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PREVALENCE AND MORPHOLOGICAL DIFFERENTIATION OF MOLD CONTAMINANTS (*ASPERGILLUS NIGER* AND *PENICILLIUM CHRYSOGENUM*) IN READY-TO-EAT BAKERY PRODUCTS (PIZZA AND SHAWARMA)

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Abstract

The changing diet habits of the community had made burdensome to prepare a meal at home. Choices have moved towards baked and fast food. Pizza and shwarma are the foremost preferable items and major source of balanced diet. The ease of availability of bakery products are one sided benefit on the other hand these are potent source of transmitting the pathogens. Penicillium chrysogenum and Aspergillus niger mostly cause spoilage of these bakery products (pizza, shwarma) and reduce the shelf life. The case is here to find about the microbial spoilage of the bakery products by Molds (Aspergillus niger, penicilliumchrysogenum). Our study will provide a safety standerd to the regulatory bodies and also provied awerness to the people to check the safety level of food before its consumption. In present study, use spread plate to check the microbial load and morphological characterization was done on the basis of colony color and shape and used lactophenol cotton blue (LPCB) for staining of the fungal spores. The obtained data results have showed that the highest and lowest count of mold (Aspergillus niger) among local bakeries was 3.2×10^7 and 2.0×10^2 CFU/g. Among branded bakeries the highest and lowest count observed was 2.3×10^3 CFU/g and 6.62×10^2 respectively. The obtained results showed that the lowest count of penicillium spp recorded in local bakeries was 2.3×10^3 CFU/g while highest count observed in bakery product samples among local bakeries was 4.29×10^5 CFU/g among the branded bakeries the lowest count was 6.0×10^2 CFU/g whereas in branded bakeries the highest count was 2.8×10^5. Our study will provide a safety standerd to the regulatory bodies and also provied awerness to the people to check the safety level of food before its consumption.

INTRODUCTION

Merriam-Webster first time introduced the term fast food in year 1951. Ready to eat food products are vital portion of nutritious intake, pizza in particular is one of the most widely consumed fast foods in the world (Beigi *et al.*, 2015). Bakery products contain low preservatives, salt and sugar provide favourable medium for microorganisms to grow. As compared to other food products bakery products are manufactured through different process so the chances of contamination are enhanced (Kotzekidou, 2013). Cooling, slicing and transport of bakery products in addition with contamination increase the spoilage of the bakery stuffs (Gray and Bemiller, 2003). When pizza is placed in freezer for maximum time period, chances of pathogenic prevalence increase (Cabo *et al.*, 2001). Novel exploration on the nutritional feature of pizza due to its preference and acceptability have showed that production and consumption of pizza has increased (Singh and Goyal, 2011). Shawarma is originated from Turkey where it is known as cervime which means turning. It contains carbohydrates, protein, low water activity which resist the prevelence of *Penicillium* and *A. niger* if handled properly.

Spoilage can be physical, chemical and microbial. Sufficient amount of moisture for microbial growth in commercially produced bakery products is very much low and since no microorganism can grow except mould. Temperature required for normal cooking of bakery products destroys fungal spores but post process contamination must be prevented to avoid spoilage of such products. Spoilage mainly occur due to filamentus fungi include *Penicilliumsp, Rhizopussp, Aspergillus sp, Mucorsp, Moniliasitophilia* and *Eurotium sp. Rhizopus stolonifera* also called bread mould is one of the most common fungus involved in bread spoilage. Mould growth can be retarded by storing bread under low humidity condition. In addition, another most important factor of economic loss associated with bakery products is mycotoxin production. The first fungi which colonize on bakery products is Eurotium and then allow other species to grow. *Penicillium* and *Aspergillus* can make toxins. Mould spoilage losses bakery between 1-5%. The use of preservatives is therefore required to reduce the spoilage and ensure food security. Mould growth is the more vital factor of reducing shelf life of baked items. For most molds the value of available water is >0.8 while for some xerophilic molds this value is 0.065. Economic loss due to molds is a serious problem. Ropy spoilage is caused mainly by the mold, bacteria and yeast. These pathogenic microorganisms can stay alive during cooking. Spoiled foods can be eatable if they may not produce disease because at this stage there are no contaminants and pathogens are present but a little bit changes in appearance, taste and smell make them to forbidden to eat (Smith *et al.*, 2004). It is very tricky to calculate the hazards of street vended foods due to mould. Toxigenic mold in food causes human illness (Guynot *et al.*, 2005). *Aspergillus* belongs to kingdom fungi phylum Ascomycota. *Aspergilusniger* is pathogenic and causes disaeses in human including illness and allergic reactions. *A.niger* causes black mold in food stuff. *Penicillium*, genus of blue or green mold belongs to kingdom fungi. These molds contaminate grains which causes illness with symptoms headache, diarrhea, vomiting, blurred vision dizziness. These mold produce mycotoxins which are carcinogenic and neurotoxic in nature. The mycotoxin "Ochratoxin A" is main contaminant of food and beverages. It is a secondary metabolite of *Penicillium*, and *Aspergilus niger.* Ochratoxin A inhibits the protein synthesis. *Aspergilus niger* causes reduced immunity (Alpha nutrition, 2006). Food related microbial hazards are the biggest threats to human health. Diarrhea illnesses are world widely caused by food spoilage. In Asia ,every year about fifty thousand children have been become sick and sixty million died and in Pakistan more than 60% people suffered with diease due to the eating of unhygienic food (Campos *et al.*, 2015). Main objectives of the study is to evaluate the prevalence of *Aspergillus niger*and *Penicillium chrysogenum*in Bakery products (Pizza, Shawarma). And also give

awareness to the against the mold contamination in bakery product because nowadays fast food in more eatable product worldwide. Our study help the regulatory bodies and conumser to produce save and nutrition foods and help to reduce food burden worldwide.

MATERIAL AND METHODS

Sampling plan & Chemicals procurement

The prevelance of *Penicillium crysogenum,* and *A.niger* in bakery products sold in different bakeries were checked by getting representative and random samples from 10 bakeries (5 local and 5 branded). Microbial evaluation of data was done in Food Microbiology and Biotechnology Laboratory, National Institute of Food Science & Technology (NIFSAT)), University of Agriculture Faisalabad. For samples collection sterilized Polyethylene zip bages were used (shwarma and pizza) and put in ice box for transportation. Potato dextrose ager (PDA) media was used for *Penicillium*and *A .niger*. Peptone water and all other chemicals were bought from Oxoid (UK).

Sample preparation

Bakery products Samples (shwarma and pizza) were collected from various bakeries and 25 g sample was placed into stomacher bag with 25-30 ml of peptone water and bag is then placed in stomacher (Model 400 Circulator, Seward, UK). Stomacher was adjusted for two minutes a 200 rpm purpose to complete homogenization of sample ingredients. The aim behind homogenization is to detach fungus from solid to liquid form (Yousef and Carolyn, 2003).

Microbiological examination (*Aspergillusniger*, *Penicilliumcrysogenum)*

Potato dextrose agar was used to isolate *A.niger* and *Penicillium crysogenum* by following spread plate method described by (Eman *et al*., 2015) with a little bit modifications.

Media preparation

For microbial count the method as described by (Prescott *et al*., 2002) was followed. About 11.5 g of Potato dextrose agar with 500 ml of distilled water was mixed and sterilization is carried out at 121 °C at 15 psi for 15 minutes, then it was kept in already set water bath to keep at the temperature of 55-57 °C, after sterilization. Pouring was done in laminar flow under sterilized conditions; Bunsen burner was used to avoid microbial contamination. Sterilized prepared potato dextrose ager was poured in petri plates at 56 ° C and was kept for solidify. Petri Plates were then placed in inverted position and stored in refrigerator at 4 – 6 °C for further use. Test tubes were filled with 9 ml saline solution and labelled (8.9 g NaCl/1000ml of distilled water) and subjected to sterilization process by autoclaving at for 15 minutes, 15 psi at 121 °C in order to make the serial dilution. Ten-fold serial dilutions were made by using micropipette. Then one mL homogenized sample was taken with the help of micropipette and transferred into test tube labelled as 1 and thoroughly mixed. Further 1 ml sample was taken from test tube 1 and transfered to test tube 2 and carried so on this process up to 5 dilutions. For the purpose of inoculation of samples on petri plate 0.1 ml sample from test tube 1 was taken and poured on petri plate 1 and was spread on petri plate by heat treated sterilized spreader. Similarly this process was repeated, 0.1 ml was taken from test tube 2 and poured on petri plate 2 spreading was done with heat or ethanol treated spreader and procedure continued so on. To avoid contamination inoculation was done in laminar air flow under sterilized conditions by burning. Bunsen burner to disinfect the surface area of laminar flow. Petri plates were incubated then at 37 °C for 5 to 7 days in order to take microbial growth. The analysis was performed in triplicate to obtain accuracy. Colonies were counted from the petri plates with help of colony counter by using this formula:

CFU/g = Average No. of Colonies × Dilution number/Volume plated × Dilution factor

Dilution factor = Sample volume/diluent Volume + Sample Volume.

Morphological Examination

The slides were prepared and examined under microscope colony shape and color were observed using lactophenol cotton blue (LPCB). Slide was prepared by putting a drop of 70% etanol and with the help of wire the culture was set on the slide the wire was heated to red hot before transfer of culture. Before the fungus dries out add one or two drops of LPCB that stain the fungus structre that could be visualized readily under microscope (Leica DM5000 B) firstly with lower lens then with resolution of 40X for the detailed examination of spore of the fungus.

Statistical Analysis

All analysis were performed in triplicates and the figures were reported as means. Significant difference among treatments was evaluated through analysis of variance (ANOVA) under completely randomized design (CRD) according to the method described by (Campos *et al.*, 2015). For the analysis of likert scale IBM SPSS Statistics 22 was used.

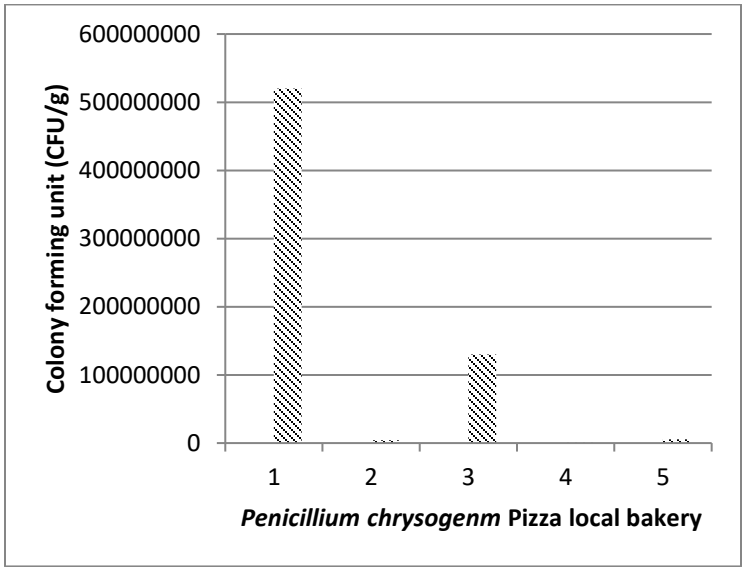
RESULTS AND DISCUSSION

Prevelanceof *Penicilliumchrysogenum* in pizza and shawarma from Local and Branded bakeries

15 Pizza samples from local bakeries were taken and the observed prevelance ratio was 11/15. The highest and lowest count observed was 5.2 x 10^9 and 3.7 x10^4 for *penicillium*in Pizza for local bakery. Present research work is also resemble with Mashak *et al.* (2014). Who stated that bakery products due to excess of water phase, salts and other factors provides a favourable medium for growth of microbes. Although during cooking at high temperature 80-100°C maximum number of microbes are destroyed. But in branded bakeries the ratio was 9/15 with highest count 8.2x10^5 and lowest count was 5.2x10^2. Presented results are in accordance with Stephane Dagnas (2017). He augmented that heat treated food products are susceptible to mold spoilage due to fluctuations in temperature and water activity. In case of **Shawarma of Local bakeries** the highest and lowest count observed was 3.2 x 10^7 and 1.1x 10^2 here by ratio 15/15. Present research findings are also resemble with Saranraj and Geetha (2012). Who concluded that *Penicilliumspp, Aspergillus sp. Rhizopusstolonifer* is a common mold that spoile bakery products and bread. They stated that bakery product spoilage can be controlled by proper handling, cooking and reducing water activity, as normal cooking destroys the mold. Shwarma from branded bakeries the highest count of *Penicillium chrysogenum* was 7.24 x10^5 the smallest count was 9.6 x10^2 with ratio of 10/15. The present results obtained are similar with Sicuia *et al* .(2014). Who studied the microbial spoilage of bakery products. They further stated that fungi is the spoilage agent of cereal based products. In the bakery products its level should be below from 10^2 CFU /100g (Saranraj and Geetha (2012).

Table 1. Enumeration of *Penicilliumchrysogenum* in Pizza and Shawarma

LOCAL BAKERIES		BRANDED BAKERIES	
PIZZA	SHAWARMA	PIZZA	SHAWARMA
5.2 x 10^9 a	1.1x 10^2 d	5.2x10^2 d	9.6 x10^2 c
4.0x10^5c	3.2 x 10^7 a	3.0x10^3 c	Not found d
1.3 x10^7 b	3.0 x10^3c	8.2x10^5 a	1.16×10^5 a
3.7 x 10^4 d	Not found e	Not found b	7.24 x10^5 b
5.5x10^5 c	2.0 x 10^5b	Not found f	6.7 x 10^5 ab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Colony forming unit (CFU/g)
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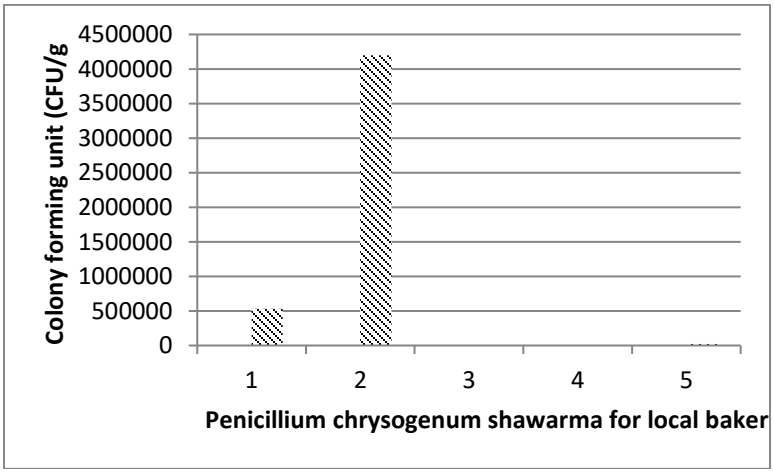
Penicillium chrysogenm Pizza local bakery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Colony forming unit (CFU/g
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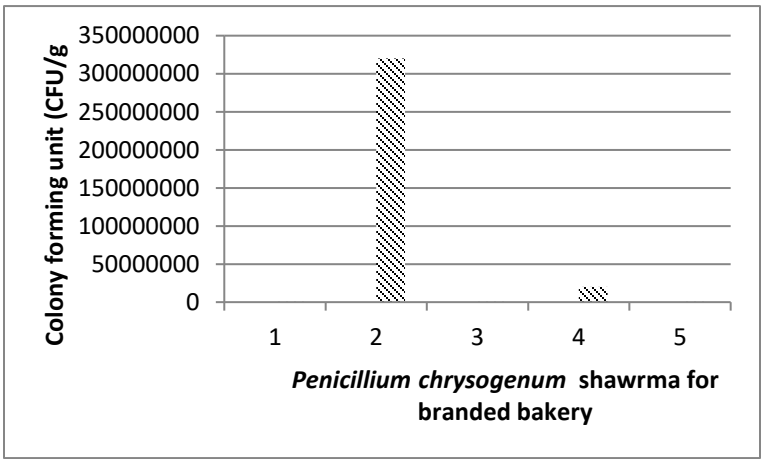
Penicillium chrysogenum shawarma for local baker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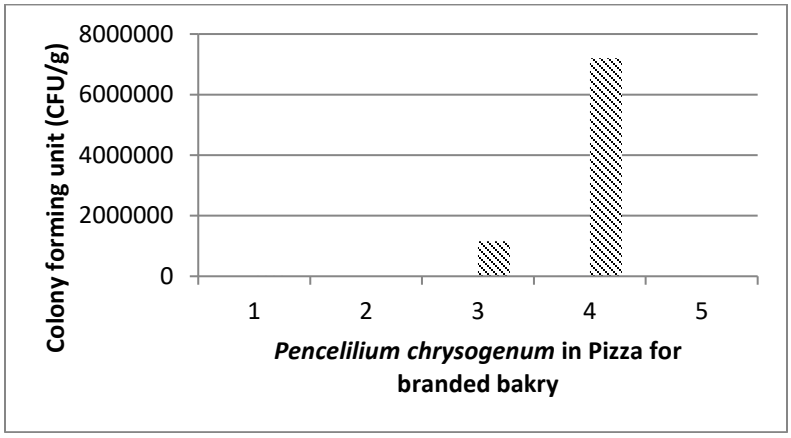
Penicillium chrysogenum shawrma for branded bakery



Characterization of *Penicilliumchrysogenum* in Pizza and Shawarma

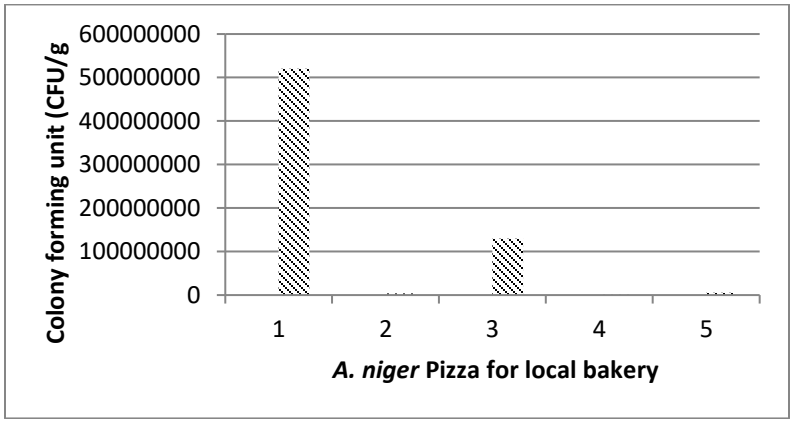
Prevelance of *Aspergillus niger* in pizza and shawarma from Local and Branded bakeries

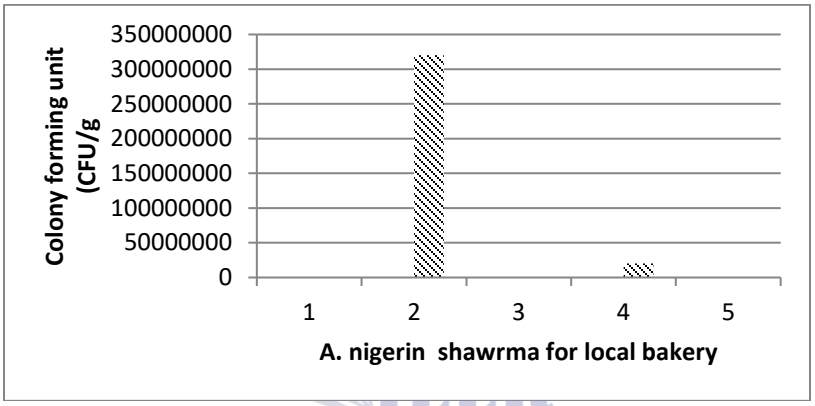
Out of all the collected samples of Pizza from local bakeries the observed lowest and highest count was 3 x 10^3 and 5.36 x10^4 with ratio of prevelance was 9/15. These results are resembled with Lore *et al.* (2005) findings. He concluded that the presence of A. *niger*in bakery products (pizza) showed a dreadful state of poor hygienic and sanitary practices adopted during packaging and processing. He declared that the toxins are already present in the food product they have the ability to multiply and cause food borne illness, when stored at room temperature (25-30 °C) for longer period of time. Whereas Pizza from branded bakeries, ratio was same but the highest count of *A. niger* was 2.8x10^5 and the smallest count was 6.0x10^2. The obtained results are in similar with Hocking (2008). He isolated mold (*Penicillium, A. niger*) and other microbes from the bakery products and concluded that mold spoilage is very tricky for the loss of bakery economy Shawarma from local bakeries and *A.niger* prevalence ratio was 10/15. The highest count of *A. niger* in shawarma was 3.2 x 10^7 whereas the lowest count was 2.0 x 10^2. Presented results are in accordance with Stephane Dagnas (2017). He augmented that heat treated food products are susceptible to mold spoilage due to fluctuations in temperature and water activity.

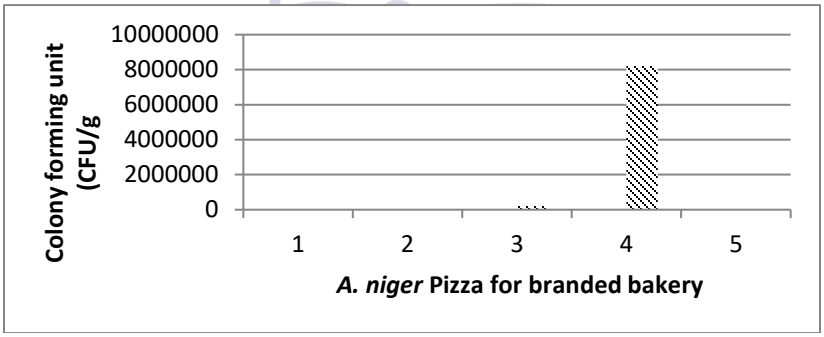
Branded bakeries shwarma *A. niger* prevalence remain same with that of local bakery shawarma but the lowest count of A. niger observed was 6.62x10^2 and the highest count was 2.3x10^3. In the bakery products is acceptable below the level of 10^2 CFU /100g (Saranraj and Geetha (2012).

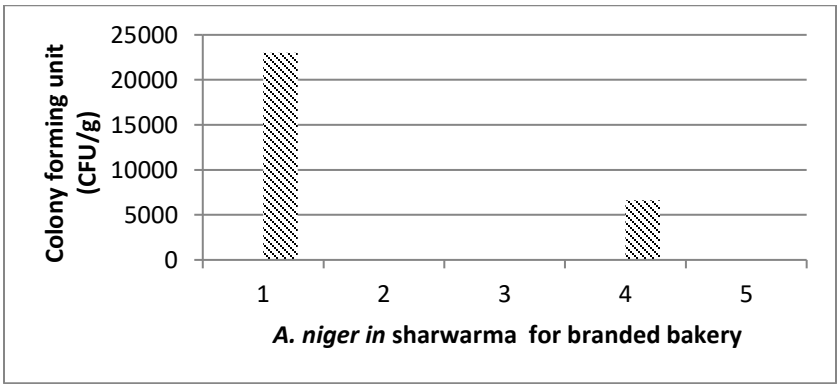
Table 2. Enumeration of *A.niger* in Pizza and Shawarma

LOCAL BAKERIES		BRANDED BAKERIES	
PIZZA.	SHAWRMA	PIZZA	SHAWRMA
5.36 x10^4 b	1.1x 10^3 c	6.0x10^2 c	2.3x10^3a
4.29x10^5 c	3.2 x 10^7 a	3.6x10^3 b	Not found b
3 x 10^3 c	3.0 x10^3c	2.8x10^5 a	Not found b
Not found d	2.0 x10^6 b	1.6x10^5a	6.62x10^2 b
Not found d2	2.0 x 10^2 d	Not found d	Not found b









Characterization of *Aspergillus niger* and *Penicillium chrysogenum*

Identification and Morphological characterization findings are with Pundir and Jain, (2011). Who identified morphological characterization of food contaminant molds. The microbial spoilage caused due to *A. niger* and identified on the basis of morphology.

Identification and Morphological characterization

Isolate	CULTURAL CHARACTERISTICS	MORPHOLOGICAL CHARACTERISTICS
Aspergillus niger	Very common black colony	Conidia covers the upper 2/3 of the conidiophores, they have rose flower like appearance.
Penicilliumchrysogenum	White colony cover the whole surface	Branched non septate hyphea they produce brownish black Ceridian in chains

The present work shows that pizza sold in branded bakeries less contaminated with *penicillium* spp. as compare to local ones. The presence of *Penicillium* spp. in food is also a spoilage issue and identified on colony colour. The *Penicillium* itself destroyed at high temperature but toxins are temperature resistant and do not eliminated by heat. If such contaminated product are served to consumer results in food borne illnesses. In order to control the *Penicillium chrysogenum* in pizza, packaging under sterile conditions furthermore proper cooking time temperature and cooking factors must be ensured.

Conclusion and recommendations

The present study concludes that the pizza and shwarma sold in various bakeries are contaminated with *Aspergilus Niger* and *Penicillium.A.niger* was found to slightly higher in case of branded bakery products while *Penicillium* was higher in local bakeries. The presence of such type of mycotoxin in food products needs special care in term of preparation, hygienic and raw material quality. The preliminary practice in this context includes, self-inspection programs, educating and training food personnel,) and introducing hazard analysis critical control point (HACCP) and ensuring good manufacturing practices (GMP) concepts. All over Pakistan these critical steps can impart their role, to check the pathogenic agents which can transfer and spread food spoilage in the food stuffs. Too many legislative expertises, industrial and agricultural departments are required to enhance safety as well as quality of food products. Punjab Food Authority is doing hard work to implement food safety measures to ensure safe and healthy diet.

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Conflict of interest

The authors have no conflict of interest.

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