Evaluation Of Proximate Chemical Components, Nutritional Value, Antinutritional And Antioxidant Properties In Yellow Mustard Seeds.

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ABSTRACT: The yellow mustard seeds was analysed for the Proximate composition, Mineral content, Antinutrient composition, Antioxidant properties and the Amino acid profile. The result obtained showed that yellow mustard seeds had a high content of fat (51.60%), followed by protein (23.11%) and fiber (9.34%) and low contents of total carbohydrates (8.23%). It is also a rich source of Potassium (K), Magnessium (Mg), Calcium (Ca), Sodium (Na) and Iron (Fe). Also, the antinutritional composition of yellow mustard seed shows that phytates, phytic acid, oxalates, tannins and phenols were 42.74mg/g, 12.04mg/g, 5.79mg/g 1.38mg/g, 14.44mg/g respectively and its antioxidant properties, phenol, flavonoid, and FRAP in this particular seed were 3.97mg/g, 0.54mg/g and 46.96mg/g respectively and the result on chelating Fe^{2+}, 1,1-diphenyl-2-picryhydrazyl (DPPH) and total antioxidant (T.A) were 42.17%, 63.13% and 25.06% respectively. Furthermore, both essential and non essential amino acids were found in yellow mustard seeds in high amounts, which contained a higher contents of Glutamate, Proline and Aspartate. In addition the yellow mustard seeds under investigation contained considerable amount of aromatic amino acids (Phenylalanine and Tyrosine), sulphur containing amino acids (Methionine and cystine), Leucine, Valine, and Lysine. The result showed that Mustard seeds powders had high nutritional value due to its high dietary fiber, antioxidant properties, mineral content, protein and amino acids. Its also possesses high antinutritional components such as phytates, phytic acid and tannins.

Keywords: Proximate Chemical components, Antinutrient, Antioxidants Properties, Aminoacid Profle, Mustard Seeds.

1.0 Introduction

Mustard seeds are small round seeds of various mustard plants. The seed are usually about 1 to 2mm in diameter. The mustard plant belongs to the *Cruciferae (Brassicaceae)* family. Mustard used in food is often a mixture of seeds from two or more species of *Brassicaceae*, for example *Sinapis alba L.* (white or yellow mustard), *Brassica nigra* (black mustard) and *Brassica juncea L.* (Brown or oriental mustard) [1]. *Sinapis alba* is commonly known as white or yellow mustard. *Brassica juncea*, commonly called brown or oriental mustard. Mustards are functional foods having beneficial physiological effects in humans. *Sinapis alba* can be used as a source of wide range of active components including isothiocyanates, phenolics, dithiolthiones and dietary fiber [2].Mustard plant at different types have been widely cultivated and used as spice, medicine and a source of edible oil since ancients times[1]. The mustard seed is rich in protein and the protein is of excellent nutritional quality, being rich in lysine with adequate amounts of sulphur containing amino acids-limiting amino acids in most of the cereals and oilseed proteins [3]. The use of protein rich full-fat and defatted flours shows promise in improving the nutritive value of the final product as well as optimum utilization of the flour. protein fortification of food of current interest because of increasing awareness is consumer towards health and quality of food [4]. Mustard is used on some meat products, such as hotdogs and burgers, but often an added ingredient in sauces, salads and other foods; for example mayonnaise, salad dressing, barbecue and related products as well as ketchup, may contain mustard. Mustard is also used in various traditional remedies to stimulate appetite and as a laxative, expectorant and antiseptic agent for treatment of various gastrointestinal, respiratory and skin diseases [5]. Oil mustard seeds had been shown to impart several beneficial effects of which the antioxidant effect is most pronounced [6]. It had been found that mustard had higher antioxidant activity as compared to fruits, cereals and nuts. The active components in spices phthalides, polyacetylones, phenolic acids, flavonoids, coumarins and terpenes were reported as powerful antioxidants [7]. Mustard had not only been found to be effective antioxidant in vivo and vitro to deal with oxidation stresses but also quite active in stabilizing the edible oils and fatty food against rancidity and oxidative deterioration [8]. mustard seeds are an excellent source of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids which had been reported to show good antioxidant activity [9]. Studies had suggested that preventive or putative therapeutic properties of mustard seeds [10] had also been considered to be associated with their antioxidant property, because free radical-mediated peroxidation of membrane lipids and oxidative damage of DNA and proteins are believed to be associated with a variety of chronic pathological complications such as cancer, atherosclerosis, neurodegenerative diseases, and aging [11]. Mustard seeds are thought to play a vital role against oxidative-stress-mediated pathological conditions. Mustard seeds [12] have a wide spectrum of biological actions, including its anti-inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertility, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hypotensive and hypocholesteremic activities. The study was carried out in an attempt to evaluate the proximate chemical

components, nutritional value, antioxidants properties, antinutrients and amino acid profile of a yellow mustard seeds.

2.0 Materials And Method

2.1 Materials
The yellow mustard seeds were obtained from Israel, in the middle East. The seeds were cleansed to remove any foreign agents, dried and grounded into powder and store in a screw capped plastic container prior to analysis.

Dried and processed yellow mustard seed

2.2 Methods

2.2.1 Proximate Analysis
These were determined with respect to the moisture content, crude protein, crude fiber, fat, ash content and carbohydrate according to standard methods [13]. The crude protein content was obtained by multiplying the nitrogen content by 6.25 (conversion factor) and the carbohydrate content was determined by the difference.

2.2.2 Determination of Mineral composition
A wet digestion method was used to eliminate all organic matter from the sample before the sample was analyzed for various minerals. About 1ml of the sample was measured into a 250ml beaker. 25ml concentrated HNO3 was added and the beaker was cover with a water glass. The sample was digested with care on a hot plate in a fume chamber until organic matter had been oxidized (20-30min). The pale yellow solution cooled 1ml 70% HCl04 was added with care digestion was continued until the solution was almost colourless (until all the HNO_3 was removed). The solution was then cooled slightly after digestion process, and about 30mls distilled water was added and allowed to boil for about 10mins, the filtered when hot through size no.4 whatman filter paper into 100mls volumetric flask. The beaker was washed well with distilled water and filtered. Then the flask was then cooled and made up to the 100mls mark. The solution was used for all minerals analysis. Sodium(Na) Potassium(K), Calcium(Ca), Magnesium(Mg), iron(Fe), Manganese(Mn), Copper(Cu), Zinc(Zn) were all determined using the perkinEliner Atomic spectrophotometer (AAS; Model A Analyst 400, Mineapolis, U.S.A) and results were recorded in milligram (Mg).

2.2.3 Antinutrient properties

2.2.3.1 Determination of Oxalates
1g of the sample was weighed into 100ml conical flask. 75ml of 1.5 NH_2SO_4 was added and solution was carefully stirred intermittently with a magnetic stirrer for about 1hour and then filtered using whatman no 1 filter paper. 25ml of the sample extract (filterate) was collected and titrated hot (80-90^oC) against 0.1N $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30seconds [14].

2.2.3.2 Determination of Tannins
Finely milled processed samples (250mg in 10cm^3 of 70% aqeous acetone) were extracted for 2 hours at 300^oC in water-bath using Gallenkamp orbital shaker (surrey U.K) at 120 revolutions per minute (rpm). Pigments and fat were first removed from the samples by extracting with diethyl ether containing 1% acetic acid. Therefore, the total polyphenols (as tannic acid equivalent) was determined in 0.5, 0.2 or 0.5cm^3 aliquot using folin ciocalteau reagent (sigma) and then 2.5ml of the sodium carbonate solution. The tubes were vortexed and absorbance recorded at 725nm after 40mins as described by [15]. The amount of total polyphenols (as tannic equivalent) was calculated from the standard curve. Duplicate samples of each processed leaves were analysed.

2.2.3.3 Determination of Phytic Acid
An indirect calorimetric method of [16] was used for phytate determination. On an iron to phosphorous ratio 4:6. A quantity of 5g of the test sample was extracted with 3% trichloro acetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide. The precipitate was then dissolved in hot 3.2N HNO_3 and the colour read immediately at 480nm. The standard solution was prepared from $Fe(NO_3)_3$ and the iron content was extrapolated from $Fe(NO)_3$ standard curve. The phytate concentration was calculated from the iron results assuming a 4:6 iron:phosphorous molecular ratio.

2.2.3.4 Determination of Phytate

Phytate were determined using anion-exchange method as described by [17]. Samples were accurately weighed (1.0 – 2.0g) and transfered into 100ml conical flasks. A total of 40-50ml of Na_2SO_4 (100g/l)-HCl (1.2%) was added. Flasks were then capped and shaken vigorously for 2hours on a rotator at ambient laboratory temperature. The supernatant was then filtered through qualitative filter paper no 4. A total of 10ml of filtered extract was diluted to 30ml with distilled water after mixing with 1ml of 0.75M NaOH and then passed through an anion resin column, the column was washed before use with 0.5M NaCl solution and deionised water. After sample application, the column was washed with 15ml of distilled water and 20ml of 0.05M NaCl solution in order to remove the inorganic phosphate. Then the retained phytic acid was eluated with 0.7M NaCl. The post column reagent was made up as a 0.03% $FeCl_3$ solution containing 0.3% sulphosalicylic acid. A total of 4ml of the reagent was added into 5ml of collected eluate and centrifuged at 3000rpm for 10minutes.

2.2.4 Antioxidant Properties

2.2.4.1 Determination of Total Phenol

The total phenol content of the extract were determined by the method of [18]. 0.2ml of the extract was mix with 2.5 of 10% Folin ciocalteau's reagent and 2ml of 7.5% sodium carbonate. The reaction mixture was subsequently incubated at 45^oC for 40 mins, and the absorbance was measured at 700nm with a spectrophotometer, garlic acid was used as standard phenol.

2.2.4.2 Determination of Total Flavonoid

The total flavonoid content of the extract were determined using a calorimeter assay developed by [19] 0.2ml of the extract was added to 0.3ml of 5% $NaNO_3$ at zero time. After 5mins, 0.6ml of 10% $AlCl_3$ was added and after 6mins, 2ml of 1M NaOH was added to the mixture followed by the addition of 2.1ml of distilled water. Absorbance was read at 510nm using a spectrophotometer against the reagent blank and flavonoid content was expressed as mg rutin equivalent.

2.2.4.3 Determination of Ferric Reducing Property

The reducing property of the extract was determined using the method described by [20]. 0.25ml of the extract was mixed with 0.25ml of 200M of sodium phosphate at buffer P^H 6.6 and 0.25ml of 1% KFC. The mixture was then incubated at 50^oC for 20mins, therefore 0.25ml of 10% TCA was also added and centrifuged at 200rpm for 10mins, 1ml of the supernatant was mixed with 1ml of distilled water and 0.1% of $FeCl_3$ and the absorbance was measured at 700nm.

2.2.4.4 Determination of Free Radical Scavenging Ability

The free radical scavenging ability of the extract against DPPH (1,1-diphenyl-2-picryhydrazyl) using the method described by [21]. About 1ml of the extract was mixed with 1ml of the 0.4mM methanolic solution of DPPH, the mixture was left in the dark for 30mins before measuring the absorbance at 516nm using a spectrophotometer.

2.2.4.5 Determination of Fe^{2+} Chelation

The ability of the extract to chelate Fe^{2+} was determined using a modified method [22]. Briefly, 150mM $FeSO_4$ was added to a reaction mixture containing 168ml of 0.1M Tris-HCl P^H 7.4, 218ml of saline and extract and the volume is made up 1ml with distilled water. The reaction mixture was then incubated for 5min before the additional of 13ml of 1,10-phenantroline, the absorbance was then read at 510nm using a spectrophotometer.

2.2.4.6 Determination of Total Antioxidant

The assay is based on the reduction of Mo(VI)-Mo(V) by the extract and the subsequent formation of a green phosphate/Mo (VI) complex at acidic P^H as described by [23]. 0.1ml of different concentration of the extract was combined with 3ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes was then incubated at 95^oC for 90min. The absorbance was measured at 695nm using a spectrophotometer after the mixture has cooled to room temperature against blank. The antioxidant activity was then expressed as galic acid equivalent.

2.2.5 Amino Acid profile

Extraction and instrumentation analysis were carried out by following the modified method AOAC method 982.30, 2006 and Danka et al in the " Simultaneous indentification and determination of total content of Amino acid in food supplement- Tablets by Gas chromatography as published by : Danka petrova Obreshkova, Dobrina Doncheva Tsvetkova and kallin Valentinov Ivanov; Asian journal of pharmaceutical and clinical Research, Vol 5, Sppl 2, 2012. The dried sample and pulverized sample was made to be free of water by ensuring constant weight for a period of time in the laboratory. The sample of 10.0g was weighed into the 250ml conical flask capacity. The sample was defatted by extracting the fat content of the sample with 30ml 0f the petroleum spirit three times with soxhlet extractor that was equipped with thimble. The sample was hydrolysed three times for complete hydrolysis to be achieved for the totality of amino acids recovery. The pulverized and defatted sample was soaked with 30ml of the 1M potassium hydroxide solution and was incubated for 48 hours at 110^oC in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydolysate was neutralized to get P^H in the range of 2.5-5.0. The solution was purified by cation exchange solid-phase extraction. The amino acids in purified solution were derivatised with ethylchloroformate by the established mechanism.

2.2.5.1 Derivation mechanism

The derivatisation of the extracted amino acids for the volatility sake in the gas chromatography with ethychloroformate is as described with the reaction below:

The derivatising reagent was removed by scavenged with nitrogen gas for proper mop up of the excess reagent. The derivatised amino acids that is free of derivatising reagent was made up to 1ml in a vial for gas chromatography analysis.

2.2.5.2 GC Condition For Amino Acids

GC: HP 6890 powered with HP chemstation Rev. A 09.01 [1206] software.
Injection Temperature: split injection
Split Ratio: 20:1
Carrier Gas: Hydrogen
Flow Rate: 1.0ml/min
Inlet Temperature: 25°c
Column Dimensions: 10m x 0.2mm x 0.25m
Oven Program: Initial at 110°c
First: Ramp at 27°c/min to 320°c
Second: Constant for 5mins at 320°c
Detector: PFPD
Detector Temperature: 320c
Hydrogen Pressure: 20psi
Compressed Air: 35psi

3.0 Result And Discussion

The result of proximate composition , mineral compositions, antioxidant compositions and amino acids profile of the yellow mustard seed powder are shown in the following tables:

Table 1: *Proximate composition (g/100g) of a Yellow mustard seed*

Moisture	Crude Protein	Crude Fat	Crude Fiber	Ash	NFE
4.51 ± 0.05	23.11 ±0.07	51.60±0.14	9.34 ± 0.37	3.22 ± 0.08	8.23 ± 0.13

Table 2: *Mineral composition (mg/kg) of the yellow mustard seeds*

Sodium (Na)	Potassium (k)	Calcium (Ca)	Magnesium (Mg)	Iron (Fe)	Manganese (Mn)	Copper (Cu)	Zinc (Zn)
25.00 ±0.01	144.00 ±0.03	52.00 ±0.03	83.00 ±0.01	13.20 ±0.01	0.55 ±0.02	0.59 ±0.01	0.68 ±0.01

Table 3: *Amino Acid composition (g/100g) of the yellow mustard seeds.*

AMINO ACID	AMOUNT
Glycine	5.55
Alanine	3.54
Serine	2.76
Proline	10.03
Valine	5.48
Threonine	2.83
Isoleucine	3.73
Leucine	7.68
AMINO ACID	AMOUNT
Aspartate	8.03
Lysine	6.33

AMINO ACID	AMOUNT
Methionine	1.81
Glutamate	18.10
Phenylalanine	4.15
Histidine	2.60
Arginine	5.79
Tyrosine	2.54
Tryptophan	0.91
Cystine	2.37

Table 4: *Antioxidant compositions (mg/g) of yellow mustard seed*

PARAMETERS	RESULT
Total Antioxidants	25.06 ± 0.95
Phenol	3.97 ± 0.05
FRAP	46.96 ± 0.15
Flavonoid	0.54 ± 0.02
Fe^{2+} %	42.17 ± 0.32

DPPH %	63.13 ± 0.19

3.1 Proximate Analysis

The proximate compositions of the seeds are shown in Table 1. The seed have a lower percentages of moisture (4.51%), ash (3.22%) and total carbohydrate (8.23%) while higher percentages of protein (23.11%), fat (51.60%), and crude fiber (9.34%). The data were in good agreement with [24], [25] and [26]. The present study showed that with respect to the crude protein and crude fiber, the seed could be added to low protein nutritional foods like pap (Ogi). Also from the result, the observed low moisture content in the mustard seed sample is an indication that the activity of the microorganisms would be reduced and thereby increases the shelf life of the seed powder. The seed sample had higher fat content of 51.60%, this high crude fat in the seed sample suggested that mustard seed is a good source of quality vegetable oil for both domestic and industrial purposes.

3.2 Mineral Compositions

The result of the mineral analysis are shown in table 2. The result showed that mustard seeds had relatively higher value of Potassium (K), Magnesium (Mg), Calcium (Ca), Sodium (Na) and little of Iron (Fe) which are known to be macro element and are required in large quantity in human body. Also it is relatively lower in Copper (Cu) and Manganese (Mn) which are micro element required in smaller quantity. The data are in good agreement with [27].Also the elements, copper (Cu) and manganese (Mn) act as cofactors of antioxidant enzymes to protect the body from oxygen free radicals that are produced during oxidative stress [28].

3.3 Amino Acid Prrofile

Amino acid profile result are shown in Table 3. The result revealed that the yellow mustard seed powder had a relatively high amounts of Glutamate, Proline, Leucine and had lower amounts of aromatic amino acids (Phenylalanine and Tyrosine), sulphur containing amino acids- (Methionine and Cystine) and other amino acids in the seed. Glutamate was the major amino acids in yellow mustard seeds (18.10%). The result showed that all indispensable amino acids were found in yellow mustard seeds powder which is in accordance with the FAO/WHO recommended value. The result indicate that yellow mustard seed powder are highly rich in both the essential and non essential amino acids which is in conformity with the trend in the amino acids composition conducted on fractions obtained by other researchers in which they reported that the amino acids composition of mustard protein is well balanced, it is rich in essential amino acids and the balance of amino acids found within the seed of mustard crops compares favourably with the required for human nutrition [1].

3.4 Antioxidants Contents

The antioxidant properties in Table 4 above shows the presence of phenol, flavonoid , 1,1-dipenyl-2-picryhydrazyl (DPPH), ferrous reducing antioxidant property (FRAP), and chelate Fe^{2+} and the result showed total antioxidant(25.06mg/g), phenol (3.97mg/g), FRAP (46.96mg/g), flavonoid (0.54mg/g), Fe^{2+} (42.7%) and DPPH (63.13%) were present in the seed. From the stated result, it shows that yellow mustard seed contains highest percentage value of DPPH with flavonoid as the lowest percentage value. Mustard seed is then said to contain elevated level of phenolic compound. The values in FRAP assay express the corresponding concentration of electron donating antioxidants. In recent years, phenolic compounds have attracted the interest of researchers because of it high powerful antioxidant ability which can protect human body from free radicals. The antioxidants activity of of phenolics is mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, free radical scavenger and metal chelators. DPPH is frequently used in determination of free radical scavenging ability of various food components.

3.5 Antinutrient composition

The fatty acid compositions of the yellow mustard seed is presented in table 5 below indicating the values of phytate (42.74mg/g), phenols (14.4mg/g), phytic acid (12.04mg/g), oxalates (5.798mg/g), and tannins (1.38mg/g). High values of tannins in food usually affect it nutritive value and also digestability of proteins, carbohydrate , fats and bioavailabilty of minerals. The low values of tannins present in the seed being investigated will definitely not affect the nutritive values of the end food product derived from the seeds having the value of 1.38mg/g. Also, the seed possess low content of oxalate since diet with high level of axalate content increase the risk of renal calcium absorption and has been implicated as a source of kidney stone [29]. The seed also contains high level of phytate, phenol and phytic acids and presence of phytic acids in food can bind with some essential minerals in the digestive tract and can result in mineral deficiencies.

Table 5: *Antinutritional compositions (mg/g)of yellow mustard seeds*

PARAMETERS (mg/g)	RESULTS
Phytates	42.74 ± 0.10
Phytic Acid	12.04 ± 0.03
Oxalates	5.79 ± 0.02

Tannins	1.38 ± 0.003
Phenols	14.44 ± 0.04

4.0 Conclusion

In all, the study clearly shows that the yellow mustard seeds had high antioxidants properties, high phytates, phytic acid and phenols. Also, the high level of protein, dietary fiber, macro mineral element and its richness in amino acids shows that the seeds is nutritionally important. It is therefore recommended to use the seeds in food fortification especially when defatted as a good and available inexpensive source of fiber, ash, high content of useful minerals and protein characterized with favourable balance of both essential and non-essential amino acids. In addition, due to it high content of antinutrient especially the phytate, it is also suggested that the seeds are subjected to processing so as to reduce the antinutrient level.

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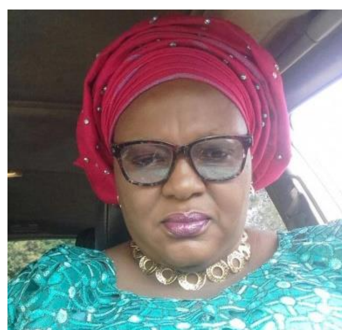

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