MICROBIOLOGICAL INDICATOR OF MEDIUM FILTH *(NAJS MUTAWASSITAH)* IN READY TO EAT FOOD: TOWARDS STANDARDIZATION OF *TOYYIBAH* FOOD

BY

AININ AZWANI BINTI ABDUL RAFA

A thesis submitted in fulfilment of the requirement for the degree of Master of Health Sciences

Kulliyyah of Allied Health Sciences
International Islamic University Malaysia

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ABSTRACT

This study was conducted to evaluate the severity of medium filth contamination in ready to eat food (RTE) as to confirm the definition of *halal* food that supposedly not contaminated with *najs mutawassitah*. A total of 52 human stools samples were collected from voluntary healthy subjects according to method as explained by Chessbrough (1987) and the screening of bacteria in the human stools samples were done according to traditional microbiological analysis methods. Determinations of bacterial growth curves were performed using NanoDrop 1000 UV-VIS Spectrophotometer at 630nm where the initial and end of lag times for each of bacteria was determined. The growth evaluation of faecal borne bacteria in RTE food was performed using prepared fried rice samples. The prevalent study of food-borne/faecalborne bacteria was performed in 120 RTE fried rice collected from four different types of food premises in the town of Kuantan, Pahang. The results showed that healthy human stools which fall under *najs mutawassitah* contained high amounts of presumptive pathogenic bacteria specifically *E. coli*, *S. aureus*, *B. cereus*, *Aromonas* spp. and *Salmonella* spp. at different mean values. Total plate count (TPC), coliform and F. coliform were used as indicators in detecting the presence of pathogenic bacteria in human stools as well as for contamination of *najs mutawassitah.* Average lag phase time for faecal borne bacteria was around 60 minutes. Thus consumption of food within one hour should not give any significant health effects. Consuming food which contains faecal borne bacteria within one to two hours would give either low risk health effect or none at all. Consuming food after two hours has medium risk. Consuming food after three or four hours has the highest health risk. If the contamination of human stools in the food is in small quantity (1-2 drops), it may have no health risk at all. The small amount of bacteria in food may need more time to adapt with the new environment. If the human stools are in higher volume (more than 2 drops or about 1 ml) then it will start to contaminate the food and could then lead to health risks. If RTE food were contaminated with small amount (about 0.1 ml) of human stools and were left over at ambient temperature (about 37^{o}C) for a certain period of time (about 4 hours), it would start to have bacteria contamination and may cause health risks. If the level of health risk was translated according to *Shariah* law, RTE foods which were contaminated with higher amount (more than 2 drops) of human stools or contaminated with small amount (1-2 drops) of human stools and were left exposed at ambient temperature for more than 4 hours can be considered as *shubhah/makhrooh* to be eaten. The study also indicated that RTE fried rice sold at markets have medium to high health risks. Fresh or just cooked fried rice which are sold at night markets have less health risks compared to those that are sold at other type of food premises.

قصالخ البحث

وقد أجريتهذه الدراسةلتقييمشدةالتلوثالقذارةالمتوسطةفي الطعاماالمستعدلتناول (RTE) تأكيدتعريفالطعام الحلالالذي لم تلوث بالنجس المتواسطة.وقد تمّ جمعما مجموعه52عيناتبرازالإنسانمنالأصحاءالطوعيةوفقا لطريقة وكما أوضحها جزبروغ Chessbrough (1987)وتمّ القيام بفحص البكتيريا فيعيناتالبرازالإنسانوفقا لأساليب التحليل الميكروبيولوجيةالتقليدية.تم تنفيذقراراتمنمنحنيات النموالبكتيريةباستخدامعمل NanoDrop 1000UV-VIS الطيففي 630nm حيثتم تحديدالأوقاتالأولية ونهاية التأخرلكل منالبكتيريا.وتم إجراءتقييمنمو البكتيرياالبرازيةتنقلهافي المواد RTE باستخدامعيناتالأرز المقلي .وقد أجريتالدراسةالسائدةالتي تنقلها الأغذية/البكتيريافي RTE120 عينات من الأرز المقلياليتي تم جمعهامنأربعة أنواع مختلفة منأماكنالطعامفي مدينةكوانتان، باهانج.وأظهرتالنتائج أنبرازالإنساناليسليمالتي تندرج تحتبالنجس المتواسطةتحتوى على كميات كبيرةمن البكتيرياالمسببة للأمراضالظنيوعلى وجه التحديد *E. coli*, *S. aureus*, *B. cereus*, *Aromonas* spp. and *Salmonella* spp.في قيممتوسطمختلفة.واستخدمتالعد الكلي من اللوحة (TPC) ،القولونيةوبكتريا القولون البرازية كمؤشراتفيالكشف عن وجودالبكتيرياالمسببة للأمراضفيبرازالإنسانفضلا عنتلوثبالنجس المتواسطة.وكانالوقت المتوسط للبكتيرياتنقلهاالبرازحوالي60 دقيقة.وبالتالي, فاستهلاكالغذاءخلال ساعة واحدةيجب أن لاتعطيأي آثارصحية كبيرة .استهلاك المواد الغذائيةالتي تحتوي علىالبكتيرياالتي تنتقل عن طريقالبرازداخل 2-1ساعاتتعطيإماالمخاطر المنخفضةفي الآثار الصحيةأو لا شيء علىالإطلاق.استهلاكالطعام بعدساعتينلديهامخاطر متوسطة .و استهلاكالمواد الغذائيةبعدثلاث أو أربع ساعاتلديها أعلىالمخاطر الصحية .وإذا كانتلوثالبرازالإنسان فيالغذاءبكمياتصغيرة (2-1قطرات), فإنه قد لا يكون لها أيمخاطر صحيةعلى الإطلاق .كمية صغيرة منالبكتيريافيالطعامقدتحتاج إلى مزيد منالوقت للتكيفمعالبيئة الجديدة .وإذا كانبرازالإنسانهيفيحجم مرتفع(أكثر من2قطراتأوحوالي 1مل)بعد ذلك سوفتبدأ فيتلوثالطعاموبمكن بعد ذلك أن يؤدي إلىمخاطر صحية .وإذاتلوثت RTE الطعاممكمية صغيرة(حوالي 1،0مل) منبرازالإنسان وتركت عند درجة حرارة الغرفة(حوالي^{o}C 37) لفترة معينةمن الزمن(حوالي 4 ساعات)، فإنه يبدألتلوث البكتيرياويمكن أن يتسببالصحةالمخاطر.وإذاترجمتمستوى المخاطرالصحيةوفقا للقانونالشريعة، فالأطعمة RTE التيكانت ملوثةبكمية مرتفعة (أكثر من 2قطرات) منبرازالإنسانأ والملوثة معكمية صغيرة (2-1قطرات) منبرازالإنسان وتركت يتعرضفيحرارة الغرفةلأكثرمن 4 ساعاتفيمكن اعتبارتناولها مكروهاأو فيه الشبهة .وأشارت الدراسة إلىأن RTE الأرز المقلياليتي تباع فيالأسواقلديهاالمتوسطلمخاطر صحيةعالية .فالأرز المقلي المطبوخة بعد قليل فقطالتي تباعفيالأسواق الياليلد يهممخاطر صحيةأقلمقارنة بتلكالتي تباعفينوع آخر منأماكنالطعام

APPROVAL PAGE

I certify that I have read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Nutrition Science).

.....
Ibrahim Abu Bakar
Supervisor

.....
Norrakiah Abdullah Sani
Co-supervisor

I certify that I have read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Nutrition Science).

.....
Norazlanshah Hazali
Internal Examiner

.....
Norazmir Md Nor
External Examiner

This thesis was submitted to the Department of Nutrition Science and is accepted as a fulfilment of the requirement for the degree of Master of Health Sciences.

.....
Nor Azwani Mohd Shukri
Head,
Department of Nutrition Science

This thesis was submitted to the Kulliyyah of Allied Health Sciences and is accepted as a fulfilment of the requirement for the degree of Master of Health Sciences.

.....
Wan Azdie Mohd Abu Bakar
Dean,
Kulliyyah of Allied Health Sciences

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Ainin Azwani Binti Abdul Rafa

Signature …… Date ……..

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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Affirmed by Ainin Azwani Binti Abdul Rafa

……..……..…… ……..
Signature Date

To my beloved family, may they always be blessed by Allah S.W.T.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
BPA	Baird Parker Agar
BPW	Buffered Peptone Water
BSA	Bismuth Sulphite Agar
cfu	Colony Forming Unit
e.g.	(example gratia); for example
EMB	Eosine Methylene Blue
et al.	(et alia); and others
Fig	Figure
g	Gram
h	Hour
LTB	Lauryl Tryptone Broth
min	Minute
ml	Mililitre
MPN	Most Probable Number
nm	Nanometer
OD	Optical Density
P.B.U.H	Peace Be Upon Him
PCA	Plate Count Agar
PW	Peptone Water
RVS	Rappaport Vasiliadis Soy
S.D.	Standard Deviation
SC	Selenite Cystine
spp.	Species
S.W.T.	Subhanahu Wa Ta'ala
TSI	Triple Sugar Iron
TW	Tryptone Water
USA	United States of America
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate
µl	Microlitre

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

It is obligatory for Muslims to consume *halal* food and solely use *halal* products. *Halal* is a Quranic term which means allowed, permitted or lawful. This has been highlighted in the Qur'an (*al-Baqarah*) by Allah S.W.T. where it has been stated that:

> O you mankind! Eat of what is on earth, lawful and good; and do not follow the footsteps of the devil, for he is to you an avowed enemy (168).

In this verse, Allah S.W.T. asks all of mankind on whether they are a Muslim or not and to consume food which are lawful and good. This is because the food consumed by a person will influence their health and actions in their daily life.

The importance and significance of *Halal* food in Malaysia began in the year 1974 through the involvement of the Department of Islamic Development Malaysia (JAKIM) in confirming the status of *Halal* food products and goods. It was initiated when the research centre of the Islamic Affairs Division of the Prime Minister's Office (before the establishment of JAKIM) started to issue *halal* certification letters for products that met the *halal* criteria (JAKIM, 2011). Malaysia is among the few countries in the world whereby the government provides full support in promoting the *halal* certification process on food and consumer products and services. Another example of a country where *halal* certification scheme is directly under the control of the government is Brunei (Brunei *Halal*, 2012).

To ensure these aspects are not taken lightly, *halal* standards and Malaysian Standard (MS) has been published where it not only covers food and beverage products, but also refers to proper standards for consumer's goods, food premises and slaughter houses and to serve as a basic requirement for *halal* food product and food trade or business in Malaysia. *Halal* was defined by SIRIM (2009) as things or actions permitted by *Shariah* law without punishment imposed on the doer which involved production, preparation, handling and storage that are based on general guidelines of MS 1500:2009. With the strict implementations of *halal* certification in Malaysia, it provides assurance to all Muslim consumers.

The opposite of *halal* is *haram* or non-*halal* which means forbidden and prohibited. Any food or drink which lies within the grey area and does not fall clearly under the *halal* or non-*halal* category is classified as *shubhah*, alternatively known as questionable or dubious. In this category, until the status becomes clear, it is obligatory for Muslims to avoid consuming *shubhah* food or drinks. To the non-Muslims, *halal* products mean quality products where it is simply due to the concept of *halal*an *thoyyiban*. *Halal*an *thoyyiban* merely means allowed and permissible for consumption in relation to *Shariah* law as long as they are safe and not harmful. The food safety factor plays a significant role in determining the *thoyyiban* of the food based on aspects such as its safety, cleanliness, nutritional values and quality. The conditions of food to be acknowledged and categorized as *halalan thoyyiban* include that they do not contain or are exposed to any ingredients that are *najs* (filth or unclean) and are not prepared, processed or manufactured using equipments which are contaminated with things that, according to *Shariah* law, are *najs*. *Shariah* law is defined by Malaysia's law as the law of Islam based on the *Mazhab* of Shafie or the laws of Islam based on any of the *Mazhab* of Maliki, Hambali or Hanafi which are

approved by the Yang di-Pertuan Agong. These laws must be enforced in the Federal Territory or the Ruler of any State, which would then make it compulsory to be enforced in the state, based on *fatwa* approved by the Islamic Authority.

In Islamic teachings, *najs* have been classified into three types which are *muqaffafah*, *mutawassitah* and *mughallazah*. *Najs muqaffafah* or very light *najs* refers to urine of a male child below the age of two years old who dependent on only breast feeding. In regards to *najs mutawassitah*, there are regarded as a medium filth that fall into the second level which is after the severe *najs* (e.g.: pig and dog), and are sometime considered as light *najs* such as vomit, pus, blood, khamar, carrion, liquid and objects discharged from the orifices. *Najs mutawassitah* can include human faeces, which is one of the sources of food contamination. The sources of these *najs mutawassitah* may come from unclean food handlers or from unhygienic practices in preparing food. Moreover, *najs* may also be carried by animals or pests in the food premises. Even though *najs mutawassitah* are lighter when compared to *najs mughallazah,* they may originate from wider sources and are more difficult to be controlled through normal human senses when they are in small amounts.

The *najs* samples in this study were taken from human stools. Human stool contain normal flora and pathogenic bacteria such as *E.coli* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Campylobacter* spp., and *Aeromonas* spp. as well as intestinal parasites (Benschop et al., 2010). Detection and enumeration of these bacteria in food, that may be more than certain microbiological limits, may predict the probable contamination of food. The presence of the bacteria may originate from various factors such as the environment, human and animal waste. Therefore, it can be harmful to the human body and could lead to various food-borne diseases.

Food-borne diseases can be defined as a disease caused by consuming food which are contaminated with pathogenic bacterium or chemicals. The main sources of food-borne diseases are through bacterial contamination, followed by physical factors (preparing and handling methods) and chemical usage (Khir, 1998). In contrast, food and water borne diseases, namely cholera, typhoid, dysentery, viral hepatitis A and food poisoning, are several examples of communicable diseases. Examples of food poisoning symptoms include nausea, diarrhea and stomach cramps. The outbreaks are due to consumption of contaminated food and water that are related to environmental hygiene.

In Malaysia, the main contributing factor to food-borne diseases are identified as insanitary food handling procedures which accounted for more than 50 % of food poisoning cases (MOH, 2007). For instance, in January 2008, 30 incidents of food poisoning and a single food chemical intoxication were reported. As mentioned, most of the implicated food settings occurred in schools' and academic institutions' food preparation premises due to inappropriate food handling practices, meals being prepared too early and were then kept at ambient temperature until served as well as other unhygienic practices which were deemed as causes for the food poisoning cases (Soon et al., 2010).

1.2 SIGNIFICANCE OF THE STUDY

As stated in the Quran, the consumption of *Halal* food is compulsory for all Muslims. Our beloved Prophet Muhammad (P.B.U.H) has stated:

> What is *halal* is clear and what is *haram* is clear. In between those two is a dubious area in which many people do not know about. So whoever distanced himself from it, he has acquitted himself (from blame) and those who fall into it, he has fallen into a state of *haram* (Narrated by Bukhari).

The underlying guidelines of *Halal* foods are that the food that are prepared and processed in a hygienic manner as well as being free from specific types of contamination.

Even though the foods are initially confirmed by JAKIM as *Halal* through its *Halal* logo, but before or during consumption, it might be contaminated by bacteria or *Najs Mutawassitah* due to poor food handling practices, cross contamination and so forth. In addition, the smell, taste and colour of *Najs* cannot be easily identified by human senses especially if the amount of *Najs* is relatively small. The problem is that there are no specific food microbiological measurements or standards used to indicate *Najs* contamination of *Halal* food in Malaysia, especially towards RTE food. Hence, this research was designed to evaluate the level of medium *Najs* contamination in RTE food as one of the sources of food contamination which can also be used as a reference in order to identify the source of food-borne diseases that could either originate from human faeces or not (Toh & Birchenough, 2000).

1.3 OBJECTIVE

1.3.1 General Objective

The main objective of this research project was to evaluate the severity of medium filth contamination in ready to eat food as to confirm the definition of *halal* food which is not contaminated with *najs mutawassitah.*

1.3.2 Specific Objectives

1. To detect and enumerate indicator and presumptive pathogenic bacteria in human faeces as it is one of the main *najs mutawassitah.*
2. To determine the growth rate of presumptive pathogenic bacteria from human faeces and classify the severity of their potential contamination in ready to eat food.
3. To evaluate the risk of *najs mutawassitah* contamination in ready to eat fried rice samples from different type of food premises.

CHAPTER TWO

LITERATURE REVIEW

2.1 *SHARIAH* LAW AND *HALAL* FOOD IN MALAYSIA

The life of Muslims is guided by *Shariah* law. In general, *Shariah* law is referred to the Islamic teaching as stated in the Quran, *Hadith, Ijma'* and *Qiyas*. In Malaysia, *Shariah* law is also referred to the Islamic school of thoughts of *Mazhab* Shafie or any one of the other acknowledged *Mazhab*s (Hanafi, Maliki or Hambali). Any new issue or amendment of certain provisions in *Shariah* law are discussed in detail by the majlis *fatwa* at both the national and state levels, where the decision will then be published in the form of a *fatwa* by the relevant Islamic authority at federal territory or state level (e-*Fatwa*, 2015).

Halal food is one of the important needs of Muslims. In Malaysia, all issues about *halal* food are also discussed in majlis *fatwa* at national and state level before being released as a *fatwa*. There is a specific guideline about *halal* food in Malaysia which is the Malaysian Standard (MS) 1500:2009. In the guideline, the word *halal* is explained as an Arabic word which means 'permitted' or 'lawful'. *Halal* activities are obligatory to every Muslim and are associated with things or actions permitted by *Shariah* law without punishment imposed on the doer (MS1500:2009). The opposite of *halal* is *haram* or non-*halal* which is also an Arabic word which means 'prohibited' or 'unlawful'. *Haram* activities are forbidden for every Muslim. Any food or drink which lies within the grey area and does not fall clearly under the *halal* or non-*halal*

category is classified as '*Shubhah*' alternatively called questionable or suspected. If one does not know the *Halal* or *Haram* status of a particular food or drink, they are considered as doubtful and should be avoided.

Halal food is clearly defined in MS1500:2009 as food permitted under the *Shariah* law and those that fulfils the following conditions:

1. Does not contain any parts or products of animals that are non-*Halal* to Muslims or products of animals which are not slaughtered according to *Shariah* law.
2. Does not contain any ingredients that are *najs* according to *Shariah* law.
3. Is safe and not harmful.
4. Is not prepared, processed with things that are *najs* according to *Shariah* law.
5. The food or its ingredients do not contain any human parts or its derivatives that are not permitted by *Shariah* law.
6. During its preparation, processing, packaging, storage or transportation, the food is physically separated from any other food that does not meet the requirements stated in items i, ii, iii, iv, or v or any other things that have been decreed as *najs* by *Shariah* law.

According to MS1500:2009, *najs* include animals or things that are themselves not permissible such as pig (khinzir) and dog and all their derivatives. It also includes *halal* food that is contaminated with things that are non-*halal*. *Halal* foods that come into direct contact with things that are non-*Halal* also are considered as *najs*. *Najs* also includes any liquid and object which are discharged from the orifice of human beings or animals such as urine, excrement, vomit, pus, sperm, and ova of pigs and dogs.

Carrion or *halal* animals that are not slaughtered according to *Shariah* law are also considered as *najs*.

Besides fulfilling the *Shariah* law, which is a must for Muslims, the food in Islam also covers the aspect of quality which is referred as the concept of *toyyibah* or *toyyiban*. It includes many aspects such as cleanliness and safety of consumption. *Toyyiban* also refers to the concept of wholesome and nutritious.

In Malaysia, Jabatan Kemajuan Islam Malaysia (JAKIM), which is a government body under the Prime Ministry Department, is responsible in establishing the *halal* logo and implementing *halal* certification scheme. They are responsible in issuing *halal* certificates for the local and export markets. They are also responsible in monitoring and enforcing the *halal* guidelines together with other government agencies such as the Ministry of Domestic Trade and Consumer Affair as well as the Ministry of Health Malaysia.

2.2 *NAJS* CONTAMINATION

One of the conditions of *halal* and *toyyiban* food is that the food does not contain any ingredients that, according to *Shariah* law, are *najs*. Furthermore, the food must not be prepared and processed with things that are *najs*.

According to Islamic teachings, *najs* can be classified into three levels which are *najs mughallazah*, *najs mutawassitah* and *najs muqaffafah*. According to *Mazhab* Shafie, *Najs mughallazah*, which is considered as a severe *najs*, includes dogs and pigs, their descendants and derivatives. It also includes any liquid objects which are discharged from their orifices. Meanwhile, *najs mutawassitah* is the medium level of *najs* which includes blood, pus, vomit, faeces, urine, '*wadi*' (usually produce by those who are really exhausted), '*mazi*' (liquid that discharge from the sex organ due to high

‘*syahwat*’), carcass except fish and grasshoppers and all types of drinks or beverages that can cause intoxication (Ismail Kamus and Mohd Azrul, 2009). Lastly, *najs muqaffafah*, which is a light *najs*, refers only to urine of a male child who is below two years old and is only dependent on breast milk. Based on *Mazhab* Shafie, all types of solid which is the by-product of digestion, urine, placenta and blood are also considered as *najs*.

In general, *najs mutawassitah* is commonly referred to faeces. According to The American Heritage Dictionary of the English Language (2011), faeces mean waste matter eliminated from the bowels. Human faeces are the biggest concern due to the fact that anything which infects one human could easily infect another human being.

The main pathogens that are commonly found in faeces include *Bacteroides* spp., *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, *Aeromonas* , *Candida*, *E.coli* 0157 and, if blood is visible in the stool sample, *Cryptosporidium* and *Entamoeba histolytica* detection are possible. Stool contains intestinal bacteria and exfoliated epithelial cells that may provide useful information concerning gastrointestinal tract health. Stool sample analysis offers a non-invasive opportunity to evaluate both luminal exposures to different types of bacteria as well as exfoliated epithelial cell markers for colorectal cancer risk.

Based on a study conducted among 312 Swiss children with acute diarrheal illness, the pathogens in stool samples that were detected included Rotavirus, *Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *Cryptosporidium* spp., diarrheagenic *E. coli*, *Campylobacter* spp., *Giardia lamblia* and *Yersinia* spp. (Essers et al., 2000). Human stool comprises of various types of pathogens. This fact is supported by a research conducted by Stephen and Cummings (1980) where microscopic counts of

the bacteria of the stool showed that the microbial fraction contained 95 % of total bacteria. Diarrhea has potential to spread as the spreading of the infection may occur through handling, processing and consumption of contaminated water and food or by direct contact with infected person (Vandamme et al., 1992).

2.3 FOOD-BORNE MICROORGANISMS

Microorganisms in food comprise of bacteria, fungi, protozoa and virus. Bacteria and virus are too small to be seen with the naked eye (Adam and Moss, 2000). Bacteria are unicellular organisms measuring about 1 micron in length. They are found just about everywhere in nature including in soil, water, air and also in the intestinal tract and mucous membranes of animals and humans. They can be divided into gram-positive and gram-negative cells, according to whether they can retain crystal violet in the cell membrane during a staining procedure known as gram staining. Gram-negative bacteria have a thin cell wall and an outer membrane, while gram-positive bacteria have a thick cell wall and no outer membrane. Some bacteria are able to develop spores by coating their membranes and cell walls with extra layers of material during the sporulation process.

According to Hudler (2000), fungi are more complex than bacteria. They comprise of two types of microorganisms, which are molds and yeasts. Molds can be either unicellular or multicellular and can be found in decaying organic matter such as mycelium and candida. Some molds produce toxins, antibiotics and even enzymes that are useful in food production. The other type of fungi is yeast which is unicellular in structure. They can grow over a wide range of conditions.

Protozoa are single-celled eucaryotes that are classified by morphology, locomotion, and life cycle. The protozoa of interest to food scientists are parasites.