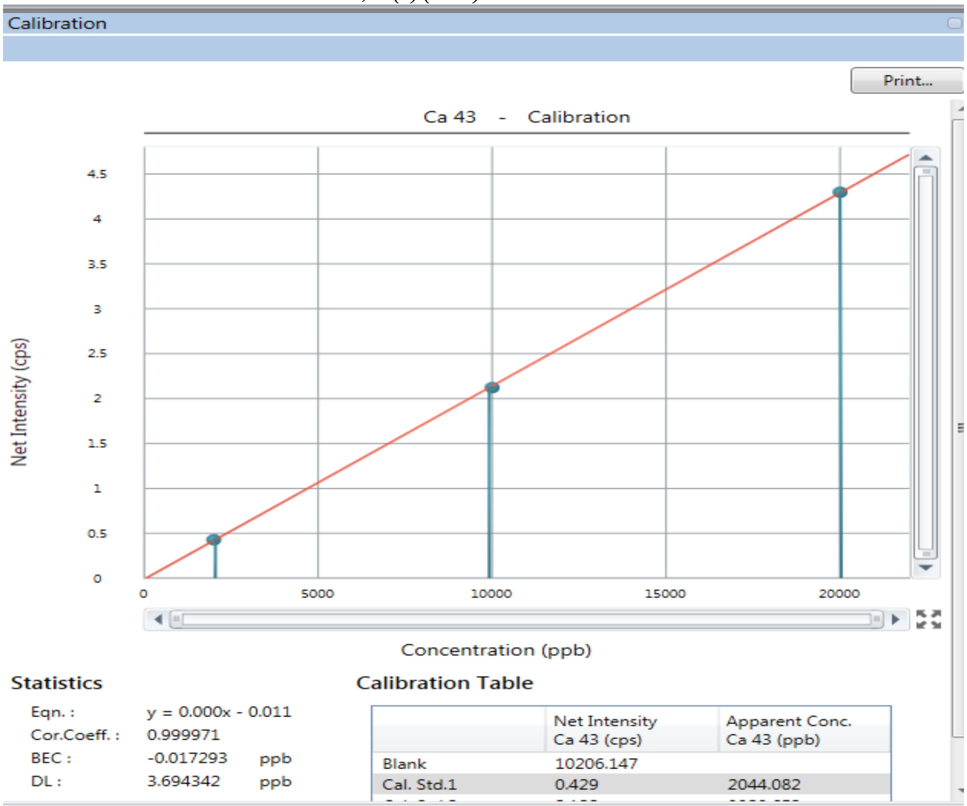
K-1 39 - Calibration
Net Intensity (cps)
2000
1500
1000
500
0
0
5000
10000
15000
20000
Concentration (ppb)
Statistics
Eqn. : y = 0x + 16
Cor.Coeff. : 0.999953
BEC : 0.026712 ppb
DL : 5.706678 ppb
Calibration Table
Net Intensity K-1 39 (cps)
Apparent Conc. K-1 39 (ppb)
Blank 3718579.486
Cal. Std.1 210.928 1943.767


Calibration
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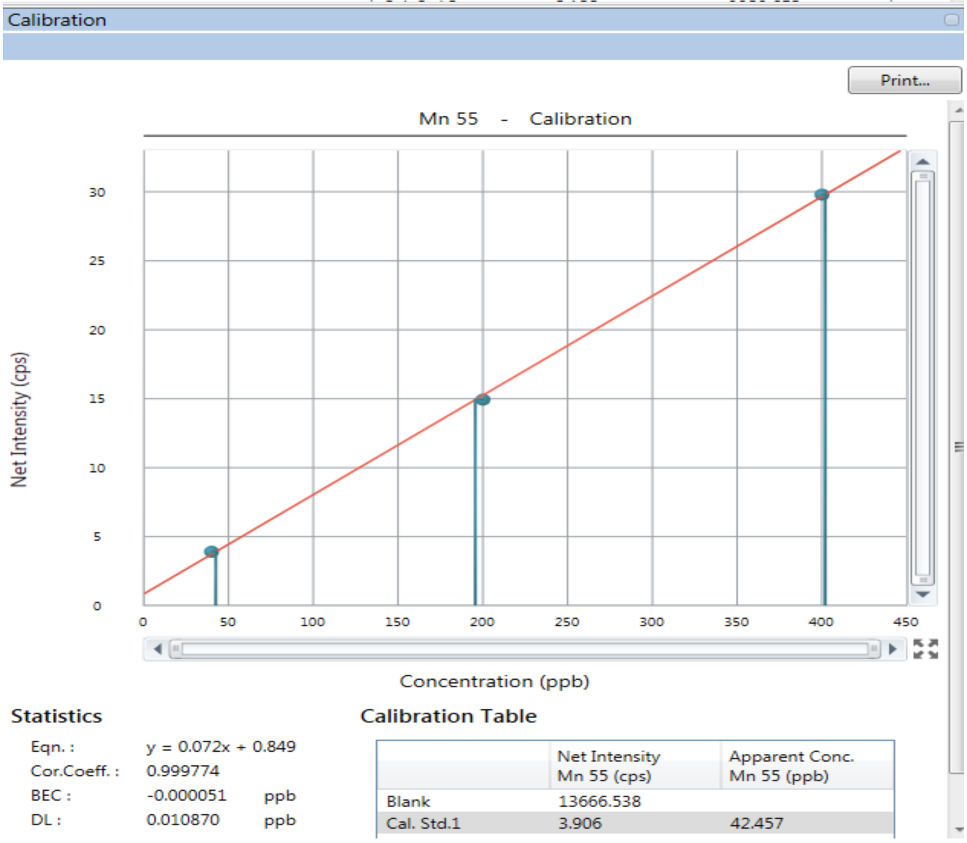
Ca 43 - Calibration
Net Intensity (cps)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
5000
10000
15000
20000
Concentration (ppb)
Statistics
Eqn. : y = 0.000x - 0.011
Cor.Coeff. : 0.999971
BEC : -0.017293 ppb
DL : 3.694342 ppb
Calibration Table
Net Intensity Ca 43 (cps)
Apparent Conc. Ca 43 (ppb)
Blank 10206.147
Cal. Std.1 0.429 2044.082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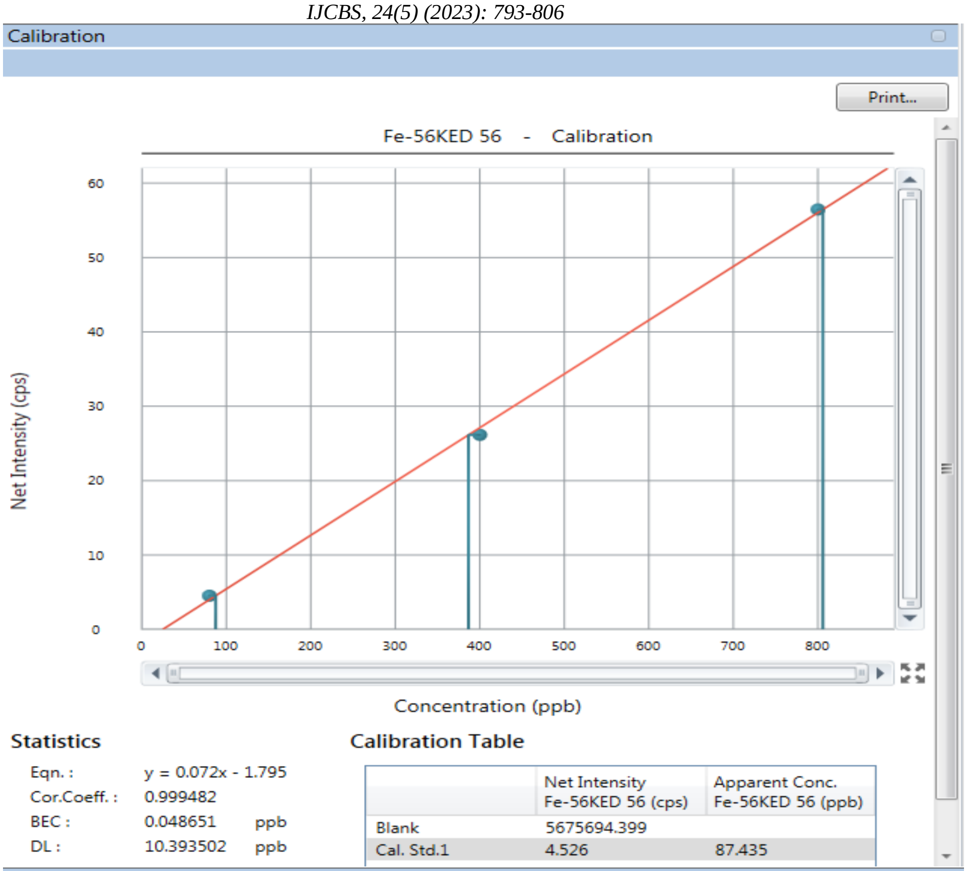
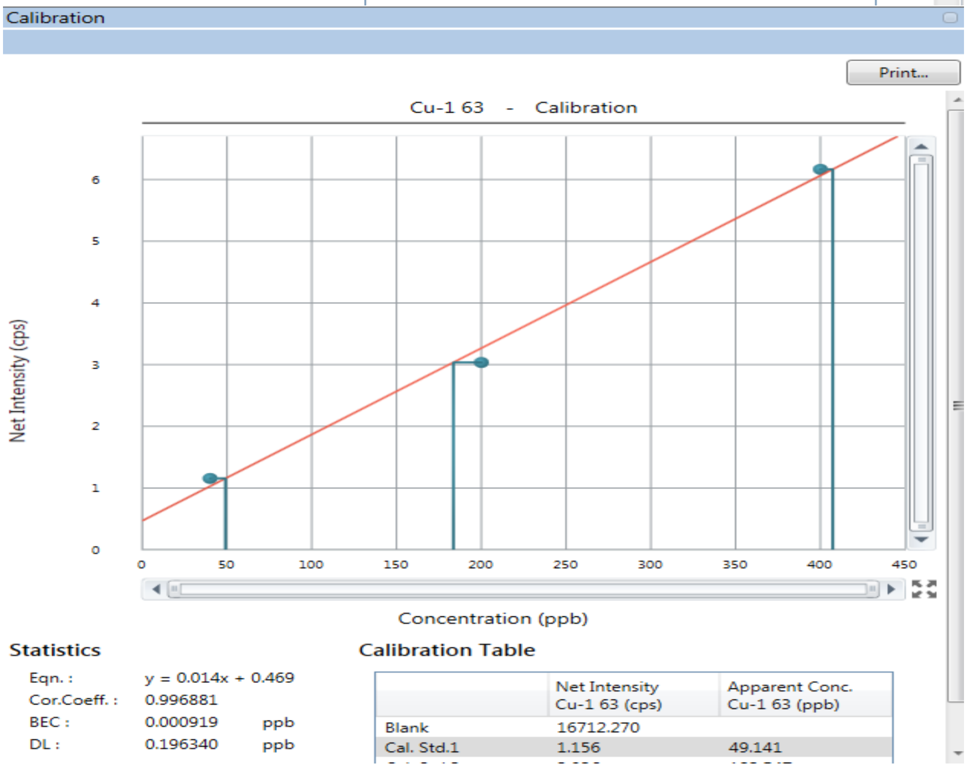
Print...
Mn 55 - Calibration
Net Intensity (cps)
30
25
20
15
10
5
0
0
50
100
150
200
250
300
350
400
450
Concentration (ppb)
Statistics
Eqn. : y = 0.072x + 0.849
Cor.Coeff. : 0.999774
BEC : -0.000051 ppb
DL : 0.010870 ppb
Calibration Table
Net Intensity Mn 55 (cps)
Apparent Conc. Mn 55 (ppb)
Blank 13666.538
Cal. Std.1 3.906 42.457


Calibration
Print...
Fe-56KED 56 - Calibration
Net Intensity (cps)
0
10
20
30
40
50
60
0
100
200
300
400
500
600
700
800
Concentration (ppb)
Statistics
Eqn. : y = 0.072x - 1.795
Cor.Coeff. : 0.999482
BEC : 0.048651 ppb
DL : 10.393502 ppb
Calibration Table
Net Intensity Fe-56KED 56 (cps)
Apparent Conc. Fe-56KED 56 (ppb)
Blank 5675694.399
Cal. Std.1 4.526 87.435


Calibration
Print...
Cu-1 63 - Calibration
Net Intensity (cps)
0
1
2
3
4
5
6
0
50
100
150
200
250
300
350
400
450
Concentration (ppb)
Statistics
Eqn. : y = 0.014x + 0.469
Cor.Coeff. : 0.996881
BEC : 0.000919 ppb
DL : 0.196340 ppb
Calibration Table
Net Intensity Cu-1 63 (cps)
Apparent Conc. Cu-1 63 (ppb)
Blank 16712.270
Cal. Std.1 1.156 49.141

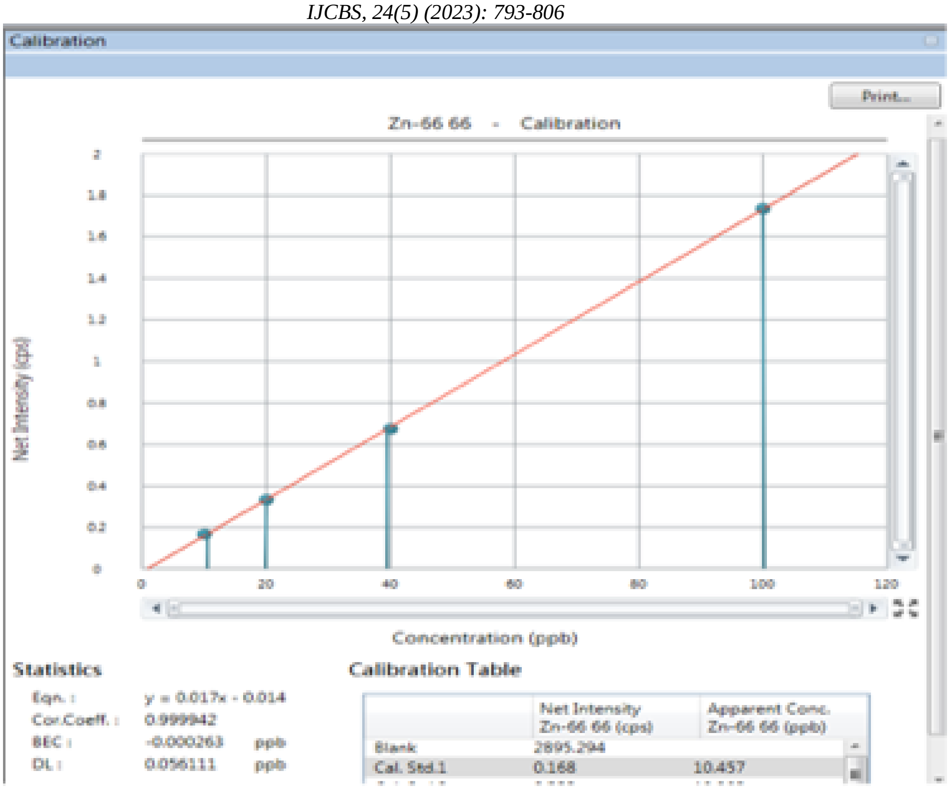


Figure 1: Calibration curves of minerals (Na, K, P, Ca, Cu, Fe, Zn, Mn & Mg) using ICP-MS Concentrations were determined at certain mass to charge ratio optimized with the minimum interference using Standard Mode and mode detectors

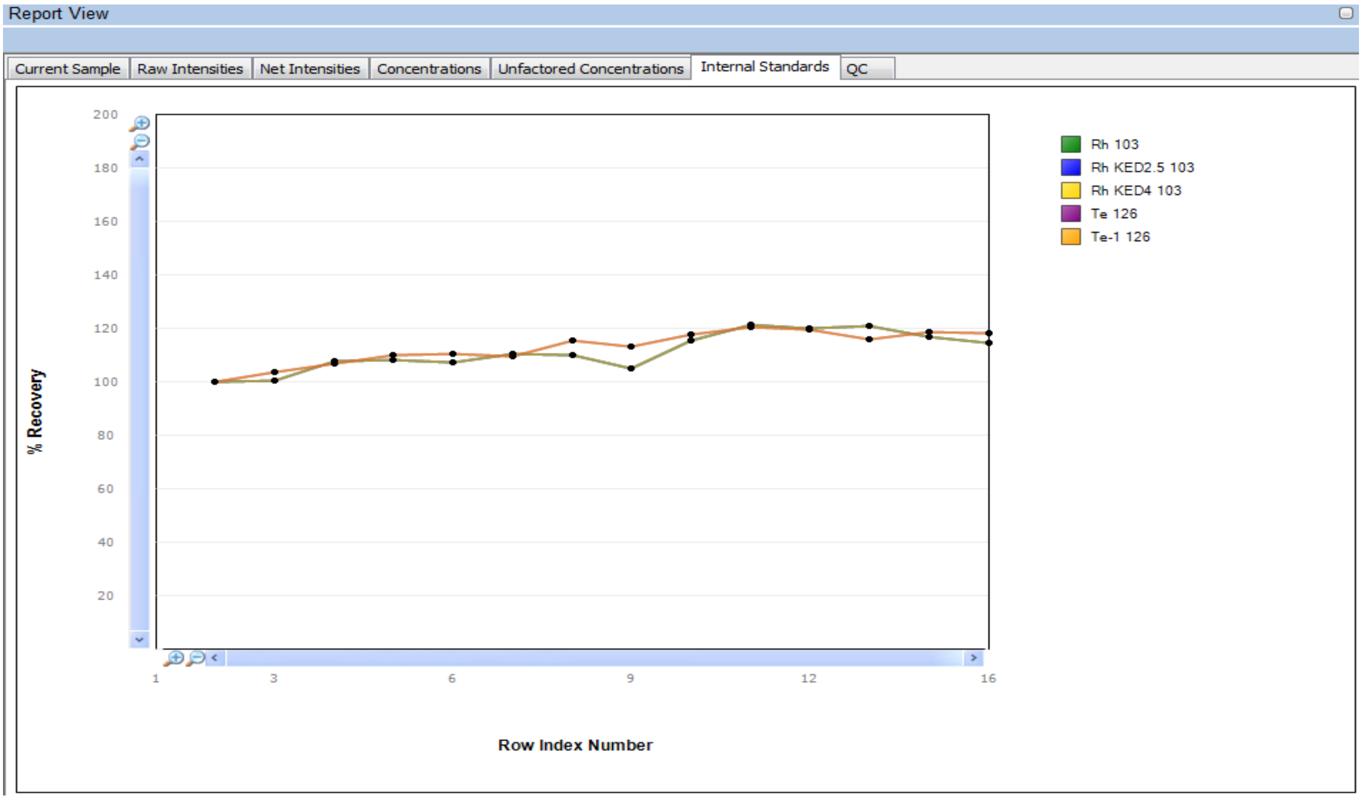
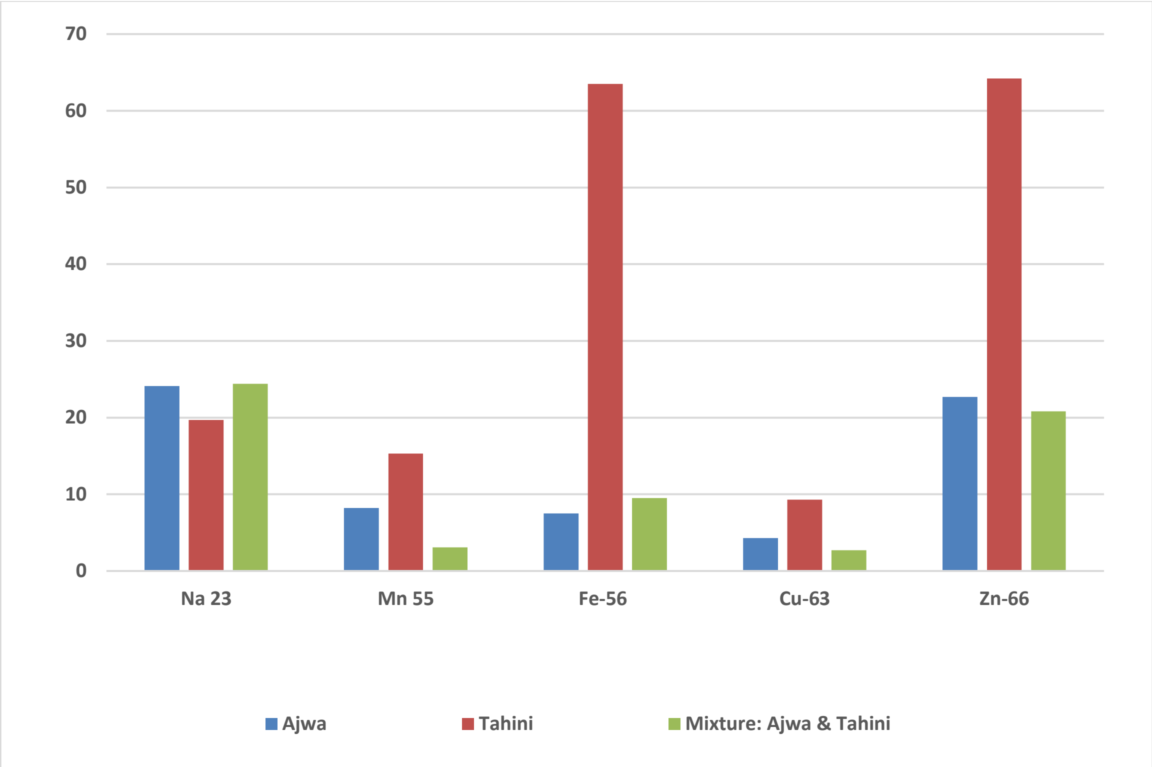


Figure 2: The recovery percentage of internal standard using ICP-MS: Minerals internal standards in gas and non-gas mode are represented in the diagram

(A)



(B)

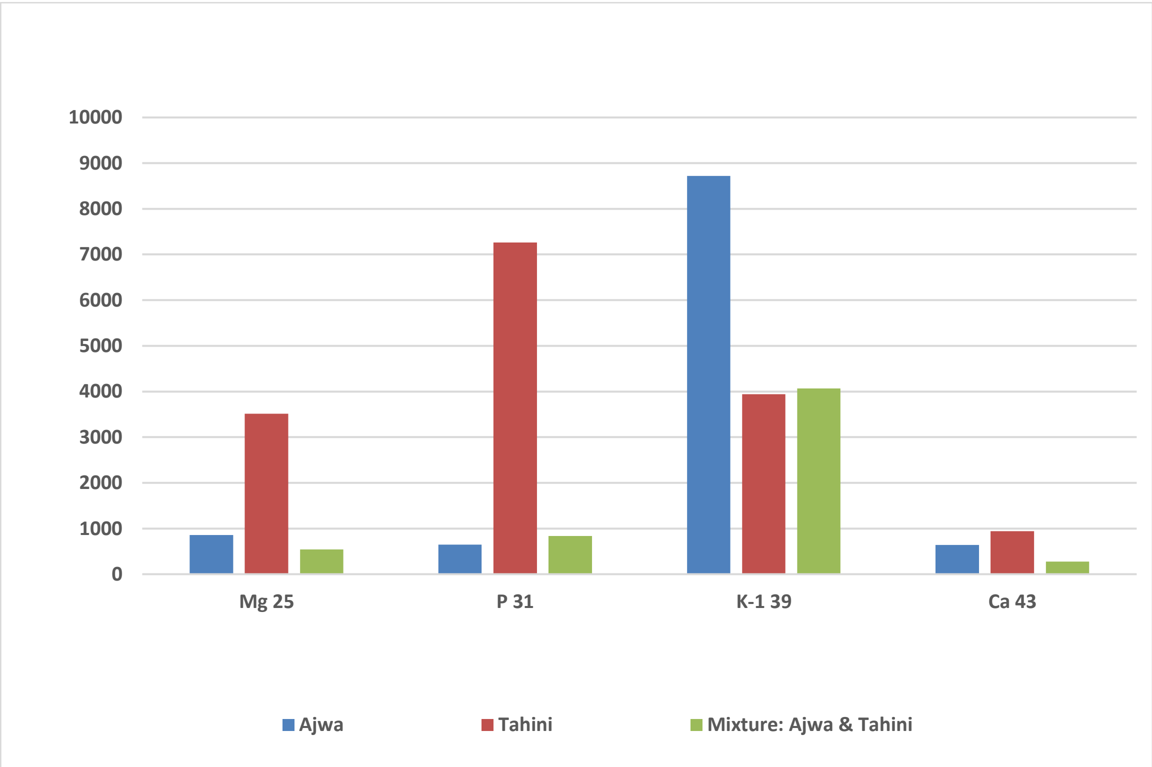
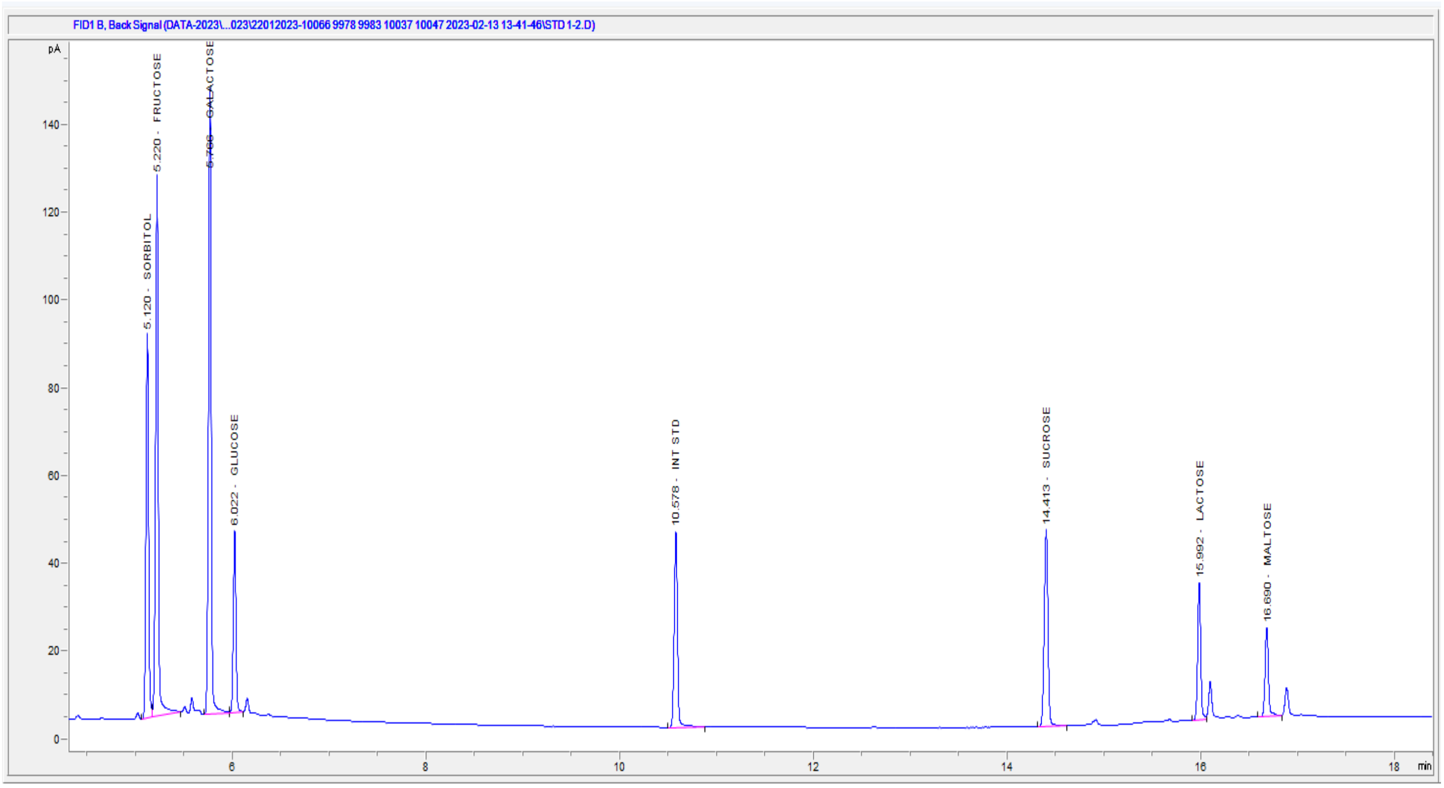
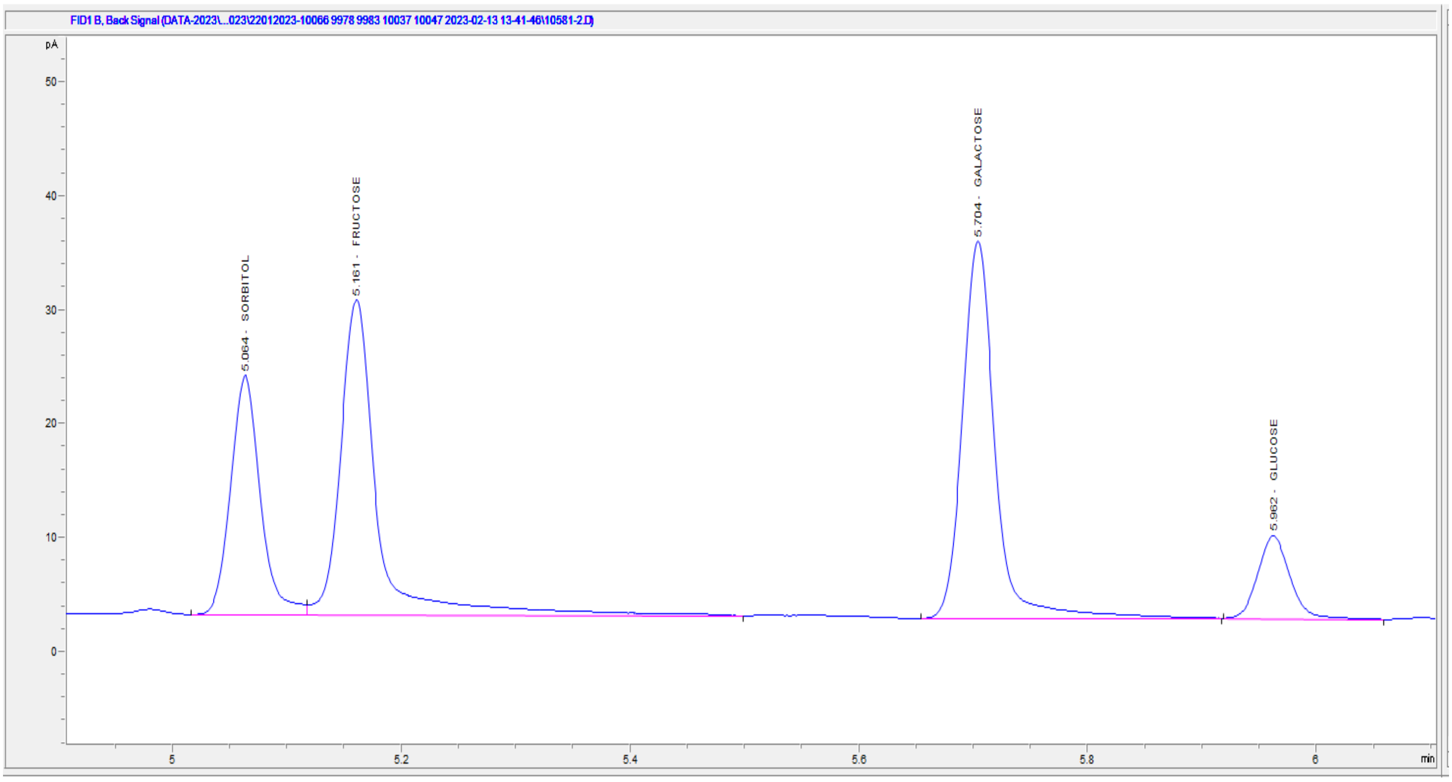


Figure 3: Overview of minerals quantified in each condition. Major different between mineral concentrations lead to present minerals comparison in tow graphs (A): concentration from 8722 to 638.7 mg/kg. The second group (B): concentration from 2.7 to 24.4mg/kg

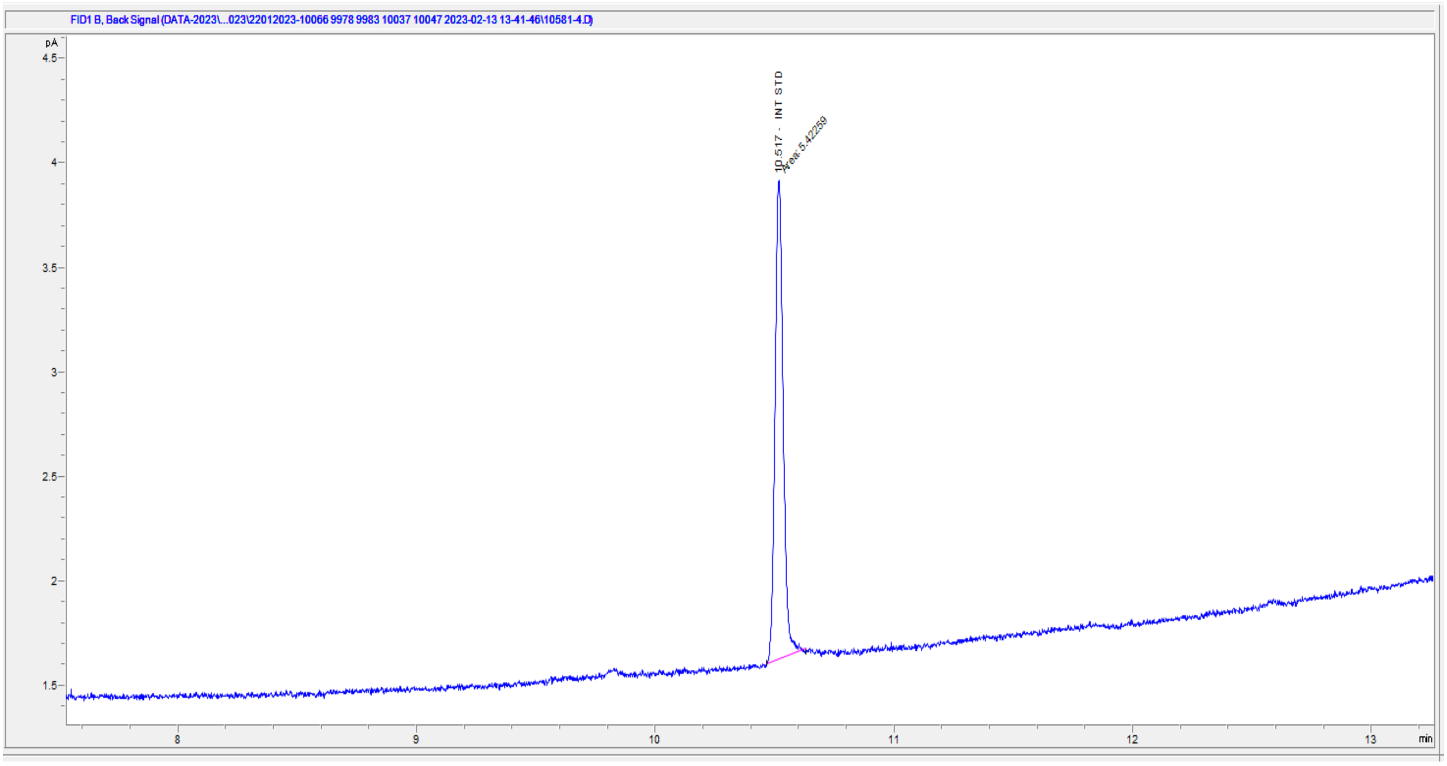
(A)


FID1 B, Back Signal (DATA-2023\...023\22012023-10066 9978 9983 10037 10047 2023-02-13 13-41-46\STD 1-2.D)
pA
5.120 - SORBITOL
5.220 - FRUCTOSE
5.768 - GALACTOSE
6.022 - GLUCOSE
10.578 - INT STD
14.413 - SUCROSE
15.992 - LACTOSE
16.690 - MALTOSE
min

(B)


FID1 B, Back Signal (DATA-2023\...023\22012023-10066 9978 9983 10037 10047 2023-02-13 13-41-46\10581-2.D)
pA
5.064 - SORBITOL
5.161 - FRUCTOSE
5.704 - GALACTOSE
5.962 - GLUCOSE
min

(C)



(D)

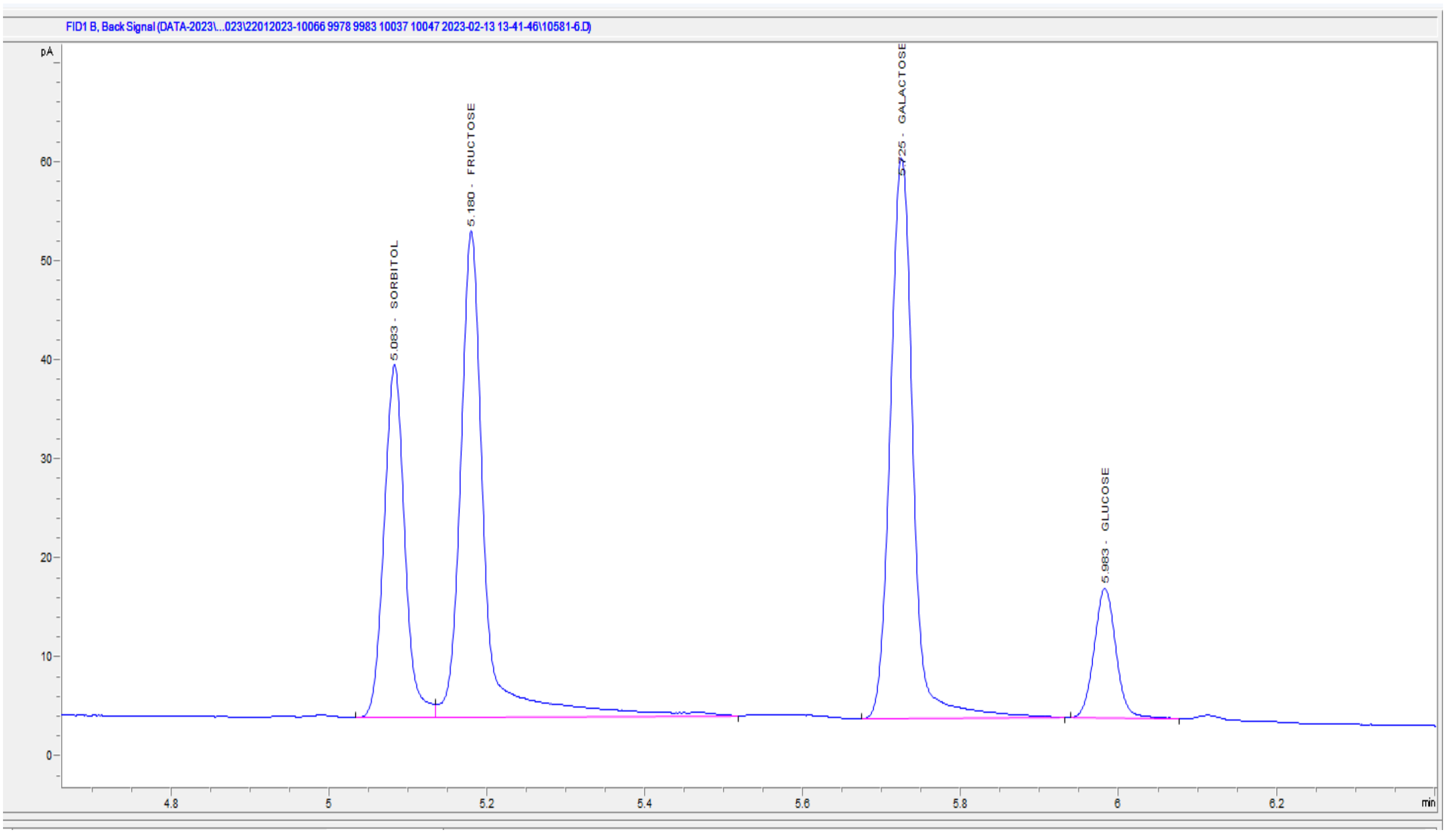


Figure 4: (A) Full Chromatogram analysis of sugar standards with Internal standard. (B) Chromatogram analysis of pure Ajwa sample. (C) Chromatogram analysis of pure Tahini sample. (D) Sugar profile for Ajwa / Tahini mixture

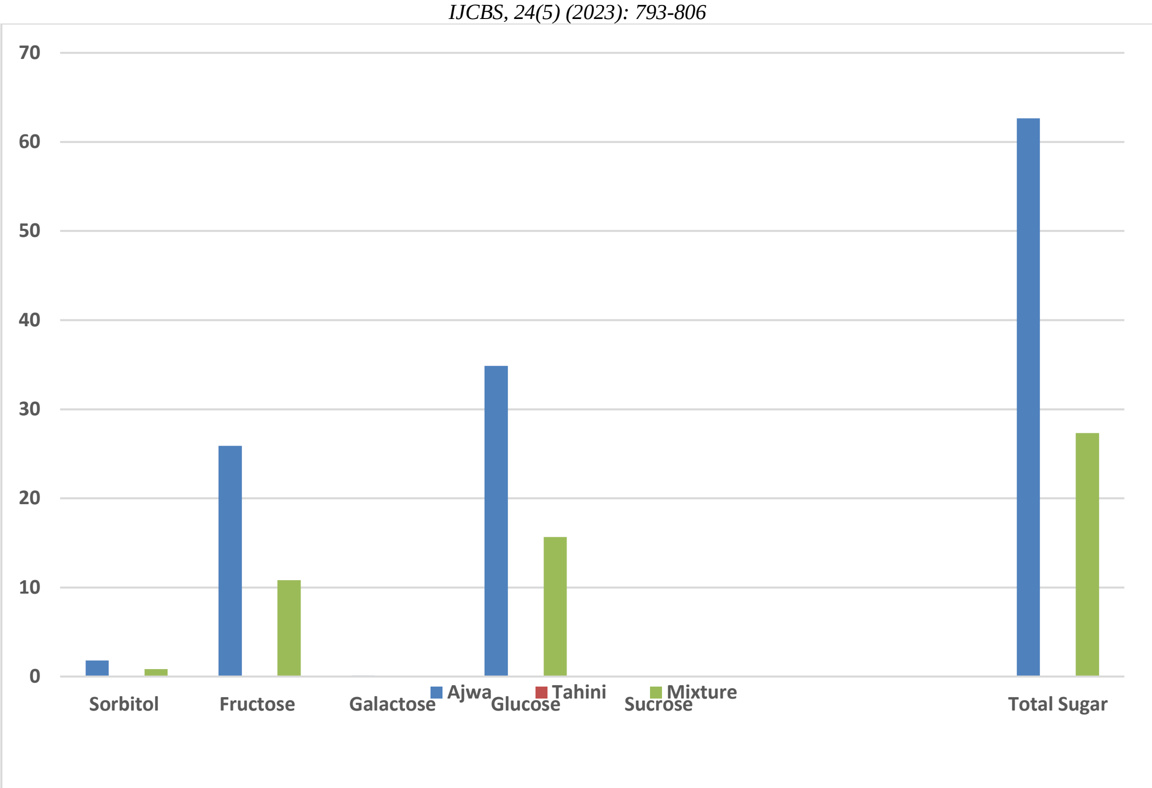


Figure 5: Overview of sugars assessed in each condition. Sucrose, lactose, and maltose not appeared in the graph due to their low concentration (<0.1%m/m) for all conditions

Table 1. Minerals assessment. Concentration of investigated minerals (mg/kg dry weight) using certified reference material TCT Inorganic Ventures and ICP-MS technique. Three pieces of ajwa weight 22g, and 5 g of tahini were used to achieve the current assessment (results are mean of triplicate)

Sample conditions	Na 23	Mg 25	P 31	K-1 39	Ca 43	Mn 55	Fe-56	Cu-63	Zn-66
Ajwa	**24.1**	**857.4**	**647.4**	**8722**	**638.7**	**8.2**	**7.5**	**4.3**	**22.7**
Tahini	**19.7**	**3511.3**	**7259.8**	**3942**	**941.7**	**15.3**	**63.5**	**9.3**	**64.2**
Mixture: Ajwa & Tahini	**24.4**	**539.7**	**839.2**	**4068**	**277.4**	**3.1**	**9.5**	**2.7**	**20.8**

Table 2. Sugar profile for sample under investigation using GC-MS. Sugar assessment findings presented for investigated conditions by % m/m dry weight. Three pieces of ajwa weight 22g, and 5 g of tahini were used to achieve the current assessment (results are mean of triplicate)

Sample conditions	Sorbitol	Fructose	Galactose	Glucose	Sucrose	Lactose	Maltose	Total Sugar
Ajwa	1.8	25.89	0.12	34.85	<0.1	<0.1	<0.1	62.66
Tahini	<0.1	<0.1	<0.01	<0.1	<0.1	<0.1	<0.1	<0.5
Mixture: Ajwa & Tahini	0.85	10.82	0.03	15.65	<0.1	<0.1	<0.1	27.32

The chromatogram of pure Ajwa sample and mixture condition demonstrated in Figure 4B&D, which determine clear sharp peaks within 8 & 7 minutes. The results reveal peaks correspond to those peaks detected for standard sugar in Figure 4A. With regards to each sugar quantity, glucose and fructose exhibited higher percentages in pure ajwa and mixture samples when compared with other sugar amounts. Obvious differences of sugar concentration in pure ajwa and tahini samples represented in Table 2. No Sugars were detected in pure tahini condition, and only Internal Std appeared in the graph (Figure 4C). Mixture condition revealed a significant decrease of sugar concentrations comparing to pure samples (Figure 4D & 5) and (Table 2).

4. Discussion

Ajwa dates and tahini are excellent natural health promoting foods. Although each of them has different taste but their co-consumed have an excellent special taste. Various types of bioactive components are found in ajwa dates and in tahini that reported previously (Najafi, 2011; Sumaina and Laban., 2021). Different dietary combination influences various changes in food component nature that favor for human health (Mariotti et al., 2021). To best of our knowledge, no previous studies has reported the potential effects of co-consuming ajwa and tahini on their nutritional contents. Thus, we studied the effects of ajwa and tahini combination as a comparative analysis of sugar and mineral quantities in pure ajwa and tahini samples with ajw/tahini mixture condition.

Overall, sugar, a vital source of natural energy to our body and minerals are necessary for growth and maintaining healthy body. Ajwa and tahini pure samples are appropriate diet for hypertension patients due to the existing of excessive amount of potassium with low sodium concentration at the same time (Ralston *et al*. 2012). Elderly people, pregnant and lactating women can rely on ajwa as a major source of potassium and magnesium because of their high concentrations (Siddeeg *et al*., 2014). Tahini contains no sugar but it full of healthy minerals providing a suitable type of diet for diabetics. In terms of mineral contents, Phosphorus is the most abundant mineral in tahini in present work, followed by Potassium, Magnesium, and Calcium, while other elements are presented in varying levels of low concentrations. These findings do not accord with work published by El-Adawy & Mansour lab which revealed that tahini samples included very high levels of Na, Mg, K, Cu, Zn, and Fe and comparatively low level of Ca (El-Adawy & Mansour 2000).

On the other hand, our results are in good agreement with those reported by (Pathak et al., 2014 & Morya et al., 2022). On the other hand, Ajwa's most prevalent mineral is Potassium. Moreover, it has a remarkable concentration of Magnesium, Phosphorus and Calcium. Variation of sugar and mineral contents among Ajwa date sections has been recorded as a function of genetic composition, soil mineral concentration, and fertilizer influence indicated to the importance of each ajwa part for human health (Sumaira et al., 2017). Another innovative result in our research was a comparison of sugar and mineral concentrations after combining ajwa and tahini. The results of the mixture analysis indicated to major changes in mineral and sugar amounts than in each pure sample. The evaluation of pure ajwa and mixture samples revealed the same order of abundant minerals in both conditions however mineral amounts reduced more than the half in the mixture. Significant decrease within almost all mineral quantitates in mixture condition than in pure tahini sample suggested excessive effect of ajwa on tahini mineral contents.

The evaluation of carbohydrates in our study for ajwa and tahini pure samples in addition to their mixture sample obtained great different of sugar contents for each condition. However, a moderate total sugar concentration was obtained from ajwa sample with abundant glucose then fructose amounts with far less level of other types, indicated to similar previous finding (Salomón-Torres *et al.,* 2019; Nadeem *et al.,* 2019). One of the important outcomes herein was the effect of ajwa and tahini on each other presented the change of sugar contents in the mixture sample which was not equivalent with pure samples analysis. The current study demonstrated that combining ajwa and tahini resulted in a considerable shift in sugar level observed in ajwa, which dropped to more than the half. Previous studies identified the relation between diabetes patients with ajwa and sesame intake.

They reported that date fruits have low glycemic index when few consumed and illustrated the improvement of insulin and glucose levels within diabetic patients after sesame intake as well (Alalwan *et al.,* 2020; Yargholi *et al.,* 2021). The recommended sugar intake per day was published by American Heart Association (AHA) indicated to 38g /day for men, and 25g / day for women. The correlation between recommendations and presented results showed that three pieces of pure ajwa (22g) or dipped in tahini (5g) is contain far less sugar amount than the recommended intake /day confirming the non-harmful effect of ajwa on diabetic people. The mixture analysis demonstrated significant points in minerals as follow:

1. For ajwa: ajwa loose more than the half of potassium, magnesium, calcium, manganese, and copper concentrations when mixed with tahini. A slight drop of zinc contents, and no significant change for sodium was achieved in the mixture. The benefit of the mixture on ajwa mineral amounts presented in the increase of iron and phosphorous concentrations than in pure ajwa.

2. For tahini: almost all mineral quantities in mixture condition were declined with different ranges than in pure tahini sample, determined the impact of ajwa on tahini mineral amounts. Mixing tahini with ajwa causing decline the beneficial role of tahini in human medical health condition. However, sodium and potassium amounts showed slight increase in mixture than in pure tahini model but still is a suitable food for hypertension individual.

5. Conclusion

Our findings show quantitative determination of mineral and sugar in Ajwa and tahini as well as their mixture. Co-consuming food has been proved by now to cause a significant alteration in its composition. Our study revealed that combining ajwa and tahini resulted in a considerable shift in sugar level observed in ajwa, as well as minerals level in both jwa and tahini separately. The future study can explore the impact of ajwa & tahini mixture on more nutritional contents such as vitamins, fibers, phenols, and fats to determine which components amount will be affected by the mixture condition. Ultimately, the assessment of more component will aid people to easily decide their dietary quantity and type.

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