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SCAN ME

*Research Paper*

# HYGIENIC QUALITY OF READY TO EAT COOKED MEAT IN RESTAURANTS AT CAIRO GOVERNORATE

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**Abstract**

The present investigation was designed to study the total mould and yeast counts and Aflatoxin residues in a total of 60 random samples of ready to eat chicken products which include Shawerma, smoked chicken breast, Pane and grilled chicken (15 of each) were collected from different localities under different sanitation levels at Cairo governorate. The incidence of mould in examined ready to eat chicken meat products samples were 12 (80%),8 (53.3%),15(100%),8(53.3%) and the total mould count ranged from 0.5 x 10 cfu/g to 1.8x 102, 4.2 x 10 ±1.5 x 10, 0.5 x 10 to 2.3 x 10 and > 10 to > 10, with mean value of 3.4 x 10± 1.5 x 10, 4.5 x 10 ±1.5 x 10, 1.4 x 10 ± 0.2 x 10 and 0.5 x 10 ±0.2 x 10 cfu/g, for chicken shawerma, smoked chicken breast, pane and grilled chicken, respectively. While the results revealed that the incidence of yeast contamination in examined ready to eat chicken meat product samples were 0 (0%),15 (100%),0(0%),1(6.7%) for shawerma, smoked chicken breast, panne and grilled chicken respectively. For moulds, the isolated species were Aspergillus, Penicillium, Acremonium, Alternaria, Aurobasidium, Cladosporium, Eupenicillium and Eurotium spp. while for yeasts, the isolated species were Candida spp, Rhodotorula spp and Debaryomyces spp. The results also indicated the mean ±SD of total aflatoxin were 1.5 x10 ± 0.2 x 10, 3.2 ± 0.5, 4.3± 0.9 and 1.2 x10 ± 0.3µg/kg for shawerma, smoked chicken breast, panne and grilled chicken respectively.

Key words: *Shawerma, Smoked chicken breast, Panne, Grilled Chicken, mould, yeast, Aflatoxin*.

## INTRODUCTION

Chicken meat is considered the main source of animal protein and a high source of iron, zinc and several vitamins, and so meat products are considered a favourable food as it

easy to buy, fast to cook, delicious to eat and also with low price .Meat products may contaminated with one of the most dangerous microbial hazard represented in moulds. Consumption of contaminated ready to eat foods have been documented to serve as vehicles for transmission of several pathogens and food-borne outbreaks [1]. Mycotoxigenic moulds such as Aspergillus, Fusarium and Penicillium play an undesirable role in the deterioration of the marketable quality and hygiene of foodstuffs by synthesizing highly toxic metabolites known as mycotoxins. The occurrence of mycotoxigenic moulds in food is potentially dangerous for public health and also constitutes a major economic problem [2].

Aflatoxins are common contaminants of foods, particularly in the diet of many countries, and they are categorized as class 1 A human carcinogen by the International Agency for Research on Cancer They have immunosuppressive, mutagenic, teratogenic and carcinogenic effects, especially on the liver. [3]. Accumulation of aflatoxins residues in human in acute cases is lethal due to intoxication, in chronic cases leads to hepatocellular carcinoma [4].

Therefore, the present study is planned to throw a light on the total mould count of some ready to eat chicken products which were represented as Shawerma, Smoked chicken breast, Pane and Grilled Chicken samples; isolation and identification of isolated mould species, yeast species and determination of aflatoxins residues in the examined chicken products.

**MATERIAL AND METHODS**

*Collection of Samples:*

A total of 60 ready to eat chicken products samples represented by (Shawerma, Smoked chicken breast, Panne, Grilled Chicken) 15 of each were collected randomly from different restaurants at Cairo governorate. The collected samples were placed in sterile ice-packed containers and conveyed to the laboratory without delaying for analysis. The collected samples were prepared according to the technique recommended by [5]. Determination of total mould and yeast counts according to [6].

Identification of mould isolates according to [7].

Identification of yeast isolates according to [8] and by using Rapid ID yeast plus system [9].

Detection of total aflatoxin residues in ready to eat chicken meat products samples by using fluorimeter method [10]. The detection of aflatoxin residues in examined ready to eat chicken meat products samples take place using immunoaffinity column method for extraction of aflatoxin and reading by VICAM fluorimeter in parallel with standard of aflatoxin[11].

1- Setup: Calibrate fluorometer. Make sure the yellow calibration standard reading is in the range listed above. Make sure that 2 mL purified water in a cuvette reads 0 ppb on a calibrated fluorometer. Prepare methanol: water (70:30 by volume) solution. Prepare AflaTest Developer solution every 8 hours. Make sure that reagent blank (1 mL methanol + 1 mL Developer in a cuvette) reads 0 ppb on a calibrated fluorometer.

2. Sample Extraction:

1. Weigh 25 g ground sample with 5 g NaCl and place in blender jar.
2. Add to jar 125 mL methanol: water (70:30).
3. Cover blender jar and blend at high speed for 2 minutes.
4. Remove cover from jar and pour extract into fluted filter paper. Collect filtrate in a clean vessel.

3: Extract Dilution:

1. Pipet or pour 15 mL filtered extract into a clean vessel.
2. Dilute extract with 30 mL purified water. Mix well.
3. Filter dilute extract through 1.5µm glass microfiber filter into a clean vessel.

4: Column Chromatography:

1. Pass 15 mL filtered diluted extract (15 mL = 1.0 g sample equivalent) completely through AflaTest column at a rate of about 1 drop/second until air comes through column.
2. Pass 10 mL of purified water through the column at a rate of 1-2 drops/ second.
3. Repeat previous step once more until air comes through column.
4. Place glass cuvette (VICAM part # 34000) under column and add 1 mL HPLC grade methanol into glass syringe barrel.
5. Elute column at a rate of 1 drop/second or slower by passing the methanol through the column and collecting all of the sample eluate in a glass cuvette.

6. Add 1 mL of AflaTest Developer solution to eluate in the cuvette. Mix well and place cuvette in a calibrated fluorometer. Read aflatoxin concentration after 60 seconds.

## RESULTS AND DISCUSSION

The total mould count is used as an index of the proper sanitation and high quality product. Moulds can assist in the putrefactive processes and in other cases they may impart a mouldy odour and taste of foodstuffs. In addition, mould can grow over an extremely wide range of temperature; therefore, one can find mould on particularly all foods at almost any temperature under which foods are held. Besides, mould can assist in the putrefactive processes and may produce toxic metabolites, namely mycotoxins, which are harmful to man and animal [12].

The result achieved in table (1) revealed that the incidence of mould in examined ready to eat chicken meat products samples were 12 (80%),8 (53.3%),15(100%),8(53.3%) for shawerma, smoked chicken breast, pane and grilled chicken respectively. While the total mould count ranged from 0.5 x 10 cfu/g to 1.8x $10^2$, 4.2 x 10 ±1.5 x 10, 0.5 x 10 to 2.3 x 10 and > 10 to > 10, with mean value of 3.4 x 10± 1.5 x 10, 4.5 x 10 ±1.5 x 10, 1.4 x 10 ± 0.2 x 10 and 0.5 x 10 ±0.2 x 10 cfu/g, for chicken shawerma, smoked chicken breast, pane and grilled chicken, respectively. According to the safe permissible limits stated by [13] for a total mould count in chicken products (Free). Table (1) indicated that 80% of the chicken shawerma samples, 53.3% of smoked chicken breast samples, 100% of chicken pan e samples, 53.3% of grilled chicken samples were not in accordance with these limits.

For chicken shawerma higher figures were reported by [14], [15] and [16] who reported that the mean values of mould count in locally manufactured chicken shawerma was $1.70\times10^3 \pm 0.14\times10^2$ , [17] who recorded that The mean values for mould count in examined chicken shawarma samples was 1.9 $x10^2$ ±3.9x10 cfu/g. and [18] who reported that the mean total mould count in the examined Shawarma samples was 3.2$x10^2$ ±6.9x10 cfu/g while the mean count in smoked chicken breast were lower than that were reported by [19] , [20] , [21]and [22] who detected mean total mould count in chicken luncheon 2.01 $\times10^2 \pm 0.53 \times10^2$, [23] who reported that the mean values of mould count of examined samples of chicken luncheon was 6.8 x $10^4 \pm 2.0 \times 10^3$ cfu/g. [24] who reported that he mean values of mould count of examined samples of chicken luncheon was $6.9\times10^3 \pm 3.5\times10^2$ cfu/g and [25] who

detected that the mean value of mould count was $2.2\times10^5 \pm 3.6\times10^4$ in examined samples of chicken luncheon while similar result was reported by [26] who reported that mean values of 5.8 x 10 ±1.5 x 10 of mould contamination was detected in examined chicken luncheon.

Results of chicken pane was lower than that recorded by [27] who reported that the mean value of mould count in fried light yellow colour chicken strips 3 × 10 ± 0.3 × 10 while in fried dark yellow colour mould count were not detected . [28] who reported that mean total mould in chicken pane meal was $3.4 \times 10^4$ cfu/g. [29] who reported that the total mould counts in examined chicken pane $7.4 \times 10^2 \pm 5.4 \times 10^2$ cfu/g, [29] and [16] recorded that the mean values of mould count was $1.35\times10^4 \pm 0.22\times10^4$ for chicken pane. [30] who recorded that total mould count in chicken pane was $2.8x10^2 \pm 1.4x10^{2.}$ [31] who concluded that the mean total mould count in examined coated chicken fillet was $2.34 \times10^2 \pm 0.78 \times10^2$ [23] who reported that the mean value of mould count in examined chicken pane was $6.8 \times 10^4 \pm 2.0 \times 10^3$ and nearly similar result detected by [32] who showed that the mean value of mould count in examined chicken pane was 8.80×10± 6.19×10 cfu/g.

But for grilled chicken, these result was higher than that reported by [16] who declared that the mould count was 0 for grilled chicken breast.

According to the microbiological standards of the public health laboratories (PHLS, 1996) of the Advisory committee for food and dairy products, the mould count/g for ready-to-eat foods not mentioned anything about fungi. Adequate temperature in cooking and storage of foods is important to minimize the growth of mould and the food that cannot maintained within the safety temperature zone may act as incubator for mould.

From obtained results, the presence of mould contamination may be attributed to use of contaminated spices (untreated food additives) which usually carry mould spores [33]. Chicken Luncheon and Chicken pane are exposed to heat treatment during their processing and any microbial activities may be recorded as a result of post processing contamination, unhygienic storage as well as long storage life which may lead to spoilage. Also, spices have been used in many industries, with the food industry and catering being predominant users. Having been dried material from plant origin, spices are commonly heavily contaminated with xerophilic storage moulds and bacteria. The most frequent fungal contaminants of spices are species from the genera

Aspergillus and Penicillium. Some species that belong to these genera are known as potential producers of different toxic substances such as aflatoxins, ochratoxins and sterigmatocystine, i.e. mycotoxins that exhibit toxic, mutagenic, teratogenic and carcinogenic effects in humans and animals [34].

The results achieved in table (2) revealed that the incidence of yeast contamination in examined ready to eat chicken meat product samples were 0 (0%),15 (100%),0(0%),1(6.7%) for shawerma, smoked chicken breast, panne and grilled chicken respectively. Higher result was recorded by [15]. Also, it is evident that the mean total yeast count was 0 for all shawerma, pane and grilled chicken samples. While in smoked chicken breast mean total yeast count was $9.2 \times 10^3 \pm 7.8 \times 10^3$ .The higher figures were showed by [14] and [15] who reported that the total yeast count in the examined chicken shawerma samples was $5.2 \times 10^5$ cfu / g and [17] who declared that the mean values for yeast count in examined chicken shawerma samples was $9.6 \times 10^2 \pm 3.0 \times 10^2$ cfu / g. Comparatively most of the previous researches had higher counts due to the differences between the chicken processing plant at this time (sanitation program – computerized machines – educated workers) and manufacturing in the past.

According to table (3) which showed the number and percentage of aspergillus isolated from examined samples it seems that *A. flavus* was 4(26.7%), 2(13.3%), 4(26.7%) and 2(13.3%) from chicken shawerma, smoked chicken breast, chicken pane and grilled chicken samples, respectively , followed by *A. niger* 3 (20%), 1 (6.7%), 0 ( 0%) and 0(0.9%), respectively, While *A. ustus* 1(6.7%), 1 (6.7%), 0 ( 0%) and 0(0%) Then *A. niveus* detected in chicken panne only 2(13.3%). then *A. ochraceus* which detected only in chicken shawerma 4(26.7%). The obtained results declare that the chicken shwerma and chicken panne samples had the highest number of Aspergillus spp. 12 and 6 isolates respectively, followed by smoked chicken breast (4 isolates) then grilled chicken (2 isolates). Nearly these results are in agreement with those found by [35], [26], [36], [30], [33], [22], and [18].

Identifications of Penicillium spp. isolated from the examined chicken meat products in Table (3) revealed that *P. candidus and P. citreonigrum*, *P. concentricum, P. oxalicum* and

*P.corylophilum* were the most predominant species isolated from the chicken shawerma, smoked chicken breast, chicken pane samples with different percentage, while grilled chicken samples Penicillium species not detected. These results

substantiate what has been reported by [26], [30] and [18] who reported that isolated Penicillium spp in chicken pane samples was *P. decumbens* 1 (100%). However, in chicken luncheon samples were *P .citrinum* 1 (50%) and *P. coryphilum* 1 (50%).

Regarding the results tabulated in table (3) shows the incidence of other mould species isolated from different examined samples the predominant mould species isolated was Acremonium strictum 2(13.3%),0(0%), 2(13.3%),0(0%) from chicken shawerma, smoked chicken breast, chicken panne and grilled chicken samples respectively, then *Alternaria alternate* 0(0%),1(6.7%), 0(0%),0(0%) respectively. while *Aurobasidium pullulans* and *Eurotium repens* were isolated from chicken pane only by 4(26.7%), ,1(6.7%) respectively then *Cladosporium cladosporidiae* which only isolated from chicken shawerma by 1(6.7%) finally *Eupenicillium cinnamopurpureum* which was isolated only from grilled chicken by 1(6.7%) .

Mould species can grow on a wide variety of meat products, as they can tolerate elevated temperature and reduced water activity , as well as, their spores are longer lived and more resistant to chemicals [7]. Aspergillus growth is influenced mainly by temperature, moisture content and storage time. Human infections are usually acquired by inhalation of airborne spores from inanimate sources. Pulmonary aspergillosis can present as different forms, including pulmonary Aspergilloma, chronic necrotizing pulmonary aspergillosis, invasive pulmonary aspergillosis and allergic bronchopulomnary aspergillosis, depending on the atopic and immune status of the host and the site of involvement within the respiratory system [37]. Some species of Penicillium have been associated with pulmonary infection, urinary tract infections and yellow rice disease syndrome which are responsible for several cases of death in man. Also, some isolates of genus Penicillium may induce endocarditis, external otomycosis, mycotic keratitis and pulmonary infection in the immunocompromised patient. For example, penicillic acid and sterigmatocystin are mycotoxins produced by some isolates of Penicillium species had a carcinogenic effect [38].

Regarding the results tabulated in table (4) the incidence of yeast species isolated from examined chicken products samples it is obvious that smoked chicken breast samples were heavily contaminated with several yeast species the most predominant yeast genera isolated from such smoked chicken breast samples was *Rhodotorula mucilaginosa* 9 (60%), followed by *C. guilliermondii* 6(40%), *C. famata*

5(33.3%) and *C. krusei* 4 (26.7%).while *Debaryomyces hansenii* was only isolated from grilled chicken samples by 1(6.7%).

These results nearly are similar to that recorded by [39] and [40] who showed The frequencies of isolated yeast genera in examined chicken luncheon samples which were; *C. albicans* (7.9%), *C. zeylanoides* (15.8%), *C. parapsilosis* (23.7%), *C. tropicalis* (21.0%), *Rhodotorula mucilaginosa* (7.9%), *Rhodotorula glutinis* (15.8%) and *Saccharomyces cerevisiae* (7.9%)., [30] and [41] who reported that the yeast isolates represented 4 genera were isolated from chicken luncheon, pane and nuggets samples in the following number and percentage, Candida 61(58%) (with the highest incidence) followed by Rhodotorula 7 (29.1%) then Saccharomyces 10 (9.5) and finally Trichosporon 3 (2.9%), respectively. Relatively few yeast species cause significant spoilage in processed foods, most being adventitious contaminants from natural sources.

It is evident from the results presented in table (5) that the average concentration of total aflatoxin residues µg/kg (ppb) in examined chicken shawerma samples was ranged from 3 to 40 with mean values of 1.5×10 ±.2×10 ppb this result was higher than that was revealed by [18] who showed that there were not any type of aflatoxin residues detected in shawerma samples. These finding may these finding may be attributed to the heat treatment of shawerma. Meanwhile aflatoxin residues in examined smoked chicken breast samples was ranged from .7 to 6.8 with mean values of 3.2 ± 0.5 ppb lower result was recorded by [33] who revealed that the mean value of the total aflatoxins residues in the examined chicken luncheon samples was 0.87 ± 0.14 ppb but higher result was reported by [42] who ascertain and quantify the presence of aflatoxins (fungi metabolites) residues in luncheon samples and the result was 5.44 ± 0.39 ppb. While in chicken pane samples the aflatoxin residues was ranged from 0.9 to 13 with mean values of 4.3 ± 0.9 ppb. Lower result was recorded by [33] who revealed that the mean value of the total aflatoxins residues in the examined coated chicken fillet was 2.53 ± 0.31 ppb. Finally, in grilled chicken samples the aflatoxin residues was ranged from 8 to 20 with mean value of 1.2×10 ± 0.3 ppb. According to the safe permissible limits stated by Worldwide Regulations for mycotoxins 20 ppb [43] indicated that 2 (13.3%) of chicken shawerma samples were exceeded this permissible limits while all examined smoked chicken breast ,chicken panne and grilled chicken samples were within these permissible limits .

These variations may be related to the amount of contaminated additives used in processing of such products, most of meat additive and spices used in Egypt in meat processing factory imported by shipping which provide suitable condition for mould growth and production of aflatoxins as: presence of oxygen, temperature between 4°C and 40°C, pH-value between 2.5 and 8 (with an optimum between5 and 8), minimum water activity of 0.80, maximum salt concentration of 14% [44]. Aflatoxins not only the cause of liver cancer, but they have additional important toxic effects. Chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and multiple micronutrients that are critical to health. The presence of aflatoxins in foods is a potential threat to the health of consumer. In developing nations, many people are exposed to aflatoxin through food and food do not routinely test for the presence of aflatoxin. As a result, an estimated 4.5 billion people living in developing countries may be chronically exposed to aflatoxin through their diet [45].

## CONCLUSION AND RECOMMENDATION

From this study, it could be concluded that the mould and yeast strains are potentially presented in the chicken products. The quantitative accumulation of moulds and yeasts in such chicken meat products varied according to the hygienic measures involved during processing, workers and/or equipment due to bad hygienic precautions. Addition of preservatives as food additives which known to be inhibitors for mould and yeast growth should be under control. Food additives must be stored in suitable conditions and examined periodically for mycotoxins production and fungal contamination.

Table (1): incidence and total mould count (cfu/g) from the examined chicken meat products samples (n= 15).

| Chicken meat products | examined Samples | | | Total mould count (cfu/g) | | | EOSQC |
|---|---|---|---|---|---|---|---|
| | No. | +ve | % | Min. | Max. | Mean(±SE) | |
| *Shawerma* | 15 | 12 | 80 | $0.5 \times 10$ | $1.8 \times 10^2$ | $3.4 \times 10 \pm 1.5 \times 10$ | Free |
| *Smoked chicken breast* | 15 | 8 | 53.3 | $4.5 \times 10$ | $2.0 \times 10^3$ | $4.2 \times 10 \pm 1.5 \times 10$ | Free |
| *Panne* | 15 | 15 | 100 | $0.5 \times 10$ | $2.3 \times 10$ | $1.4 \times 10 \pm 0.2 \times 10$ | Free |
| *Grilled Chicken* | 15 | 8 | 53.3 | > 10 | > 10 | $0.5 \times 10 \pm 0.2 \times 10$ | Free |

Table (2): Incidence and total yeast count (cfu/g) from the examined chicken meat products samples (N= 15).

| Chicken meat products | examined Samples | | | Total yeast count (cfu/g) | | | EOSQC |
|---|---|---|---|---|---|---|---|
| | No. | +ve | % | Min. | Max. | Mean(±SE) | |
| *Shawerma* | 15 | 0 | 0 | 0 | 0 | 0 | Free |
| *Smoked chicken breast* | 15 | 15 | 100 | $5 \times 10^2$ | $5.6 \times 10^3$ | $9.2 \times 10^3 \pm 7.8 \times 10^3$ | Free |
| *Pane* | 15 | 0 | 0 | 0 | 0 | 0 | Free |
| *Grilled Chicken* | 15 | 1 | 6.7 | > 10 | 0 | 0 | Free |

Table (3): incidence of isolated mould species from chicken meat product examined samples (N=15)

| Products / Isolated mould | *Shawerma* | | *Smoked chicken* | | *Panne* | | *Grilled Chicken* | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| *A. flavus* | 4 | 26.7 | 2 | 13.3 | 4 | 26.7 | 2 | 13.3 |
| *A. niger* | 3 | 20 | 1 | 6.7 | 0 | 0 | 0 | 0 |
| *A. niveus* | 0 | 0 | 0 | 0 | 2 | 13.3 | 0 | 0 |
| *A. ochraceus* | 4 | 26.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. ustus* | 1 | 6.7 | 1 | 6.7 | 0 | 0 | 0 | 0 |
| *P. candidus* | 0 | 0 | 4 | 26.7 | 0 | 0 | 0 | 0 |
| *P. citreonigenum* | 2 | 13.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| *P. concentricum* | 0 | 0 | 0 | 0 | 1 | 6.7 | 0 | 0 |
| *P. oxalicum* | 0 | 0 | 1 | 6.7 | 0 | 0 | 0 | 0 |
| *P.corylophilum* | 0 | 0 | 1 | 6.7 | 0 | 0 | 0 | 0 |
| *Acremonium strictum* | 2 | 13. | 0 | 0 | 2 | 13. | 0 | 0 |
| *Alternaria alternata* | 0 | 0 | 1 | 6.7 | 0 | 0 | 0 | 0 |
| *Aurobasidium pullulans* | 0 | 0 | 0 | 0 | 4 | 26. | 0 | 0 |
| *Cladosporium cladosporidiae* | 1 | 6.7 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Eupenicillium* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6.7 |
| *Eurotium repens* | 0 | 0 | 0 | 0 | 1 | 6.7 | 0 | 0 |

N = number of examined samples

Table (4): incidence of yeast species isolated from chicken meat product examined samples (N=15).

| Yeast species | *Shawerma* | | *Smoked chicken breast* | | *Pane* | | *Grilled Chicken* | |
|---|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | No. | % |
| Candida species<br>*C. famata* | 0 | 0 | 5 | 33.3 | 0 | 0 | 0 | 0 |
| *C. krusei* | 0 | 0 | 4 | 26.7 | 0 | 0 | 0 | 0 |
| *C. guilliermondii* | 0 | 0 | 6 | 40.0 | 0 | 0 | 0 | 0 |
| *Rhodotorula mucilaginosa* | 0 | 0 | 9 | 60 | 0 | 0 | 0 | 0 |
| *Debaryomyces hansenii* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 6.7 |

Table (5) Average concentration of total aflatoxin residues µg/kg (ppb) in examined samples.

| *Chicken meat products* | No. of examined samples | +ve samples | | Total aflatoxin residues (µg/kg) | | | Accepted samples | |
|---|---|---|---|---|---|---|---|---|
| | | No. | % | Min. | Max. | Mean ±SD | No. | % |
| *Shawerma* | 15 | 15 | 100 | 3 | 40 | 1.5 x10 ± 0.2 x 10 | 13 | 86.7 |
| *Smoked chicken breast* | 15 | 15 | 100 | 0.7 | 6.8 | 3.2 ± 0.5 | 15 | 100 |
| *Pane* | 15 | 15 | 100 | 0.9 | 13 | 4.3± 0.9 | 15 | 100 |
| *Grilled chicken* | 15 | 15 | 100 | 8 | 20 | 1.2 x10 ± 0.3 | 15 | 100 |

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