

A Study on the Mycoflora of Fried, Roasted and Raw Peanuts available in Local Markets of Riyadh (Saudi Arabia)

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The aim of this study was to evaluate the mycoflora of peanuts (*Arachis hypogaea*) locally available in Riyadh markets. Infection of peanuts by fungi and their toxic metabolites is a serious problem, as it has adverse effects on human health and also causes loss of economy. Fried - salted, roasted - salted, raw peanuts were analyzed for moisture content (%), peroxide value and mycoflora. Moisture content was found highest in the raw peanuts, next to it was the roasted- salted peanuts and it was found to be lowest in the fried- salted samples. Peroxide value was highest in the fried -salted peanuts followed by roasted-salted and lowest in the raw samples. The most dominant fungal genus was *Aspergillus*. Results showed that the *Aspergillus flavus* was the most dominant fungus in all tested samples followed by *A. niger* and *Rhizopus* sp. Also, *Alternaria alternata*, *Aspergillus terreus*, *Cladosporium* sp. and *Penicillium chrysogenum* were isolated from some samples. It was found that the roasting, frying and salting of peanuts have enhanced the lipid oxidation, however it showed a decrease in moisture content and fungal infection. Our results indicate that the peanuts are vulnerable to fungal infection; especially to aflatoxin producing fungi therefore a quality check is required at every stage right from production to consumption of the peanuts. Further research is needed to explore the other means of preventing mould infection; the measures may include the prevention of infection in the field through the use of biological control agents, use of natural preservatives, proper handling of peanuts during storage, processing and transportation.

Key words: Mycoflora, peanut, fungus, peroxide value, *Arachis hypogaea*.

Peanut (*Arachis hypogaea*) is a major world oil seed crop. These are sold and consumed in various forms such as raw, roasted, fried and in a variety of the other processed forms¹. In Saudi Arabia peanuts are normally eaten as raw or after some processing such as frying, boiling, oven-roasting, microwave-roasting etc. As per the nutritive value of peanut is concerned, it is rich source of energy, fat and protein^{2, 3, 4}. It contains

many essential vitamins and minerals necessary for proper health⁵. In comparison to other nuts it contains less total fat⁶ and mostly consists of unsaturated oleic acid. It has been found useful in dietary regimes designed to reduce blood cholesterol levels in postmenopausal women, without resulting in problems associated with oxidation of low density lipoproteins⁷, reduces risk in developing type II diabetes^{8, 9} and cardio vascular disease^{10, 11}. It also provides an inexpensive source of high quality dietary proteins and oil. Its incorporation in various products improves the protein level of product and also helps in reducing malnutrition especially in overcoming protein deficiency in many underdeveloped and developing countries.

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Mercer *et al.*,¹² defined the term shelf life as “the number of days before the onset of oxidative rancidity, either by exposure to heat or air”. Since the peanuts contain high amount of oil, its quality deteriorates quickly¹³. This happens due to lipid oxidation which depends on various factors such as oxygen, light, moisture and high temperatures. The peroxide value is a measure of the content of hydro peroxides, which are primary oxidation products. This results from rapid decomposition of hydro peroxides to secondary oxidation products at elevated temperature.

In peanuts, fungal infections can cause loss of germination, mustiness, mouldy smell¹⁴ and aflatoxin production^{15, 16}. Fungi can grow both on simple and complex food products and can produce various metabolites such as aflatoxins, cyclopiazonic acid, fumonisin, zeralenone etc¹⁷. These micro organisms exist in the environment and get distributed by wind, insects and rains¹⁸. Aflatoxin and fumonisins have been reported to cause many diseases in humans¹⁹ and have been found to be responsible for causing esophageal cancer²⁰, mutagenic and hepatocarcinogenic effects²¹.

Food safety is an important criterion for addressing current health problems in any country. Infection of peanuts by fungi and their toxic metabolites is a serious problem, as it has adverse effects on human health and also causes loss of economy. These fungi can infect the nuts in fields, post harvest or may contaminate the nuts during food processing and packaging. Therefore a quality check is required at every stage right from the production to consumption of the peanuts. The present study will help in expanding the existing knowledge and also in developing different strategies to prevent the fungal infection.

MATERIALS AND METHODS

Sample Collection

Fried -salted⁵, dry roasted - salted⁷ and raw⁵ peanut samples were purchased from the local shops of different regions of city Riyadh. Nuts were packed in sterilized polybags and stored under dry and cool condition till the analysis was carried out.

Moisture content

The vacuum oven-air dried method was used for moisture content test. It was carried out

according to the procedure mentioned by AOAC²². Briefly, 10g of sample was crushed in a mortar and pestle 5g bits was placed in a crucible. The sample were kept in hot air oven (Haraeus, VT 5042EK, Germany) at $103 \pm 5^\circ\text{C}$ till the constant weight of samples were obtained. Weight of the samples was recorded after samples were cooled in the desiccators. The percentage moisture content on dry basis was calculated using the formula

$$\text{Percent moisture content (dry basis)} = (\text{change in weight/weight of dry matter}) \times 100.$$

Peroxide value

Peroxide value was carried out according to the AOAC method number 965.33²³. Oil was extracted from peanut by soxhlet extraction (standard extraction technique) with little modification. Sample was mashed, grinded and homogenised. Two gram of the sample was placed in a cellulose thimble and plug of glass wool was placed on top of the sample to prevent spillage. Twenty five ml of chloroform (Sigma Aldrich, USA) was used as solvent for each extraction. Five replications were made for each material extracted (Raypa, Spain). One gram of sample was weighed into a 250 ml Erlenmeyer flask and then 30 ml acetic acid (Winlab, UK) - chloroform (3:2) solution was added (under the fume hood). The flask was swirled until the sample was dissolved and 0.5 ml saturated potassium iodide (Sigma Aldrich, USA) solution was added. The solution was allowed to stand with occasional swirling for one minute and then 30 ml distilled water was added. After that it was titrated with 0.01 N sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) (BDH, England) by constant shaking until the colour changed to light yellow. The 0.5 ml of 1% soluble starch indicator (Winlab, UK) was added, it gives blue colour. Titration was continued by shaking the flask vigorously near the endpoint.

Peroxide value was calculated in milliequivalents/kg (meq)

$$\text{Peroxide /kg of oil} = \frac{S \times M \times 1000}{\text{weight of sample in grams}}$$

Where S = volume of $\text{Na}_2\text{S}_2\text{O}_3$, and M = 0.01, the concentration of the $\text{Na}_2\text{S}_2\text{O}_3$ solution.

Isolation of Fungi

Fungi were isolated from the whole (uncrushed) peanuts under laboratory conditions using both agar and blotter test methods. Fungi were also isolated on agar plate after crushing the samples in sterilized mortar and pestle (Dhingra

and Sinclair²⁴). All samples were divided into three parts for fungal analysis. Three replicates were used for each set up. Before carrying out experiment all samples were surface sterilised with 3% (v/v) sodium hypochlorite (Sigma Aldrich, USA). For blotter test, surface sterilised ten seeds were placed in each petri dishes layered with sterilised blotter papers and moisten with sterilised water. To carry out agar test for isolation of fungi from whole and crushed peanuts, the set up was similar to that of blotter test except Petri dishes containing 20 ml potato dextrose agar, PDA (Scharlau Chemie, Spain) was used. All Petri dishes were incubated at 23°C±2 for 5-7 days. After incubation, the number of seeds infected with fungus, colony/cultural characteristics of fungi were recorded. The individual colonies of fungi were transferred to new PDA plates in order to obtain pure cultures. All isolates were maintained on PDA and kept at 4°C for the identification.

The taxonomic identification of fungi (based on morphological macro and micro characteristics) was done with the help of microscopic observations, identification keys and illustrated manuals^{25,26}.

All experiments were performed in triplicates. Results were expressed as mean ± standard deviation.

RESULTS

Table 1 shows the moisture content of different types of peanuts and it was found that moisture content of raw samples was maximum followed by roasted-salted peanuts and minimum was in fried-salted samples. Highest peroxide value was found the in fried-salted sample followed by

roasted-salted and was lowest in raw samples.

Results presented in Table 2-4 clearly showed that the irrespective of type of peanut the most dominant genus was *Aspergillus*.

Results showed that the *Aspergillus flavus* was the most dominant fungus in all tested samples followed by *A. niger* and *Rhizopus* sp. The other fungi isolated from the peanuts samples were *Alternaria alternata*, *Cladosporium* sp. and *Penicillium chrysogenum*. The most frequently isolated fungus was *A. flavus*, whereas *Cladosporium* was isolated only from some samples (14.2 %). Occurrence of *Rhizopus* sp. was highest in the raw peanut samples (80%) and 60 percent fried - salted samples were found infected with the same fungus and 57.14% roasted- salted peanuts were infected with *Rhizopus* sp. Whereas, all raw samples were found infected with *A. flavus* followed by fried - salted peanuts (60%) and roasted- salted peanut samples (42.86%). Similarly, *A. niger* was also isolated from all raw peanut samples followed by roasted -salted (71.43%) and fried -salted (20%). *A. terreus* and *A. alternata* each accounted for 14.2 % occurrence in roasted - salted samples and 50% in raw samples, but these fungi were not isolated from fried –salted nuts. Other fungus isolated from the samples was *Penicillium chrysogenum*. Isolation of *P. chrysogenum* was more from salted samples than other samples. The frequency percentage of fungi isolated from peanuts is presented in Fig. 1. *A. flavus*, *Rhizopus* sp., *A. niger* and *P. chrysogenum* had the highest frequency percentage irrespective of sample type. Frequency percentage of *A.alternata* and *A.terreus* was moderate and that of *Cladosporium* sp. was least (Fig 1).

Table 1. Moisture content and peroxide value of peanuts

	Roasted -salted	Fried - salted	Raw
Moisture Content (%)			
Average	3.93	1.92	4.106
Standard Deviation	1.154	1.588	1.569
Range	2-5.3	1.3-2.6	2.6-6.33
Peroxide Value (meq)			
Average	4.98	6.97	3.83
Standard Deviation	2.330	4.826	3.006
Range	1.7-8.67	4.1-14.6	1.5-8.9

(meq) milliequivalents/kg

Table 2. Fungi isolated from roasted- salted peanuts

Sample No		Fungi						
		<i>Rhizopus</i> sp.	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Alternaria alternata</i>	<i>Cladosporium</i> sp.	<i>Penicillium chrysogenum</i>
Sample 1	A	+	-	-	-	-	-	-
	B	+	+	+	-	-	-	-
	C	+	+	+	+	-	-	-
Sample 2	A	+	-	-	-	-	-	-
	B	+	-	-	-	-	-	-
	C	-	-	-	-	+	+	-
Sample 3	A	-	-	-	-	-	-	+
	B	-	-	-	-	-	-	-
	C	+	+	+	-	-	-	+
Sample 4	A	-	-	-	-	-	-	+
	B	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-
Sample 5	A	-	+	-	-	-	-	-
	B	+	+	-	-	-	-	+
	C	+	+	-	-	-	-	+
Sample 6	A	-	+	-	-	-	-	+
	B	-	+	-	-	-	-	-
	C	-	+	-	-	-	-	-
Sample 7	A	-	+	+	-	-	-	+
	B	-	+	+	-	-	-	+
	C	-	+	-	-	-	-	-

(+) present; (-) absent A= Blotter Test for whole samples, B= Agar Plate Technique for whole samples, C= Agar Plate Technique for crushed samples

Table 3. Fungi isolated from fried -salted peanuts

Sample No		Fungi						
		<i>Rhizopus</i> sp.	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Alternaria alternata</i>	<i>Cladosporium</i> sp.	<i>Penicillium chrysogenum</i>
Sample 8	A	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-
Sample 9	A	-	-	-	-	-	-	+
	B	-	-	+	-	-	-	-
	C	+	+	-	-	-	-	-
Sample 10	A	-	-	-	-	-	-	-
	B	-	-	-	-	-	-	-
	C	-	-	-	-	-	-	-
Sample 11	A	+	-	-	-	-	-	+
	B	-	-	-	-	-	-	-
	C	-	-	+	-	-	-	+
Sample 12	A	+	-	-	-	-	-	+
	B	+	-	+	-	-	-	-
	C	+	-	+	-	-	-	+

(+) present; (-) absent A= Blotter Test for whole samples, B= Agar Plate Technique for whole samples, C= Agar Plate Technique for crushed samples

Table 4. Fungi isolated from raw peanuts

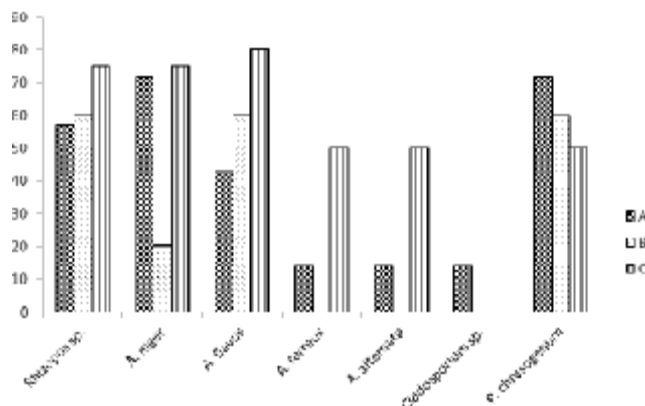
Sample No	Sample	Fungi						
		<i>Rhizopus</i> sp.	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Aspergillus terreus</i>	<i>Alternaria alternata</i>	<i>Cladosporium</i> sp.	<i>Penicillium chrysogenum</i>
Sample 13	A	+	+	-	-	+	-	-
	B	-	+	+	-	-	-	-
	C	+	+	+	-	-	-	-
Sample 14	A	+	+	+	-	-	-	+
	B	-	+	+	+	-	-	+
	C	+	+	+	-	-	-	+
Sample 15	A	+	+	+	+	-	-	-
	B	+	+	-	+	-	-	-
	C	+	+	+	+	-	-	-
Sample 16	A	-	-	+	-	-	-	+
	B	-	-	+	-	-	-	+
	C	-	-	+	-	+	-	+
Sample 17	A	+	-	-	-	-	-	+
	B	-	+	-	-	-	-	+
	C	+	+	-	-	-	-	+

(+) present; (-) absent

A= Blotter Test for whole samples, B= Agar Plate Technique for whole samples, C= Agar Plate Technique for crushed samples

From Table 2, 3 and 4 it has been found that maximum number of fungal species was isolated from Agar Plates of crushed samples (C)

however occurrence of *P.chrysogenum* was highest on blotter plates (A). *A. terreus* was isolated only from Agar Plates of whole nuts.



A=Roasted -salted, B= Fried-salted, C= Raw

Fig. 1. Frequency percentage of fungus isolated from peanuts

DISCUSSION

Providing nutritious food of right quality which is also free from contaminants is very challenging task. Peanuts are nutrient dense

agricultural product but due to its improper processing, packaging, transportation and storage; peanuts and its products get infected with fungi. Moisture content is one of the most important quality factors to the peanut industry²⁷ and

increased moisture content is responsible for enhanced chemical reactions²⁸. As expected, the moisture content of raw samples was highest followed by roasted and was lowest in fried-salted peanuts. The present result is in line with the result obtained by Youssef *et al.*,²⁹ they found higher moisture content in untreated sample than roasted and salted samples. Abdul Hafez *et al.*,³⁰ reported that high moisture content of seeds/grains increases the fungal growth. Higher moisture content of peanuts leads to significant deterioration of quality as compared to lower moisture content. To prevent the mould growth, moisture content should be lower than 10%³¹.

Lipid oxidation is the main cause of flavour deterioration and off flavour formation in peanuts³². These off flavours and off odours render the roasted peanuts less acceptable. Lipid oxidation can be accelerated by a variety of factors such as fatty acid composition, oxygen concentration, temperature, moisture, antioxidants etc. The peroxide value (PV) of the roasted peanuts decreases with decreasing oil concentrations. Divino *et al.*,³³ reported that PV has a direct correlation with oxidation. Low moisture content in peanuts and lower storage temperature reduces the rate of nuts deterioration³⁴. St. Angelo and Ory³⁵ have reported that the quality of the peanuts varied greatly before and after roasting/processing; therefore it had different level of peroxidation at different stages of processing. Mate *et al.*,³⁶ showed that at high concentration of oxygen in peanuts, both dry and oil roasted peanuts had higher PV and hexanal content than low oxygen concentration peanuts. Peroxide value of fresh oils are less than 10 milliequivalents /kg, when the peroxide value is between 30 and 40 milliequivalents/kg, a rancid taste is noticeable. Vercellotti *et al.*,³⁷ described good quality roasted peanuts with PV = 1.4 mEq/kg and oxidized PV= 111 mEq/kg. None of our sample was rancid; however the Pv value of peanuts was higher than the value mentioned as the parameter of good quality of peanuts. Damame *et al.*,³⁸ reported that the oil roasting is more detrimental and hazardous to nutritional quality and storage stability of peanuts as compared to dry roasting.

The most important factor responsible for the deterioration of peanut is number and types of microbes present in the product. The most

commonly found microbes are species of *Aspergillus* and *Penicillium*^{29, 39 and 40}. The results show that the *A. flavus* was the most dominant fungus and all raw peanut samples were contaminated with the same, followed by of fried-salted samples (60%) and roasted-salted samples (42.86%). In a survey on the quality of peanuts, researcher reported that the 93.7% samples were infected with *A. flavus* whereas, 60% salted samples were infected with the same⁴¹. This is also in line with result obtained by Pitt *et al.*,⁴² with 95% contamination by this fungus. In a study it has been reported that one of the important reason for the contamination level of groundnuts are shell damage and kernel splitting apart from poor harvesting and drought⁴³. In another study it has been documented that *A. flavus* can infect and multiply in *A. hypogaea* at both pre and post harvest stage⁴⁴. It has been reported that the peanut is one of the most vulnerable crops to *A. flavus* infection⁴⁵. The other dominating species of *Aspergillus* found in this study was *A. niger*, 75% raw peanut samples were found to be contaminated with this fungus. Whereas, 71.43 percent roasted-salted peanut samples were infected with the same fungus and only 20% samples of fried-salted peanuts were infected with the *A. niger*. Similar results were also observed by Rostani *et al.*⁴¹.

Other dominating fungal contaminants were of the genera *Penicillium* and *Rhizopus*. Results of this study shows that fungal infection by *P. chrysogenum* in salted peanut samples was more than that of raw peanuts. This result is again in line with the result of survey of peanut fungal infection⁴¹.

Our results are in full agreement with the previously records which states that *Aspergillus* and *Penicillium* are most commonly isolated fungi from peanuts^{46, 47, 48}. The presence of *A. niger*, *A. flavus*, *Rhizopus* sp. and *P. chrysogenum* may be due to early stage infection of crop, improper processing and handling. From the Table 2-4 it is clear that raw peanut samples were more infected with the fungi than processed peanut samples. There are some studies which reported that peanut processing methods such as roasting reduce aflatoxin levels⁴⁹. Nawar⁵⁰ in a study reported that sodium chloride affects the growth of the *A. flavus* and *A. niger* on pistachio nuts and found that with

the increase in NaCl concentration the growth of these fungi decreases. He also observed that NaCl prevents invasion and colonization of the fungi on pistachio nuts. Similar result was obtained by Thanaboripat *et al.*,⁵¹.

Most of the nuts are generally observed to be infected with *A. flavus*. This fungus causes spoilage and aflatoxin production. It is also reported to be responsible for pulmonary infections in immunocompromised patients⁵² and various other health hazards^{53,4}. Species of *Aspergillus* are known as the source of ochratoxins, cyclopiazonic acid, gliotoxin and sterigmatocystin⁵⁵ and they are hepatotoxic and carcinogenic^{56, 57}. *Penicillium* species are reported to produce cyclopiazonic acid, Penicillic acid, patulin, ochratoxins⁵⁵ Petulin and Penicillic acids are carcinogenic^{58,59}.

CONCLUSION

The present study showed that almost all peanut samples were infected with one or more than one fungal species. It clearly indicates that peanuts are vulnerable to fungal infection especially to aflatoxin producing fungi. It was found that the roasting, frying and salting of peanuts have increased the lipid oxidation, however the moisture content and fungal infection have reduced. The peanuts are good medium for fungal infection and for producing various toxic metabolites. Therefore a quality check is required at every stage right from production to consumption of the peanuts. Further research is needed to explore other means of preventing mould infection. These could include prevention of infection in the field through the use of biological control agents, proper handling of peanuts during processing and transportation, use of natural preservatives such as NaCl.

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