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Microbiological Quality of Wheat Cultivated in Many Different Regions of Iraq

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Abstract

Microbiological quality (total plate count, yeast and molds, coliform, and *E. coli*) of wheat cultivated in seven provinces of Iraqi were determined and compared to Standard Quality of Iraq (IQS) and to the standardization of Food and Drug Administration (FDA). The samples were collected from fields, and some samples taken from near of a street (reachable by people) and far of a street as well as rural fields. The regulation has two limits, which are good and acceptable limits, and the samples within the acceptable limit should be two samples of five. The results showed that all analyzed samples were within the acceptable limit that approved by IQS and FDA. There was one field exceed IQS regulation and there were several fields exceed the FDA regulations related to the number of samples within the good limit. The main reason for exceeding was total plate count (APC) followed by yeast and molds. Enhancing the microbiological quality of wheat cultivated in Iraq should be truly considered to be within IQS and FDA regulations.

Keywords: Wheat, Microbiology, E. coli

محتوى الاحياء المجهرية للقمح المزروع في بعض المحافظات العراقية

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قسم السيطرة النوعية، الشركة العامة لتجارة الحبوب، وزارة التجارة، بغداد، العراق.

الخلاصة

تم قياس عدد الاحياء المجهرية (العدد الكلي للبكتريا، عدد الخمائر والاعفان، عدد بكتريا القولون، وعدد بكتيريا E. coli) للقمح المزروع في سبع محافظات عراقية وتم مقارنتها مع اعداد الاحياء المجهرية المقرة من قبل المواصفة القياسية العراقية the Standard Quality of Iraq (IQS) والمواصفة الدولية الموضوعة من قبل إدارة الغذاء والدواء الامريكية Food and Drug Administration (FDA). جمعت عينات سنابل الحنطة من حقول قريبة وبعيدة من حركة الناس وكذلك من حقول ريفية. ان المواصفة القياسية العراقية والمواصفة الامريكية اقرت حدين، أحد الحدين للكمية الجيدة والأخر للكمية المقبولة على ان يكون نموذجين كحد اعلى من أصل خمسة نماذج ضمن الحد المسموح والثلاثة الأخرى ضمن الحد الجيد. اظهرت النتائج ان كل العينات تحوي اعداد احياء مجهرية اقل من الحد المسموح به المقر من قبل IQS و FDA. كما أظهرت النتائج ان أحد الحقول كان اعلى من حدود IQS وعدد من الحقول كانت اعلى من الحدود المقرة من ال FDA فيما يخص عدد النماذج المسموح بها ضمن الحد الجيد. ان العدد الكلي للبكتريا اعتبر أكثر مسبب لارتفاع العينات عن الحد الجيد يليه عدد الخمائر والاعفان. تقليل العدد المايكروبي للحنطة المزروعة في

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العراق يجب ان يؤخذ بنظر الاعتبار من خلال السيطرة على المحتوى المايكروبي للحقول لجعل اعداد الاحياء المجهرية في الحنطة المزروعة ضمن المواصفة القياسية العراقية وال FDA.

Introduction

Cereal and cereal products are a substantial food resource for people around the world [1]. Wheat, rice, and maize are the most important crops in the world [2]. Wheat is the leading grain used for human consumption because of its nutrition value, easy harvesting, storing and transportation, and processing compared to other cereal crops. Wheat consists of approximate 12.6% protein, 59.2% starch, 1.6% fat, 1.9% ash, and 9% other carbohydrates on 14% moisture base [3]. Wheat and rice are the most important crops that serve as a staple food for most people in Iraq. Annually, Iraq government via Grain Board of Iraq (GBI) imported about 696.000 and 366.000 ton of rice and about 350.000 and 152.000 ton of wheat in 2015 and 2016 respectively in addition to domestic cultivated crops to distribute a monthly ration to Iraqi people (Data from GBI). Quality Control Department (QCD) in GBI is responsible for inspection imported crops of wheat and rice chemical, microbiological, physical, and rheological properties.

Microbial contamination affects most foodstuff, therefore food microbiological assessment is often a mandatory step in the food production chain [4]. Plants might be an important carrier for human pathogens, such as *Escherichia coli* and *Salmonella* spp. than previously thought [5]. Recently, tomatoes have been detected to be as a source of Salmonellosis; *Salmonella enterica* in tomato caused 12 outbreaks in the United State for period 1998 to 2008. The contamination could be originated to tomato fields and/or the packaging step [6]. Beuchat [7] mentioned that many outbreaks by *Escherichia coli* O157:H7 have been reported by raw vegetables, while such outbreaks were associated with ground beef historically. Therefore, plants can be pathogen contaminated from fields. Micro-organisms can growth on cereal grains and ultimate products that store under improper conditions [1]. Food microbiological is one of the safety assessment of cereal crops, which is determined routinely for all the imported amounts of wheat and rice by QCD. Conversely, for the domestic cultivated wheat, microbiological inspection has not been conducted by QCD during receiving wheat from farmers. Therefore, the aim of this survey is to detect the microbiological quality of wheat cultivated in different provinces of Iraq and compare with the IQS and FDA regulation. This survey is also focusing on wheat cultivated near people fields and far from people reaching.

Materials and Methods

Wheat cultivated in different fields of Iraqi provinces were collected and used. Violet red bile agar, peptone water, sabouraud dextrose agar (Oxoid Ltd, Wade, Basingstoke, Hants, RG24 8PW, UK), nutrient agar (MicroMedia Trading House, Torbagyi str. 134. Paty, Hungary), and tartaric acid (Fluka AG, CH-9470 Buchs, Switzerland) were purchased.

Study Area

The study area involved seven provinces of Iraq, which covered middle, east, and south of Iraq. Figure-1. illustrates all the provinces used to gather wheat spikes, which were Al Taji (north Baghdad), Wasit, Babel, Al Basrah, Al Najaf, Al Qadisiyah, and Al Muthanna.

Sample Collection

Wheat spikes were collected from different fields in different provinces of Iraq during harvest season (May and June) of 2017. Wheat spikes have been collected by using gloves to avoid contamination. Samples were taken from the first rows (reachable by people) and the other samples were taken from about 1km far from the beginning of the fields of Wasit, Al Basrah, and Al Qadisiyah. Other fields were used in Al Qadisiyah, Al Najaf, and Al Taji (north Baghdad) that were in a rural area. Another field in Al Najaf was close to a street (≈30m). In Babel and Al Muthanna, one field was used for each province to collect wheat spikes that were closed to a highway, and samples were collected from first rows. All collected samples were triplicate for each position.

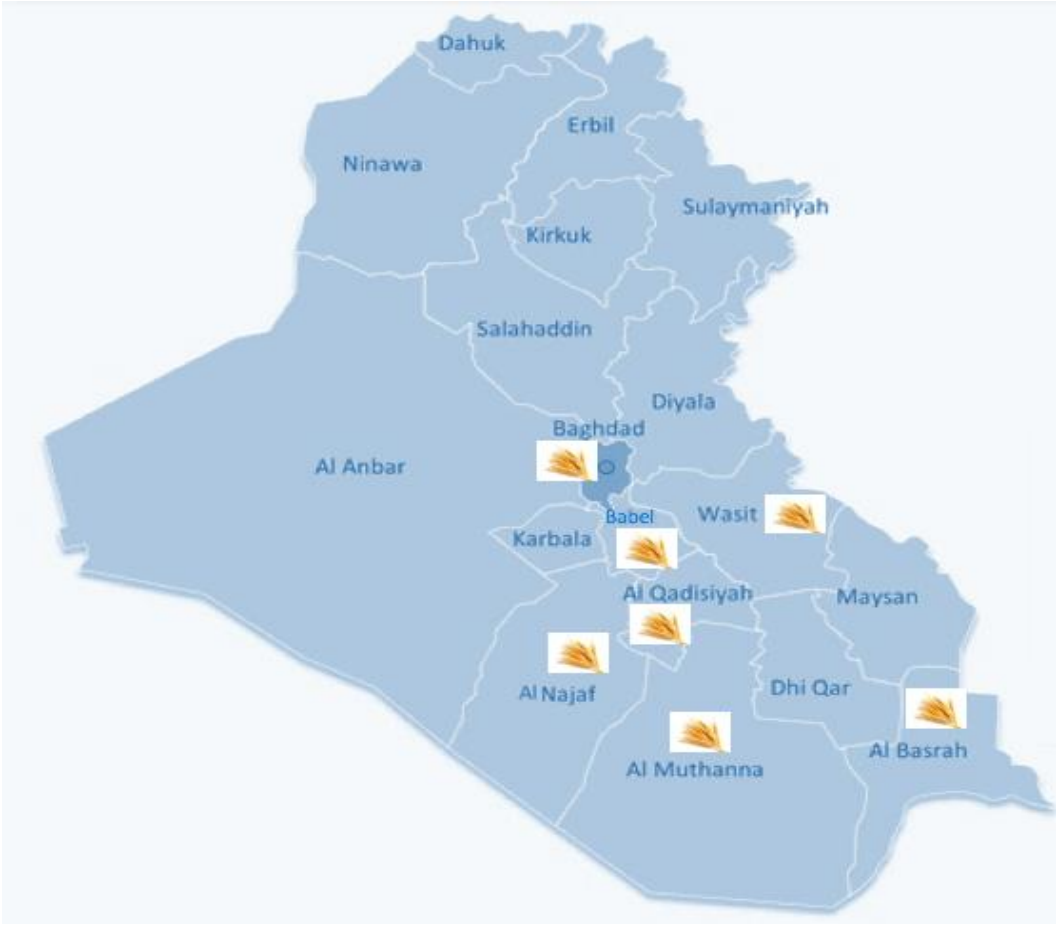

Figure 1-Provinces of Iraq used to collect wheat spikes marked with spikes photo.

Microbiological Analyses

Wheat spikes were peeled manually by crashing spikes in a sterilized atmosphere. Ten grams of wheat samples were placed in 90 mL sterilized peptone water (15 g/L) to make first dilution and further dilutions were done by taking 1 mL from the first dilution to 9 mL of peptone water.

Nutrient agar, ready to use powder medium, was used to APC, the powder medium was prepared based on the manufacturer's instructions, and the pH was adjusted to 7.2 ± 0.2. Then the dissolved mixture was sterilized by an autoclave at 121 ℃ for 15 min. Plate pour method was used from the second dilution for APC.

Sabouraud dextrose agar was used for yeast and molds count. The powder medium was added to distilled water, mixed, and autoclaved at 121 ℃ for min 15 min. Then the pH was adjusted to 3.5 by sterilized tartaric acid (10%) before pouring to Petri dishes. Plate pour method was used to molds and yeast count started from the first dilution.

Violet red bile agar was used to count coliform and *E. coli* bacteria. The powder medium was prepared according to the manufacturer's instructions, and the pH was adjusted to 7.4 ± 0.2. The powder medium was completely dissolved and bring to boil; no further sterilization was necessary. Coliforms were counted after incubated at 37 ℃ for 24 h, and *E. coli* was counted after incubated at 44 ℃ for 24 h; the first dilution was used [8]. All microbiological analyses were made at Microbiological laboratory, QCD, GBI.

Results

Total Bacterial Count

Total bacterial count of all wheat samples is presented in Table-1. All samples had a lower count than the accepted quality limitation of wheat that approved by FDA. Some samples cultivated from near street had a higher range of total bacterial count than samples taken from far of a street, for example, Wasit, the first field, Al Qadisiyah, and Al Basrah. However, there were no differences between samples taken from near and far of a street for the second field of Wasit. The number of

samples has total bacterial count more than the good quality count was presented in Table -2, and there were many fields exceed the limit that was approved by FDA, which were the first field in Wasit, the first field in Al Qadisiyah, and the two fields in Al Najaf. Some other fields had high possibility to exceed the FDA limit, such as rural field in Al Qadisiyah, Babel, Baghdad, and Al Muthana fields.

Yeast and Molds

Yeast and molds count of wheat samples were mentioned in Table-1. All samples had lower yeast and molds count than the accepted quality that approved by IQS and FDA. In most fields, far off-street samples had higher than near of street samples (Table-1). The number of samples had yeast and molds higher than the good quality count to a total number of samples taken from a field were presented in Table-2. The only field had a number of samples (5 of 6) higher than the limit approved by IQS and FDA was in Wasit (first field). All other samples were within the limitation except Babel and Al Qadisiyah fields, which were might have higher than the limitation (2 of 3 samples).

Coliform and *E. coli*

The range of coliform and *E. coli* count of wheat samples were presented in Table-1. All the count range of coliform was within the acceptable quality level, while all the count of *E. coli* was less than the limit of the good quality count. Coliform count of far street samples was mostly higher than far street samples of the same field Table-1.

Table 1-Microbiological range (cfu/g) of wheat cultivated in different fields of different provinces of Iraq.

Province	Wheat Fields	Total Plate Count	Yeast and Molds	Coliform	*E. coli*
Wasit, Field1	Near of a Street	480-17000	100-500	<10	<10
	Far of a Street	100-3900	600-1700	<10-900	<10
Field 2	Near of a Street	<100-100	<10-100	10	<10
	Far of a Street	100-100	<10-10	<10-100	<10
	Near of a Street	100-2500	10-20	<10-10	<10
Al Qadisiyah	Far of a Street	100-1600	<10-370	<10-10	<10
	Rural Area	100-4900	400-1200	<10-100	<10
Al Basrah	Near of a Street	200-200	<10	<10	<10
	Far of a Street	<100-100	<10 - 370	<10-200	<10
Al Najaf	Near of a Street	180-2000	<10-60	<10-10	<10
	Rural	200-140000	<10-110	<10-100	<10
Babel	Near of a Street	200-200	200-600	100	<10
Baghdad	Near of a Street	<100-700	<10	<10-100	<10
Al Muthana	Near of a Street	100-120000	<10-10	<10-50	<10
FDA, Good and Accepted Q*		10^2 - 10^6	10^2 - 10^4	10^2 - 10^4	10^2 - 10^4
IQS, Good and Accepted Q**		-	10^2- 10^5	-	-

*FDA [9]

**IQS [10]

Table 2-Numbers of samples had more than the good quality count to a total number of samples taken from a field.

Province	Wheat Fields	Total Plate Count	Yeast and Molds	Coliform
Wasit	First Field	4 of 6*	5 of 6*	1 of 6
	First Field	0 of 4	0 of 6	0 of 6
Al Qadisiyah	First Filed	4 of 6*	0 of 6	0 of 6
	Rural Field	2 of 3**	2 of 3**	0 of 3
Al Basrah		2 of 6	0 of 6	1 of 6
Al Najaf	First Field	3 of 3*	0 of 3	0 of 3
	Rural Field	3 of 3*	1 of 3	0 of 3
Babel		2 of 3**	2 of 3**	0 of 3
Baghdad		2 of 3**	0 of 3	0 of 3
Al Muthana		2 of 3**	0 of 3	0 of 3
FDA Limitation		2 of 5	2 of 5	2 of 5
Iraq Limitation		-	2 of 5	-

*Fields higher than the maximum allowable number of marginally acceptable units.
**Fields with high possibility of having higher than the maximum allowable number of marginally acceptable units.

There were just two samples that had higher than the good quality limit, which was in Wasit, the first field, far of a street position (900 cfu/g) and in Al Basrah, far of a street position (200 cfu/g). *E. coli* had not been detected in all samples that implanted from the first dilution. Table-2, shows that there was no field having higher than the maximum allowable number of marginally acceptable unit that related with the coliform count.

Discussions

Numerous microorganisms are resident in the intestinal tract of human in a complex ecosystem. Microbiota causes various diseases either by direct infection or by biotransforming many of ingested or endogenously formed compounds to harmful compounds that causes toxicity, carcinogenesis, and aging [11]. Molds can cause human illness via three specific mechanisms, which are a negatively effect to immune system response, direct infection by organism, and toxic effect from their byproduct [12]. Mycotoxins are the main harmful byproducts of molds that cause adverse effect of human, animal, and crops (economic losses) [13]. Therefore, microbiological limit of food is in important issue. Microbiological limit of wheat approved by FDA was mentioned that for each 5 samples, just two of them should be within the acceptable limit, which are 10^6, 10^4, 10^4 cfu/g, and 10^4 MPN/g for APC, yeast and molds, coliform, and *E. coli* respectively, and the other three samples should be within the good quality limits which were 10^2 cfu/g of all microbes mentioned above. Standard quality of Iraq was just determined for yeast and molds which is 10^2 cfu/g for good quality and 10^5 cfu/g for the acceptable quality. All fields marked with * in Table-2. Were higher than the limitation approved by FDA for human consumption. Total plate count was the main problem that caused a high number of samples exceed the limitation of good quality followed by yeast and molds count. For IQS, just the first field of Wasit had higher than the limitation. However, other fields such as Babel field and the rural field in Al Qadisiyah had high possibility to get higher than the limitation. In comparison with other studies, Eglezos [14] mentioned that the mean and maximum number of yeast and molds of wheat taken from two mills were $2.5 * 10^6$ cfu/g and $1.9 * 10^9$ respectively, which are higher than the yeast and molds of this study and the limitation approved by FDA. For *E. coli*, they did not find it for most samples, but the maximum number was 4 cfu/g. In this study, *E. coli* count was less than 10 cfu/g, which is the detection limit.

In the two different fields of Wasit, there was a big difference in the total bacterial count and yeast and molds count Table-1. The first field was higher than the limitation of FDA for total bacterial count, and yeast and mold count, and for yeast and molds of IQS Table-2. While the second field of Wasit had no sample higher than the good quality limit Table -2. Therefore, more studies are required

to determine the reason for this difference, in addition, to include more fields to confirm the results. As well as, the reason behind high total bacterial count, and yeast and molds should be investigated to enhance the microbiological quality of wheat cultivated in many different areas of Iraq.

Although during samples collections, there were some domestic animals, such as sheep around fields or cows eating from harvested fields, the amounts of coliform were mostly within the good quality limit and all *E. coli* count was within the good quality limit too.

Conclusion

The microbiological quality of wheat cultivated in Iraq is mostly within the IQS and mostly exceed the FDA regulations. The total bacterial count was the main reason to exceed FDA regulations, and molds and yeast count came second. Wheat from tested fields was safe in term of coliform and *E. coli*. Monitoring and enhancing field microbiology quality should be considered to enhance wheat microbiological quality.

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