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Multigrain (*Glycine max*, *Sorghum bicolor*, *Avena sativa* L) taco formulation, nutritional & phytochemical investigation

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Abstract

Tacos can be described simply as a tortilla wrapped around a filling. The contents of taco can be different according to the geographical region where you are eating them. The taco can be eaten as an entree or snack. A taco is traditionally a Mexican dish and is considered staple there. Americans use flour based tacos, but Mexicans eat corn based tacos. Mexican tacos are not spicy but can be made spicy as needed. Tacos are originally meaty but vegetarian version is also available.

The tacos under study have been made using a combination of flours for the purpose of making them a nutritionally dense meal using defatted soy flour (*Glycine max*), sorghum (*Sorghum bicolor*), oats (*Avena sativa* L) along with corn (*Zea mays*) flour. They were investigated for their enhanced nutrition content. It was found the tacos contained excellent amount of phosphorous and calcium, which are good for health and a small amount of zinc. Further, they were also investigated for their phytochemical content which were present in fair amounts. The GC-MS results further signified that they are a good source of oleic acid, which has a good antioxidant property. Furthermore, vitamin E was also detected. Trans fatty acids were not found in multigrain tacos. Thus, the overall study reveals that multigrain tacos can be an interesting snack from a nutrition point of view.

Keywords: Taco, tortilla

1. Introduction

It is commonly assumed that the snack foods aren't doing any good for health. They are generally considered the reason for continually increasing obesity, heart problems, increased cholesterol level etc. problems. They receive criticism mainly owing to the presence of salt, sugar, and fat in higher amounts. Snack foods can prove to be nutritious if made from natural raw materials. However, health problems such as obesity are not necessarily due to the consumption of snack foods, but the cause is rather an unbalanced diet with excess fat, sugar, and salt. Therefore, tacos as snacks can be an important source of fiber and energy, particularly for the poor people as their diet is highly deficient in nutrients^[1].

Taco is traditionally a Mexican dish. "Taco" is family of foods, with all kinds of amusing taco relatives namely^[2]:

- **Taco**
- **Burrito:** Big taco
- **Quesadilla:** Taco with cheese
- **Gringa:** Big taco with cheese
- **Sincronizada:** 2 tacos smashed together with cheese
- **Enchilada:** Taco smothered in spicy sauce
- **Enmolada:** Taco smothered in mole sauce
- **Taco Sudado:** Steamed taco
- **Flauta:** Deep-fried taco

The formulated tacos consist of a combination of whole grains to make it nutritious and health friendly mainly to break the common myth about the bad effects of snacks. Efforts were made to develop a consumer friendly snack by using defatted soy flour (*Glycine max*), sorghum (*Sorghum bicolor*) and oats (*Avena sativa* L) along with traditional ingredient corn flour.

Soy flour (*Glycine max*) is an officially declared cholesterol-lowering by FDA (Food & Drug administration)^[3]. Many health benefitting attributes are associated with it – reduced risk of heart diseases, reduced menopausal syndrome owing to the isoflavones present in soybean, reduced risk of osteoporosis^[4]. Sorghum (*sorghum bicolor*) on the other hand is rich in

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antioxidants, dietary fiber, and iron. It may inhibit cancer growth, protect against diabetes & insulin resistance, help manage cholesterol, may reduce risk of certain cancers and promote cardiovascular disease owing to phytochemical content mainly antioxidants^[5]. The high phenolic compounds and anti-fungal protein levels are very beneficial, as they are natural barriers against molding^[6].

Oats (*Avena sativa L*) are rich in a specific type of fiber- beta glucan, which is known to lower the level of bad cholesterol and also reduce the risk of type 2 diabetes. Oats are an excellent source of protein, B vitamins, vitamin E, calcium, iron and other healthy minerals^[7]. They may reduce asthma in children, increase appetite control hormones, help cut the use of laxatives, controls blood pressure, may boost the nutrition profile of gluten-free diets^[8].

With the use of such gluten-free and health benefitting ingredients the tacos are surely going to become a nutritionally dense meal. By incorporating above flours to make the tacos, the potential of becoming a health benefitting food can be further studied. For this purpose nutritive analysis was done on the tacos. Along with this, the phytochemical estimation was done to know the hidden powers inside the snack.

Choosing the enhanced tacos will create a simpler inclusion of whole grains into one's diet along with convenience. Such a product enables consumers to obtain a nutritionally dense meal component with one simple choice instead of many.

2. Materials & Methods

Flour of *Glycine max*, *Sorghum bicolor* & *Avena sativa L* along with *Zea mays* flour, which is the main ingredient were selected for the formulation of Multigrain Taco were purchased from the market.

2.1. Formulation

Various combinations of the selected flours were tried along with corn flour keeping in mind the nutrition factor of tacos & palatability till an acceptable formulation was reached.

For the purpose of comparison, a control was formulated without using flour of *Glycine max*, *Sorghum bicolor* & *Avena sativa L*. The complete analysis was done on Control & Multigrain Taco both.

2.2. Phytochemical Analysis

The phytochemical analysis was done on the flour extract. The total phenolic content was estimated by the Folin ciocalteu reagent method^[9]. The absorbance of the standard (Gallic acid and) the combined extract was measured spectrophotometrically at 765 nm against DMSO blank. The results were expressed as Gallic acid equivalents (GAE, µg/mg extract).

The total flavonoid content was determined using aluminium chloride colorimetric method^[10]. The optical density of the standard (catechin) and the sample extracts were measured at 510 nm against DMSO blank, the total flavonoid content was expressed in mg of catechin equivalents per mg of weight of extracts (CE, µg/mg extract). Crude alkaloids was estimated using the methods already described by Harborne^[11]. The results were calculated in %. The concentration of tannins was estimated by the method described by Van- Buren & Robinson^[12]. The optical density of standard & extract was measured at

605nm spectrophotometrically against the blank. The result was expressed in terms of tannic acid equivalents (TAE, µg/mg extract).

2.3. Determination of Antioxidant Activity

The antioxidant activity of flour extract was done by DPPH & FRAP assay

2.3.1. DPPH Assay

DPPH (2, 2-Diphenyl-1-Picryl-hydrazyl) radical scavenging was done as given by the method of Blois, 2000^[13] with slight modification. Using methanol as a reference & Methanol & DPPH as blank, the optical density was measured at 517nm. The result was measured in % inhibition.

$$\% \text{ Inhibition} = \frac{B - A}{B} \times 100$$

Where,

B: absorbance of blank at 517nm.

A: absorbance of sample at 517nm.

2.3.2. Ferric Reducing Antioxidant Power (Frap) Assay

This assay is based on the methodology of Benzie & Strain^[14]. The FRAP reagent consists of 10mM TPTZ in 40 mM HCl, 20 mM FeCl₃ and 250 mM sodium acetate buffer (pH 3.6) in a ratio of 1:1:10 respectively. A 100 µl of sample and 900 µl FRAP reagent is added, followed by incubation for 4 min at 37 °C, the absorbance is measured at 593 nm against the blank. BHT was used as standard. FRAP value was calculated as mg of BHT equivalents/g extract.

2.4. Nutritive Analysis

Nutritive analysis was done on the ground tacos. Moisture & ash content was measured using the method described by AACC, 2000^[15]. The difference in weight determines the moisture content & a muffle furnace was used in ash content analysis. The testing method for protein content & dietary fiber was the AOAC method^[16]. Fat content was estimated using petroleum ether & for reducing sugar was IS 15279. Mineral content was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES). Fatty acid profiling was done by using gas chromatography^[15]. Total carbohydrates and energy content were calculated using formulae:

Total carbohydrates (% fresh weight) = 100 - moisture (%) - protein content (% fresh weight) - crude fat (% fresh weight) - ash (% fresh weight) and reported as total carbohydrates in %.

The energy value in kilocalories (kcal) was calculated according to the system of Atwater, namely: kcal = (3.36 × % protein fresh weight) + (3.60 × % total carbohydrate fresh weight) + (8.37 × % fat).

2.5. GC-MS Analysis

Other secondary metabolites in tacos were identified by analyzing 1µl of sample extract by using GC/MS analysis where helium was used as carrier gas. An Agilent 6890 GC with 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050) for all samples. Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST'05 library. The transfer line temperature was set

to 300 °C, solvent delay was 3 min, ion source and quadruple temperature were 230 °C and 150 °C, respectively [17]. The detected compounds have been identified by NIST'05 mass spectrum library and more than 90% matching value were reported.

3. Results & Discussion

3.1. Phytochemical Analysis

Phytochemicals being non-nutritive plant chemicals provide promising health benefits. They have disease preventive

properties. Plant extracts having different classes of polyphenols are very attractive in the food industry. Multigrain Taco has been found to have a moderate amount of flavonoid content. A diet containing alkaloids may prove helpful for healing wounds. Phenolics are considered to be the major bioactive compounds for providing health benefits. Tannins bind and precipitate proteins. They can have a large influence on the nutritive value of many foods. Flavonoids contribute to antioxidant activity. Results are shown in table 1.

Table 1: Phytochemical estimation of taco

Extract	Total Phenolic Content ($\mu\text{g GAE}/\text{mg extract}$)	Total Flavonoid Content ($\mu\text{g CE}/\text{mg extract}$)	Tannin Content ($\mu\text{g TAE}/\text{mg extract}$)	Alkaloids (%)
Taco (control)	34.08	58.24	5.78	0.95
Multigrain Taco	17	62.5	5.31	3.5

3.2. Antioxidant Activity

3.2.1. DPPH Radical Scavenging Activity

This is an easy & a rapid method to determine the antioxidant activity of the sample being analyzed [13]. Flavonoids contribute to antioxidant power. The result is shown in table 2.

Table 2: Antioxidant activity by DPPH

Sample	Antioxidant Activity (%)
Taco(control)	49.4
Multigrain Taco	48.1

3.2.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The principle of this method is the reduction of ferric tripyridyl-s-thiazine complex to ferrous colored form in the presence of antioxidants [14]. The calibration curves revealed highly positive linear relation between mean FRAP values and the concentration of BHT standard. The BHT equivalents for the respective samples are tabulated in table 3.

Table 3: Antioxidant activity by FRAP

Sample	BHT equivalents ($\mu\text{g BHT}/\text{mg sample}$)
Taco (Control)	132.15
Multigrain Taco	112.30

3.3. Nutritive Analysis

The dry matter (Ash) content and mineral content are very important to analyze during the nutrition analysis as the quality, nutrition, microbiological stability depends a lot on the type and concentration of minerals. The dry matter (Ash) content is a measure of the total mineral present within a food, whereas the mineral content is a measure of amount of specific inorganic components present within the food like Ca, Na & K [18]. The moisture content is most common attribute within nutrition analysis as the freshness and shelf life of foods depends on this factor [19]. The protein content measures the nutritional quality of the food. They also act as major structural components, a major energy source, as well as contain essential amino-acids which are essential to human health. The fat content parameter is necessary to estimate in order to control production processes, to ensure accurate labelling, and to monitor food safety [20]. Carbohydrates are an important source of energy and dietary fiber, also contribute to

the sweetness, appearance and textural characteristics of many foods. Thus, it's necessary to analyze them for quality and adulteration of food. The determination of amount of reducing sugar contained in food products can serve as an aid in its grading and classification [21]. The results of various nutritional tests are mentioned below. The energy value of multigrain taco was higher than control which was desired. Protein content was almost same & multigrain taco contained dietary fiber in higher amounts. The results of nutritional analysis are shown in table 4.

Table 4: Nutritive Analysis of Taco

Test Parameter	Taco (control)	Multigrain Taco
Moisture content (%)	0.1	0.4
Protein content (%)	10.82	10.03
Fat content (%)	17.26	24.95
Reducing Sugar content (%)	5.43	3.50
Dietary fiber content (%)	9.69	13.95
Total carbohydrate (%)	69.82	63.12
Energy Value (kcal)	432.17	469.76

3.3.1. Ash Content

The amount of ash content present in tacos is shown in table 5.

Table 5: Ash content estimation of tacos

Sample	Control (flour)	Control (taco)	Product (flour)	Product (taco)
Ash Content (%)	1.16	2	1.84	1.5

3.3.2. Minerals, Heavy Metals & Trace Elements

The mineral, heavy metals were estimated by ICP-OES. The results are tabulated below in table 6 & 7.

Table 6: Mineral content estimation of Taco (Control)

S. No	Analyte	Concentration (ppm)
1.	Ca	285
2.	Cr	1.00
3.	Cu	5.00
4.	Fe	36.25
5.	Mg	547.50
6.	Mn	7.50
7.	P	15800
8.	Ti	2.50

Table 7: Mineral content estimation of Multigrain Taco

S. No	Analyte	Concentration (ppm)
1.	Ca	278
2.	Cr	1.25
3.	Cu	2.50
4.	Fe	38.25
5.	Mg	532
6.	Mn	7.50
7.	P	16025
8.	Sr	2.50
9.	Zn	20

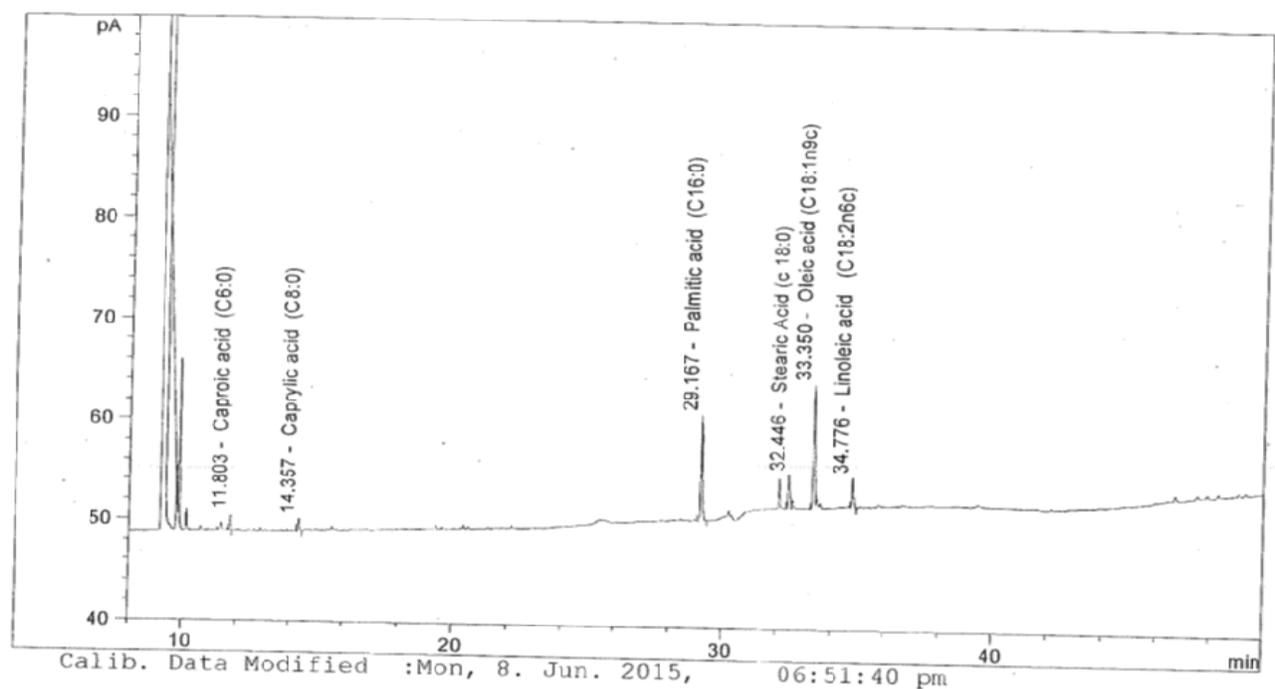
3.3.3. Fatty Acid Profiling

Fatty acid profiling is of utmost importance in the case of a snack. Fatty acids are the chemical compounds that make up fats. Trans fatty acids raise LDL (or “bad”) cholesterol levels

in the blood. While unsaturated fatty acids like omega-3 & omega-6 are essential fatty acids with lots of health benefits. The multigrain tacos were found to have no trans fatty acids & contain a fair amount of unsaturated fatty acids as evident from table 8 & the specific fatty acids present in them are listed in table 9 along with the graph.

Table 8: List of fatty acids present in multigrain taco

Fatty Acid	Amount (g/100g)
Saturated Fatty acids	12.80
Trans Fatty acids	Not Detected
MUFA	9.77
PUFA	2.38



Graph showing peaks of fatty acids present in multigrain tacos

Table 9: Name of all fatty acids detected in the sample

Peak Number	Name	RT (min)	Area (min)	Area (%)
1.	Caproic acid (C6:0)	11.80	4.36	3.54
2.	Caprylic acid (C8:0)	14.36	3.45	2.80
3.	Palmitic acid (C16:0)	29.17	42.38	34.42
4.	Stearic acid (C18:0)	32.45	12.99	10.55
5.	Oleic acid (C18:1)	33.35	48.20	39.15
6.	Linoleic acid (C18:2)	34.78	11.74	9.54

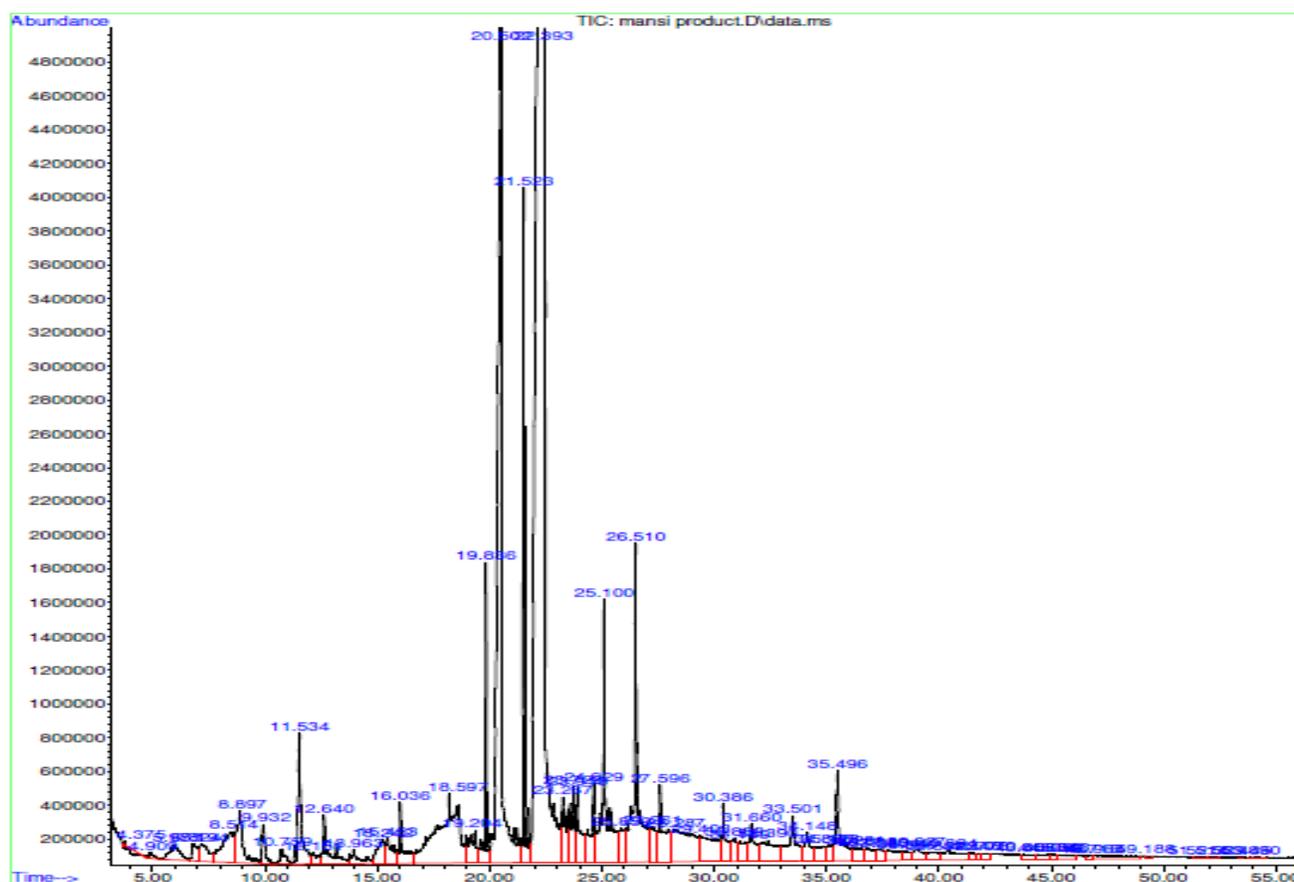
3.4. Estimation of Secondary Metabolites (GC-MS)

The analysis of secondary metabolites done on tacos as shown in table 10, both control and the product revealed that they are a good source of oleic acid, linoleic acid, which is omega-6 fatty acid etc. omega-6 fatty acid is an essential fatty acid that must be consumed to maintain brain function, regulate growth and reduce risk of heart diseases. It has good antioxidant property & is good for the skin^[22]. Vitamin E present helps in reducing cholesterol and the risk of developing cancer along with antioxidant properties^[24]. D-Hexadecanoic acid present has anti-inflammatory properties^[23]. Gamma tocopherol is the form of vitamin E^[24]. In addition to its impressive antioxidant capacity. Campesterol, a plant sterol, balances cholesterol in the body, boosts the immune system and prevents colon cancer^[25]. Stigmasterol, plant sterol has antibacterial, antioxidant, anti-inflammatory effect, antimicrobial, anti-osteoarthritis

effects [25]. Vanillin is a phytochemical in the class of phenolic acids has antimicrobial, antibacterial, antioxidant properties [26].

Table 10: GC/MS analysis of Multigrain Taco

Compound Name	CAS#	RT	% Area
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	000067-47-0	11.532	1.33
2-Methoxy-4-vinylphenol	007786-61-0	12.642	0.67
Vanillin	000121-33-5	13.966	0.32
Hexadecanoic acid, methyl ester	000112-39-0	19.831	0.89
n-Hexadecanoic acid	000057-10-3	20.504	12.22
10,13-Octadecadienoic acid, methyl ester	056554-62-2	21.525	2.54
9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	22.389	44.64
Eicosanoic acid	000506-30-9	23.914	0.85
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	023470-00-0	023470-00-0	2.60
9,17-Octadecadienal, (Z)-	056554-35-9	25.854	0.65
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	003443-82-1	26.505	3.80



Chromatogram for Multigrain Taco

4. Conclusion

The multigrain tacos, which were formulated with the idea of making them more nutritious proved that they are really an excellent source of nutrition along with consumer appeal. The nutritive analysis revealed that the multigrain tacos had more energy value than taco control which was made with traditional ingredients only. Also, Vitamin E, omega-6 fatty acids present in them were an added attraction from the health benefits point of view. They have a fair amount of antioxidant properties. The multigrain tacos may also lead to cholesterol lowering and thus reducing heart diseases. Mineral content was also high which is good for our body. The multigrain tacos had no Trans fatty acids and contained PUFA, MUFA. Thus, the

multigrain taco snack are an excellent source of health benefitting nutrients.

5. Acknowledgement

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