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Discovering Thoughts, Inventing Future

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The Place of Secretarial Education in Tertiary Institutions in Kaduna State, Nigeria, for Future Office 2020

By Dr. S. S. Amoor & Z. B. Magaji
Ahmadu Bello University, Nigeria

Abstract- A research conducted by the International Association of Administrative professionals reveals that the Future Office 2020 will be characterized with highly advanced office technology and information system that requires expertise and advanced technological skills. The administrative and technological advancement will transform the workplace and will place high premium on technical abilities and interpersonal skills of professional secretaries. Ironically, the more advanced office technology becomes, the more confidential secretaries' interpersonal skills, knowledge and competence are showcased. The paper examined the place of secretarial option of business education programme in tertiary institutions in Kaduna State for future office 2020. Two research questions were raised and two hypotheses were postulated for the study. The descriptive research design of the survey type was used for the study. A self-structured questionnaire was used to elicit responses from the respondents.

Keywords: *secretarial education and future office 2020.*

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The Place of Secretarial Education in Tertiary Institutions in Kaduna State, Nigeria, for Future Office 2020

Dr. S. S. Amoor α & Z. B. Magaji σ

Abstract- A research conducted by the International Association of Administrative professionals reveals that the Future Office 2020 will be characterized with highly advanced office technology and information system that requires expertise and advanced technological skills. The administrative and technological advancement will transform the workplace and will place high premium on technical abilities and interpersonal skills of professional secretaries. Ironically, the more advanced office technology becomes, the more confidential secretaries' interpersonal skills, knowledge and competence are showcased. The paper examined the place of secretarial option of business education programme in tertiary institutions in Kaduna State for future office 2020. Two research questions were raised and two hypotheses were postulated for the study. The descriptive research design of the survey type was used for the study. A self-structured questionnaire was used to elicit responses from the respondents. Section A dealt with the bio-data of the respondents while section B dealt with questionnaire items on the availability and usage of mobile-note-taking device and web based conferencing for teaching and learning in the secretarial option of business education programme of tertiary institutions in Kaduna State. The reliability of the instrument was ensured through the test and re-test reliability method and reliability co-efficient of 0.78 was obtained. Data collected were analyzed using Pearson Product Moment Correlation (PPMC). Findings revealed that there was significant relationship between mobile-note taking device and web-conferencing services and secretarial option of business education programme in tertiary institutions in Kaduna State. Based on these findings, the paper concludes that secretarial education has to provide adequately up-to-date knowledge, attitudes, skills and competencies to students in modern office administration, technology and information systems to meet the demands of the ever-advancing office administration, technology and information systems in future office 2020. Among the recommendations made are that the curriculum of secretarial education in tertiary institutions be reviewed to accommodate an in-depth study of information technology using modern office devices and services like mobile note taker, web conferencing service, lap-tops, I phones and I pads which is the vague internationally.

Keywords: *secretarial education and future office 2020.*

I. Introduction

In the 1980s, the traditional functions of confidential secretaries in office occupation were strictly answering of phone calls, filing, faxing, photocopying, typing of letters, memos, taking notes in shorthand, keeping of office imprest, running errands and preparing tea for the manager of executive. The educational training made available to would be secretaries at the secondary schools or commercial colleges then were courses like typewriting, shorthand, bookkeeping, office practice, English Language while the tertiary institutions offered courses like business management, economics, principles of accounts, communication skills, shorthand, typewriting, commerce, human relations (Aliyu, 2006).

In the 1999s, the International Association of Administrative Professionals, according to Fenner (2005) in a research finding, revealed that between 1999-2010, office occupation would experience an explosion of new office technology and information systems to improve productivity and enhance service delivery. This became a reality and indeed not only enhanced the functions of professional secretaries in office occupation but also elevated the status of secretarial professionals positively. Since 1999 to date, the rapid advancement of office technology and information systems, and its influx in industries, manufacturing companies, banking, educational and research institutes have in no small measure changed the structure, content and methodology of office occupation for secretarial administrators.

According to Amoor (2008), the functions of professional secretaries both in private and public sectors of the economy have gone far beyond their traditional orthodox duties. Today, professional secretaries in office occupation are charged with the responsibilities of manipulating and managing databases, creating presentations and reports using suitable computer software and digital graphics. Professional secretaries also use computer to generate, process, store, retrieve, handle and disseminate information to staff and clients as well as handle administrative responsibilities with little or no supervision. In most of the organizations, professional secretaries are called information managers. The education and training made available for these professionals in tertiary institutions to enable them handle multi-task, business communications and participate in executive decision-making processes especially in today's office occupation is far inadequate.

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Akinyemi (2001), Okoli (2006) and Amoor (2010) posit that office technology has developed very rapidly but schools and training institutions have not yet matched it up by giving the type of training required for the automated office. The education and training for these professional in Nigerian tertiary institutions include courses such as shorthand, computer appreciation, organization and management, accounting, business law, economics, communication skills, office procedure, business statistics. In some tertiary institutions, in addition to the list above, also offer computer appreciation. These courses, Amoor (2010) further stresses do not adequately expose students to the knowledge of office administration, office technology and information systems. In his views, some tertiary institutions do not teach a single computer application package to their students. In view of this, most students graduate from tertiary institutions without knowing how to operate a single computer application package. This trend poses a lot of challenges to secretarial graduates in the labour market hence their lack of skills and competences in the operation of office technology and information systems today. The minimum computer application packages needed in the labour market for secretarial graduates are Microsoft Word, Microsoft Excel and Power Point. In addition to these, the secretarial graduates are expected to handle busy and upwardly mobile executive calendar. Ironically, most of the tertiary institutions do not expose their secretarial graduates to these packages, (Amoor 2009).

II. Concept of Secretarial Education

Secretarial education is a component of vocational education that provides knowledge and skills needed to perform efficiently and effectively in the world of work. It involves acquisition of skills, knowledge and competencies and makes the recipient proficient in secretarial profession. Secretarial education prepares the secretarial students for performing all roles of the secretary, the accountant and office manager. Aliyu (2006) opines that business education (secretarial inclusive) is an education offered in Colleges of education, Polytechnics and the Universities primarily to educate and train students to acquire knowledge, skills and competence to become professional secretarial educators and administrators.

Also, according to Okolo (2001) secretarial education provides students with adequate skills and information needed to function well in office occupation. In addition to the scholars' contributions, secretarial education provides adequate training and education in office administration, office technology and information systems to would be secretarial administrators to understand complex assignments and to play a major role in the general operations of a business office. It guides individuals for suitable placement in office to earn his living, improves personal qualities and builds attitudes that are necessary for adjustment to personal and employment situation.

In 2011, the International Association of Administrative Professionals (IAAP), in Http://www.iaap-hq.org retrieved on 20th May, 2014 posits that another research finding that the Future Office 2020 will be characterized with much more sophisticated office technology and information systems and more tasking administrative responsibilities for professional secretaries. This implies that secretarial education has a responsibility to adequately prepare the graduates to face the challenges of the future office 2020.

III. Future Office 2020

The research carried out by the Office Team sponsored by the International Association of Administrative Professionals (IAAP) (2005) raised alarm in connection with the new office administration, office technology and information systems in the next ten to fifteen years. Bill (2005) posits that research findings reveals that the office of the future 2020 will be increasingly mobile, with technology enabling employees to perform their jobs virtually anywhere. The Office of the Future 2020 study warns that greater control over where and how people work will not necessarily translate into more free time. The International Association of Administrative (IAAP) stresses that forty-two percent of executive polled said they believe employees will be working more hours in the next ten to fifteen years. Daine (2010) observes that technology will continue to re-shape the work-place, change how and where business will be conducted in the next ten to fifteen years. The trend identified in 1999 is a reality today, so also the projections for ten to fifteen years shall also be a reality.

The daunting tasks and competitiveness of future office makes it mandatory for secretarial education students to be current with the trends or ever-changing office administration, office technology and information systems so as to remain relevant in the world of work and business. International Association of Administrative Professional (2005) says that the future office will be increasingly mobile with the use of multifunctional wireless office technology and information systems such as mobile note-taker, web-based conference services and telecommuting, enabling office workers to perform their duties from virtually anywhere. The IAAP(2005) stresses that the future office will create new administrative roles for professional secretaries. These include human resource coordination, workflow control, information management and virtual-meeting organization. The miniature wireless devices, WiFi, Wimax and mobile technology will

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continue to allow workers to work outside of the office with greater ease.

Additionally, virtual environments and web-based conferencing services will provide off-site employees with real-time access to meetings, reducing the need to travel. Improved wireless connectivity will allow for an increasingly flexible workforce. The study according to Daine Domeyer (2010) revealed that 87% of executives surveyed believe that telecommuting will increase in the next 10-15 years. This enables employees to work where it is most convenient. These will include entrepreneurial duties, resource coordination, workflow control, virtual meetings organizations, internet research, desktop publishing, computer training for the junior staff and website maintenance.

IV. Mobile-Note-Taker

This is a portable device designed to store handwritten data so that it can be transferred to a computer at a later date. The concept consists of an electronic pen with real ink and a memory unit with a c monochrome LCD display and 2 MB memory that when positioned above the writing surface, records all strokes on its scrolling screen. The effect is twofold; while you are making real world written notes a virtual copy of every mark you make is being recorded. The mobile note taker can be plugged directly into a private computer, allowing handwritten notes to be made within Microsoft Office documents and e-mails.

The skills needed by the graduates of secretarial education from tertiary institutions will be analysis of information and exercising good judgment, establishing rapport and facilitating team building, selecting the best technical tools and using them effectively, identifying and adapting to the needs and work styles of others and participating in business discussions that produce positive results. This is a signal to secretarial education programme in Nigerian tertiary institutions to provide secretarial training and education to professional secretaries to understand the complex assignments and to play a major role in the general operations of business office; multi-taking, expert business communication and participating in executive decision-making processes.

V. Web-Conferencing Service

This refers to a service that allows conferencing events to be shared with remote locations. These are sometimes referred to as webinars or interactive conferences or online workshops. Ukunegbu (2003) posits that web-conference is the meetings that take place with people who are at physically different sites through the use of tele-communications technology. The web-conference is the service that is made possible by Internet technologies particularly on TCP/IP connections. The services allow real time point to point communications as well as multicast communications from one sender to many receivers. It also offers data streams of text-based messages, voice and video chat to be shared simultaneously across geographically dispersed locations. Applications for web conferencing include meetings, training events, lectures or short presentation from any computer.

VI. Statement of the Problem

Office occupation in 2020 according to International Association of Administrative Professional (IAAP) (2005) will be characterized with much more sophisticated office technology and information systems and more tasking administrative responsibilities for secretarial graduates that will opt to work in office occupation. In support of this, Bill (2005) opines that the office of the future 2020 will be increasingly mobile with technology enabling employees to perform their jobs virtually anywhere. Despite the alarm raised by these scholars, the researcher observed that tertiary institutions in Kaduna States that offer secretarial option of business education programme, at best, provide only computer appreciation to the students; some of the tertiary institutions offer computer appreciation and micro-soft word only. The education and training provided to the secretarial students in office management, technology and information systems in tertiary institutions today are far inadequate for secretarial education graduate to match up with the future office 2020. This problem forms the gap this study seeks to fill.

VII. Objectives of the Study

The general purpose of the study is to determine the place of secretarial option of business education programme for future office 2020. Specifically, the study intends to:

1. To find out the relationship between mobile-note-taking device and secretarial option of business education programme in tertiary institutions in Kaduna State.
2. Ascertain the relationship between web-based conferencing service and secretarial option of business education programme in tertiary institutions of Nigeria.

VIII. Hypotheses

1. There is no significant relationship between computer mobile-note-taking device and secretarial option of business education programme in tertiary institutions in Nigeria.
2. There is no significant relationship between web-based Conferencing and secretarial option of

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business education programme in tertiary institutions in Nigeria.

IX. Methodology

Descriptive survey design was adopted for this study. The population of the study consists of seven lecturers from the Department of Vocational and Technical Education, Ahmadu Bello University, Samaru, Zaria, twenty-eight lecturers from the Department of Business Education, Federal College of Education, Zaria and twenty-two lecturers from Gidan Waya College of Education, Kafanchan, Kaduna State. The entire population of fifty-seven lecturers was used for the study hence there was no sampling technique since the population was manageable. The instrument for data collection was a self-structured questionnaire for the purpose of the study titled "Electronic Paperless World" (EPW). Before the use of the instrument, it was validated by experts in the field . With its internal level of consistency of 0.78 from the pilot study, the instrument was found reliable for the purpose in which it was constructed.

Section A of the instrument was for the bio-data of the respondents while section B was made up of the 20 items of questionnaire approved for the study. On the rating of the instrument, the researcher used a 4-point rating scale with 4 points for strongly agree (SA), 3 points for agree (A), 2 points for Disagree (D) and 1 point for strongly disagree (SD). The questionnaire was personally administered by the researcher and 48 copies of the questionnaire were duly completed and returned.

X. Analysis of Data

The research questions were analyzed using weighted mean score. In the course of answering research question, "strongly agree' and "agree" were classified as "agree". Similarly, "strongly disagree" and "disagree" was classified as "disagree" A mean score of 2.5 and above was considered as an index for agree, while the weighted mean score of less than 2.5 was considered as disagree. The opinions of the students were coded, entered into the computer and analyzed. The SSPS statistical package was employed to run Pearson Product Moment Correlation (PPMC) to test all the null hypotheses at the significance level of 0.05

a) *Data Presentation And Analysis*

Data collected in respect of the research questions were summarized and presented in table 1 and 2 as follows:

Table 1 : Respondents' opinions on the relationship between mobile note-taking device and secretarial option of business education programme in tertiary institutions of learning

Option	Total Score	Mean Score	SD	Decision	Remarks
Agree	126	3.0			Mobile-note-taking device has
Disagree	41	1.0	0.66	3.0>2.5	significant relationship with secretarial
Total	167	4			option of business education programme in tertiary institutions of learning.

Source: Result from the field work

Table 1 shows that the respondents who agreed that mobile-note-taking device has significant relationship with secretarial education in tertiary institutions of learning scored 126 points representing 3.0 weighted mean while the respondents who had contrary views scored 41 points with weighted mean of 1.0, with mean deviation value of 0.69. From the analysis, the result revealed that the calculated weighted mean of 3.0 is greater than 2.5 (3.0 > 2.5). This signified that mobile-note-taking device has significant relationship with secretarial education in tertiary institutions of learning.

Table 2 : Respondents' opinions on the relationship between web-based conferencing and secretarial education in tertiary institutions of learning

Option	Total Score	Mean Score	SD	Decision	Remarks
Agree	138	3.7			Web-based tele-conferencing has
Disagree	10	0.3	0.66	3.7>2.5	significant relationship with secretarial
Total	148	4			option of business education programme in tertiary institutions of learning.

Source: Result from the field work

Table 2 shows that the respondents who agreed that web based conferencing has significant relationship with secretarial option of business education programme in tertiary institutions of learning

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scored 138 points representing 3.7 weighted mean while the respondents who had contrary views scored 10 points with weighted mean of 0.3, with mean deviation value of 0.69. From the analysis, the result revealed that the calculated weighted mean of 3.7 is greater than 2.5 (3.7 > 2.5). This signified that web-based tele-conferencing has significant relationship with secretarial option of business education in tertiary institutions of learning.

XI. Hypothesis One

Table 3 : PPMC test analysis of the responses of lecturers on relationship between mobile-note-taking device and secretarial option of business education programme in tertiary institutions of learning.

Model	N0	$\pm$	SD	DF	r-cal	r-crit	Decision
Sec. Education							
Mobile note taking device	57	3.04	0.80	56	2.89	1.96	There is significant relationship

(*Source: Result from Field Work*

Table 3, revealed that the calculated r-value of 2.89 is higher than the table value of 1.96, the null hypothesis of the study is rejected. This then signifies that there is a relationship between the mobile note taking device and secretarial option of business education in tertiary institutions of learning.

Table 4 : PPMC test analysis of the responses of lecturers on relationship between web- based tele conferencing and secretarial option of business education programme in tertiary institutions of learning

Model	N0	$\pm$	SD	DF	r-cal	r-crit	Decision
Sec. Education							
Web conferencing service	57	3.00	0.80	56	2.64	1.96	There is significant relationship

(*Source: Result from Field Work*

Table 4, revealed that the calculated r-value of 2.64 is higher than the table value of 1.96, the null hypothesis of the study is rejected. This then signifies that there is a relationship between the web-based tele-conferencing and secretarial option of business education in tertiary institutions of learning.

XII. Discussion of Findings

The study, in tables 1-4 reveal that computer mobile note taking device and web-based conferencing services have significant relationship with secretarial option of business education programme in tertiary institutions of learning. The study discovered that business education lecturers are not conversant with these advanced learning technologies; talk less of the secretarial students in tertiary institutions of learning in Kaduna State. These findings agree with Aliyu (2010) who asserts that business education teachers are not adequately exposed to ICT for teaching and learning, and recommended that it must be made mandatory for business teachers in tertiary institutions to possess a certificate in some application software, especially Microsoft office package. Amoor (2010) posits that secretarial students in business education in tertiary institutions were not adequately exposed to the modern office technology and information systems to keep pace with ever-advancing office technology and information systems.

XIII. Conclusion

In view of the findings, the researcher concluded that the skills and competencies provided for secretarial students of business education in Nigerian tertiary institutions in Kaduna State may soon become obsolete and useless if secretarial students are not adequately provided with an up to date knowledge, skills and competence in advance office technology and information systems so as to remain relevant in the future office 2020.

XIV. Recommendation

Based on both current and future trends in office administration, office technology and information systems, it has become necessary to restructure secretarial education programme in Nigerian tertiary institutions and broaden its scope to meet the ever-advancing office administration, office technology and information systems so as to produce competent secretarial administrators that will effectively and efficiently man the future office 2020 and withstand the competitiveness of the labour market. To ensure that

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secretarial graduates withstand the competitiveness of the labour market and also function well in office occupation in Future Office 2020, the curriculum of secretarial education in tertiary institutions needs to be restructured to include courses such as:

1. *Information and Technology.* This consists of Operating systems and Utilities, Information technology and major business functions, application software, input technology, Databases management systems, programming and application development, Communication and networking infrastructures, Information and Retrieval, Systems Analysis and Design.
2. *Communication:* These include: Foundation of communication, Technological communication, Organizational communication.
3. *International Business.* This consists: Foundations of International Business International Business Communication International management.
4. The model offices for practicals should be adequately equipped with devices and services such as mobile note taker, web conferencing services and host of the latest office technology and information systems for teaching and learning. The students should be made to know how to operate them while still in the school. This will subsequently enable them to be acquainted with these ones so that they will have no difficulties in understanding the enhanced version of the equipment in the Future Office 2020.

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An Investigation on the Causes of *Escherichia Coli* and Coliform Contamination of Cheddar Cheese and How to Reduce the Problem (A Case Study at Kefalos Cheese Products)

By Amanda Kwenda
Degree in Food Processing Technology, Zimbabwe

Abstract- During the past year (2013) at Kefalos Cheese Products, pathogenic microorganisms, *Escherichia coli (E.coli)* in particular have been increasingly included in various types of cheeses including Cheddar cheese. *E.coli* outbreaks associated with consumption of different varieties of dairy products have been reported in several countries. The aim of this study was to investigate on the causes of *E.coli* contamination on Cheddar cheese through the application of some Hazard Analysis Critical Control Points (HACCP) principles. Cheese samples were analyzed for *E.coli* and coliform bacteria after production during the validation stage as well as at the verification stage and the average *E.coli* and coliform counts were statistically analyzed using the T-test. Results showed that after the implementation of the corrective measures there was a decrease in *E.coli* and coliform count which was compared at 5% significance level. Results presented in this study also showed that the manufacturing of Cheddar cheese at high hygienic standards improves the reduction of *E.coli* and other coliform related bacterial contamination of the product though the problem is not entirely eliminated.

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Chapter One

I. Introduction

a) Background of the Study

Kefalos Cheese Products is a company that specializes in the production of dairy products (cheese, ice cream and yoghurt). The production process is labour intensive and human resources are extensively used from raw material acquisition, conversion of raw materials into final product, packaging and finally dispatch. Among the wide range of products produced at Kefalos Cheese, Cheddar cheese is one of the most popular product and due to its increased demand in the market it became one of the major income generator of the company.

According to Steffen et al (1993), Cheddar cheese is widely consumed in its natural state, while smaller quantities are further processed and consumed as pasteurized process cheese. The cheese is packaged in different forms such as blocks, cuts, slices or shreds to suit the needs of the customers. It is a good substrate for the growth of certain adaptive fungal species and other bacteria due to its low pH, elevated salt concentration and low water activity (Pitt and Hocking, 1997). Growth of microorganisms can occur on cheese during its ripening period or in the distribution chain under refrigerated storage and this can result in a safety and spoilage problem.

Numerous researchers (Robinson, 1995; Kosikowski and Mistry, 1997; Beresford *et al*, 2001) have reported bacterial and/or bacteriostatic effects on pathogenic bacteria in foods because of reduced moisture, low water activity, low pH as the result of organic acid production, salt, heat treatment, competing flora, biochemical metabolites, bacteriocins and ripening, either singly or as part of hurdle technology. Refrigeration alone cannot reduce the number of pathogens, as it has been proven that *Listeria monocytogenes* and other psychrotrophic pathogens are capable of growth at these temperatures. Therefore, other factors such as diligence with regard to good hygiene practices may be shown by the lack of pathogen growth in fermented dairy foods.

Buazzi *et al* (1992) stated that during the manufacture of semi-soft, hard and very hard cheese, the cheese is subjected to relatively long exposure to ideal incubation temperature for bacteria. For example, Cheddar and other related varieties are maintained at 31-39°C during manufacture and are formed or hooped at temperatures in the 32-37°C range. Many Cheddar – type cheeses are cured or aged at temperatures up to 15.6°C. Swiss cheese is held for 4-8 weeks at a temperature of 22.2-23.3°C to develop the characteristic eyes and flavour. If storage of Cheddar and Swiss cheese at room temperature had any inherent detrimental effect on safety of these cheeses, then neither would be safe to consume (Buazzi *et al*, 1992).

Pathogens such as *Escherichia coli* 0157:H7, *Salmonella, Listeria monocytogenes* and *Staphylococcus aureus* have been found in low as well as high moisture cheese as a result of poor pasteurization (Frye and Donnell, 2005). Post pasteurization contamination also plays a role in pathogen presence in cheese that is supposed to be fit

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for human consumption. However, the main objectives of this study were to investigate on the possible ways in which *E.coli* can contaminate cheddar cheese during its processing. Further investigation is to seek possible ways which can be implemented so that contamination of cheese by this pathogen is reduced.

b) Problem Statement

At the same time that the dairy industry is becoming more diverse, dairy companies find that they must thrive against competition and the demand for quality products from consumers. Cheddar cheese being one of the major cheeses produced at Kefalos Cheese Products, because of its increased demand on the market, concern has been raised on its microbial safety. In the quest to fulfill their consumer demand on product quality and maintain guarantee on product safety on health it is important to investigate the causes of microbial contamination of this cheese paying particular attention to *E.coli*/ coliform bacteria. Therefore investigation on this issue has to be conducted so that consumer safety is assured. It is also important to understand the effects of these bacteria on the product and on consumer health.

c) Aim

To investigate on the causes of *E.coli* and coliform contamination on cheddar cheese and how to eliminate the problem.

d) Research Objectives

- To investigate the causes of *E.coli* contamination on cheddar cheese
- To explore the development of risk reduction procedures and practices at both primary level (milk screening) and the cheese production level to improve the safety of the aged cheese
- To fully explore the impact of pasteurization of milk on the microbial ecology of the aged cheese. Does pasteurization increase the susceptibility of cheese to the growth of pathogens via post-processing contamination?

e) Research Questions

- What are the possible causes of *E.coli* contamination of cheddar cheese?
- What measures can be implemented to prevent contamination of our cheddar cheese by microorganisms?
- Do acid-adapted cultures of these microbial species show enhanced ability to persist in aged cheese by withstanding salt, water activity and mild heat conditions encountered during the cheese processing?

f) Hypothesis

H_0: There is no difference in the average E.coli count measured before and after cheese process improvement.

H_1: There is a difference in the average E.coli count measured after the cheese process improvement.

g) Delimitations

The project will mainly focus on the investigation of causes of *E.coli* contamination on Cheddar cheese and ways to reduce the problem.

h) Limitations

Time to travel to and from Kefalos.

i) Justification

At Kefalos Cheese Products, cheddar cheese is producing positive results on *E.coli* and coliform tests taken to verify its microbial load. These results are positive after production and sometimes during storage of this cheese. This has therefore raised concern on the consumer safety of the cheese thus the need to investigate on the causes of the problem which will then lead in coming up with ways which can be implemented so that this contamination by *E.coli* can be reduced.

The study is important to the following stakeholders to:

a) The Industry (Kefalos Cheese Products Pvt)

The study will try to produce a trend analysis on *E.coli* survival on this cheese during storage. The advantages to the industry for this investigation are a better control of microbial contamination during and after processing, improved product quality and confidence of safety assurance of product to consumers.

b) Harare Institute of Technology

The study will provide reference material to the library for use by other scholars who wish to research on a related topic.

c) The researcher

The project will give the researcher an ability to test real life industrial problems and find solutions thereby improving information gathering and problem solving skills. It also will give the researcher a full understanding on the art of cheese product and its microbial safety implications. Lastly the study is also carried out in partial fulfillment of the Bachelors of Technology (Hons) degree in Food Processing Technology and thus it will bridge the theoretical aspect of this degree with the practical industrial real world.

Chapter Two

II. Literature Review

a) Cheddar Cheese

Cheddar cheese is a popular cheese that is consumed the world over and it is widely traded traditionally. It originated in England in the town of Cheddar in the 16th century from where it spread to the whole world (Robinson, 1995). It has colour variations from pale to deep yellow while the flavour can be mild and creamy for the mild Cheddar to strong and biting for

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the mature Cheddar. It is also described as having a slightly nutty walnut flavour (Robinson, 1995).The difference in texture and flavour of Cheddar arise as a result of the length of the ripening period (Kosikowski and Mistry, 1997). Mild Cheddar can be sold at around 3-4months and it has a texture that is close and firm yet pliable and breaks down smoothly when small portions are kneaded between the fingers (Kosikowski and Mistry, 1997). Mature Cheddar is usually 12-24months old, it has an intense flavour and the texture of the cheese at this stage is harder (Robinson, 1995). Cheddar cheese is a rennet coagulated cheese. The production of Cheddar cheese involves the mixture of milk, rennet, microorganisms and salt. These go through the process of coagulation, whey removal, acid production, salt addition and lastly ripening (Beresford *et al*, 2001).

Cheddar cheese is good substrate for the growth of certain adaptive fungal species and other bacteria due to its low pH, elevated salt concentration and low water activity (Pitt and Hocking, 1997). Growth of microorganisms can occur on cheese during its ripening period or in the distribution chain under refrigerated storage and this can result in a safety and spoilage problem

i. *Cheddar Cheese Manufacture*

In general terms, cheese is a concentrated food made from fluid cow's milk. In the process of cheese making, the casein protein component of the milk is induced to coagulate. The network structure formed entraps the milk fat globules, but allows much of the water and soluble components (whey) to drain out. Typically, casein coagulation is induced by a combination of acid production by starter culture organisms and also the action of the enzyme rennet. After coagulation, cheese undergoes a number of steps to separate the whey from the curd, followed by an aging step of variable length. The aging step allows characteristic flavour development due to microbial and enzyme activity.

The manufacturing process entails the following steps:

1. *Standardization*- Milk is often standardized before cheese making to optimize the protein to fat ratio to make a good quality cheese with a high yield. For Cheddar cheese it is standardized to a butterfat of 3.5%.
2. *Pasteurization*- Depending on the desired cheese, the milk may be pasteurized or mildly heat-treated to reduce the number of spoilage organisms and improve the environment for the starter cultures to grow. Cheddar cheese is pasteurized at 72°C for 15seconds.
3. *Cooling*- Milk is cooled after pasteurization or heat treatment to 32°C to bring it to the inoculation temperature.
4. *Renneting*- Addition of rennet will serve to coagulate the milk protein and form curds. The vat must be mixed thoroughly after the addition of the rennet to ensure equal mixing. This will also help to dilute the rennet to make it easier to spread around. In some cases pure water is used when diluting the rennet, as any pH that not near 7 will rapidly decrease the effectiveness of the rennet, as will chlorine. Thus, water with impurities will result in a vastly decreased yield of cheese.
5. *Curd setting*- Once the rennet is added, the mixture must be allowed to set and form curds. The mixture is kept around 29°C to 31°C. Temperature is controlled by flowing warm water through the jacket of the vat. Setting time varies, and allowing a proper amount of time is vital. It takes anywhere from 30 to 40 minutes to set the curd. The most common way to determine when the curd is set is by inserting a flat blade at a 45° angle into the curd and raising it slowly. If the curd breaks cleanly leaving a glassy fracture, it is ready for cutting. This may also be tested using a viscometer.
6. *Cutting the curd*- The curd is cut into 0.63-to-1.59-centimetre (0.25 to 0.63 in) cubes using stainless steel wire knives. A smaller cube size means the cheese will be lower moisture, whereas a larger cube size will result in a high-moisture cheese. It is important that cutting time is minimized and that the cuts be clean. The best way to determine how efficient the cutting job was is to determine the fat content of the whey. The curds are handled gently after cutting to prevent fat and protein loss to the whey. The curd is prevented from sticking to the sides of the vat, but minimal agitation is desired. The curds are allowed to set again for 10 to 15 minutes. Fat and protein loss may affect the ability of the cheese to be considered Cheddar cheese.
7. *Cooking the curd*- The curd is cooked by adding hot water to the jacket of the vat (up to 39 °C). The curd is stirred constantly during this step to avoid uneven cooking or overcooking, and the cooking will only take 20–60 minutes. The whey's pH will be around 6.1 to 6.4 by the end of the cooking.
8. *Whey draining*- Whey is removed from the curds by allowing it to drain out of the vat. In general, a gate is present to prevent curds from escaping. When most of the whey is gone, the curds are raked to either side of the vat, allowing whey to drain down the middle of the two piles.
9. *Cheddaring*- Cheddaring is the unique step in making Cheddar cheese. It is a multi-step process that gives Cheddar its unique flavour. The curds are allowed to set until they reach a pH of about 6.4. "Loaves" of curds are cut about 15 centimetres (5.9 in) wide along each side of the vat. After ten

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minutes, the loaves are turned over and stacking begins. Every ten minutes when the loaves must be turned over, they are stacked. The first time, two loaves are stacked together. The next time the loaves are turned, two stacks of two are put together. When the stacks get large enough (in general, 4 high), stacking stops but the loaves are still turned every ten minutes. This process is complete when the acidity of the whey is between 5.1 and 5.3, so it is checked constantly.

10. *Milling the curd*- When the turning process is complete, the loaves must be cut down into a size that fits in the mill. The mill will cut the matted curd into about 1.3 centimetres (0.51 in) pieces. During this process, the milled curds are constantly stirred to avoid re-matting.
11. *Salting*- When all of the curd is milled, salt must be added. The amount of salt varies, but it will be between 1% and 3% by weight. The salt must be mixed thoroughly. Salt helps remove some of the whey from the cheese, which lowers moisture content, adds to the flavour of the cheese, and will also stop the cheese from becoming too acidic, which imparts a bitter taste.
12. *Pressing*- The curds are placed into moulds that will be used to press the curds and form the blocks of Cheddar. After this, the cheddar cheese will be aged.
13. *Aging*- In general, Mild Cheddar is aged only for one to two months, but mature Cheddar can age for a year or more, even past twelve years. Aging time depends on the type of Cheddar being made (Rehman et al, 2008; St-Gelais et al, 2009).

According to Ryser (1999), transformation of chalky, acid-tasting curd into ductile, full-flavoured cheese is accomplished during ripening through the action of milk enzymes, rennet and various organisms in the cheese, including those with starter culture. The bio chemical changes that occur during cheese ripening are complex and involve fermentation of the carbohydrate; hydrolysis of fats and proteins with subsequent decarboxylation, deamination, and/or hydrogenation; and production of carbonyls, nitrogenous compounds, fatty acids and sulphur compounds, all of which contribute to the overall body, texture and flavour of the final product. These inherent characteristics also create a hostile environment for pathogens (Ryser, 1999).

b) *Microorganisms in Cheese*

Microorganisms are an essential component of all natural cheese varieties and play important roles in both cheese manufacture and ripening. They can be divided into two groups; starters and secondary flora. The starter flora, *Lactococcus lactis, Streptococcus thermophilus, Lactobacillus helveticus* and *Lactobacillus delbrueekii* used either individually or in various combinations depending on the cheese variety and responsible for acid development during cheese production. Starters may be either blend of defined strains or as in the case of many cheeses manufactured by traditional methods, composed of undefined mixtures of strains which are either added at the beginning of manufacture or are naturally present in the cheese milk. During cheese ripening, the starter cultures, along with the secondary flora promote a complex series of biochemical reactions which are vital for proper development of both flavour and texture (Sutherland et al, 1994). The secondary flora is composed of complex mixtures of bacteria, yeast and moulds, and is generally associated with particular cheese varieties. In many cheese varieties, the action of the secondary flora contributes significantly to the specific characteristics of that particular variety. The secondary flora may be added in the form of defined cultures, but in many situations is composed of adventitious microorganisms gaining access to the cheese either from the ingredients or the environment. During cheese manufacture and ripening, complex interactions occur between individual components of the cheese flora. Environmental factors within the cheese also contribute to these interactions (Spahr and Url, 1994).

i. *Microbiological analysis*

Cheese is a potential habitat for microorganisms such as yeast, mould and bacteria species.

a. *Pathogenic Bacteria*

Pathogenic bacteria are not supposed to be present in any successfully manufactured cheese because of heat treatment and acidity of cheese. The occurrence of enterococci, coliform, salmonella species, clostridium species and bacillus species is a sign of re-contamination.

Pathogenic organisms in milk products like cheese have been a matter of public health concern since early days of dairy industry. Many diseases such as Tuberculosis, Brucellosis, Diphtheria, Scarlet fever and Gastroenteritis are transmitted via milk product. Milk products have frequently been implicated in transmission of human pathogen (Bryan, 1990). Contamination of dairy products by coliform is attributed to three reasons. Firstly, the wide distribution of coliform in nature makes them useful indicators of the extent of re-contamination after pasteurization and also the sanitary quality of the pasteurized product. Secondly, coliform themselves cause rapid spoilage of food held under conditions conducive to the microbial growth. Thirdly, some strains are bacterial enteric pathogen which may be disseminated by dairy products (Rutzun and Marth, 1999).Coliform bacteria include all the aerobic and facultative anaerobic gram negative non sporulating bacilli that produce acid and gas from fermentation of lactose.

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b. Escherichia coli

Davis, (2003) reported that *Escherichia coli* should not be present in a good and well prepared cheese as high acidity of the fermented product should restrict their survival. Slow acidity development can allow sufficient build up of *E.coli* to give a "taint" or "off-flavour" to this retail product which is a clear indication of gross contamination.

Until the late 1970s and early 1980s, the presence of low numbers of coliforms was taken lightly because coliforms were considered indicator organisms of contamination rather than the main cause of severe illness. It wasn't until 1982 that E. coli O157:H7 was first recognized as a pathogen that is a causative agent for haemorrhagiccolitis (bleeding in colon). Later it was recognized that E. coli O157:H7infection can lead to Haemolytic Uremic Syndrome (HUS), characterized by anaemia, urinary tract infection and kidney failure. A serious multistate E. coli O157:H7 outbreak in 1993 brought a total awareness to recognize this organ-ism as an important and life-threatening food borne pathogen (Davis, 2003).

c. Characteristics of the organism

Escherichia coli are gram negative bacteria which appear as a rod when viewed under a microscope. *E.coli* has the ability to produce shiga-like toxin which is identical to the one produced by the *Shigella* species and it is pathogenic in nature (Norholt, 1984). To cause sickness, this bacterium requires highly specific receptors on the cell surfaces of intestines so that they can attach to them and enter the cells. However, humans as well as cattle and swine possess these receptors thus they can possibly harbour this bacteria without any ill effects and with cattle shedding them in their faeces. As a result they gain their entrance into raw milk. Though pasteurization can inactivate this bacterium, it is a requirement to observe sanitary practices in a dairy industry since the raw milk can be easily contaminated. Fox (2000) reported that water can be a carrier of this bacterium so any wash water which is to be used should be chlorinated before used.

d. Related foods and outbreaks

Dairy products are possible vehicles of food borne *E.coli* as is drinking water as well as ground beef to mention but just a few. A large population in the United States has reported to have been infected by this bacterium through the consumption of contaminated food as well as water. An incident reported in a daily mail of the Cheese Market News in May (2013) confirmed that more than a dozen people in California were infected by *E.coli* after the consumption of contaminated Dutch-style Gouda cheese. This information clearly shows that consumption of E.coli contaminated cheese has some health effects to the consumers.

e. Presence and control in the processing environment

Since this pathogen may originate from numerous sources, it may be nearly impossible to prevent contamination in some areas of food processing facilities. Workers may transport this pathogen into the processing environment on their shoes from the outdoor environment. This could results in tracking the organism to many locations within the building. Foods entering the facility may already contain *E.coli*, and processing these ingredients may lead to contamination of processing surfaces and any food contacting these surfaces later may become contaminated. An example is raw milk coming into a dairy processing plant. Contamination by *E.coli* in raw milk may come from the soil, air, or animal (cow, goat) udders. After pasteurization, the milk should no longer harbour *E.coli*, but the handling of raw milk before pasteurization is very important for reducing the probability of contamination of different areas within the processing facility. When this organism is introduced to equipment and preparation surfaces in a processing facility, it may survive and multiply. This may lead to subsequent contamination of foods during and after processing procedures. There are several methods currently used in food processing plants to lower numbers and/or to determine if they inhibit *E.coli* (Reddy, 2013).

f. Regulatory status of E.coli

Due to the constant changing conditions in nature and the use of antibiotics, some bacterial strains are undergoing mutations and to some extent, are changed into pathogenic bacteria. Therefore, it may be recommended to use pasteurized milk for cheese manufacture though other Codes of federal Regulations give provision to manufacture cheese using unpasteurized milk. Another possible and safer way to reduce these organisms is the use of highly active bulk starter cultures and maintenance of low pH in cheese. In addition much care is required to monitor and eliminate such organisms and thus all possible prevention measures which reduce the occurrences of food related incidences of *E.coli* should be employed. Raw milk should be protected from contamination and proliferation of *E.coli* through the implementation of strict sanitary measures. These organisms may also be growth retarded by inoculating the milk with milk silo cultures which turn on the lacto peroxidase system of raw milk causing retardation in growth for both pathogenic and non-pathogenic gram negative bacteria (Reddy, 2013).

Strict attention should also be given on the sanitary practices done in cheese and dairy plants to prevent post-pasteurization contamination in pasteurized milk cheese. The safety of cheese however, does not depend only on the microbiological quality of its milk but also on a careful assessment of the cheese-making procedure. Therefore applying the Hazard

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Analysis Critical Control Points (HACCP) principles on the processing may lead to the production of the final product which can be consumed with confidence (Early, 1998). In addition, the salting, acidification and drying stages of the process also play a role in assuring the safety of the final product. HACCP is then the preventive system which is based on the application of technical and scientific principles to a food production process and it requires the food handler to have sufficient information on the food and its related procedure so that any food safety problem may be easily identified (Leedom, 2006).

Oliver *et al,* (2009) adds on to say that HACCP is not the only method in ensuring that safe food products are manufactured. The plan will be successful when other procedures are in place such as sanitation standard operating procedures (SSOP's) and by using good manufacturing practices (GMP's). Although the Minnesota Food Code does not require them, these programs are fundamental in the development of a successful HACCP plan. SSOP's should include personal hygiene practices as well as daily sanitation of the food contact surfaces and equipment. Good sanitation practices are the foundation of manufacturing and preparing safe food.

c) *Inherent characteristics of cheese*

Most of the cheeses are made from pasteurized or heat treated milk which will leave the product free from most pathogens. The use of starter cultures develops some inherent characteristics of cheese which provide hurdles that inhibit growth of pathogens (Ahmed *et al*, 1986). Besides pasteurization, there are other practices which have significant contribution to the microbiological safety of cheese. These may include milk quality, lactic culture protocols, pH control, salt addition and controlled curing conditions. Other factors may be natural inhibitory substances, starter metabolites and fermentation by-products, including organic acids. Water activity imposes additional detrimental effects on food borne pathogens during manufacturing and ripening of cheese (Buazzi *et al*, 1992).

Cheese is exposed to ideal temperatures favourable to bacteria for long periods during the manufacture of semi-soft and very hard cheeses. Taking for example Cheddar cheese which is maintained at 31-39°C during manufacture, hooped or formed at temperatures in 32-37°C range and cured or aged at temperatures up to 15.6°C (Moustafa *et al*, 1983).fermentation is a method used to inhibit the growth and survival of pathogenic bacteria with lactic acid bacteria commonly used to produce fermented dairy products. However, lactic acid bacteria are antagonistic to food borne pathogens and will neither inhibit nor inactivate them but responsible are the action of metabolites such as lactic acid, diacetyls and hydrogen peroxide which work in synergy on pathogens such as *Salmonella, Escherichia coli, Staphylococcus aureus and L. monocytogenes* (Schaak, 1994).

In most cheese varieties, salt concentrations attain levels of 1.6-3.0% of the total weight of the cheese, which would not affect most of the pathogenic bacteria in cheese. But it must be realized that salt is dissolved in the aqueous phase of the cheese only, the actual site of bacterial growth. Given the respective calculated values, salt concentrations in the aqueous phase reach levels of 2.2-6.55 or higher and will, in fact, at least slow down the growth rate of most bacteria and even have a detrimental effect on the more sensitive ones (Spahr,1994). Where scientific data do not exist, all the inherent characteristics of cheese serve as criteria in determining growth of pathogens by the use of mathematical modelling (Sutherland, 1994). When two or more of these criteria are combined, the resultant effect is an additional hurdle to the outgrowth of pathogens of concern. It is this effect that makes it possible to store certain cheeses safely beyond either one of the two food Code criteria for date marking and refrigeration (i.e., 7 days at 5°C or 4 days at 7.2°C).

Cheddar is a hard cheese that does not support *L. monocytogenes* growth and that causes gradual death at all temperatures (Gengeorgis, 1991). This finding is confirmed by an FDA correspondence (Buchanan, 1999) and also agrees with work by Ryser and Math (1987), who reported that growth of *L. monocytogenes* during Cheddar cheese manufacture appeared to be inhibited by proper acid development resulting from an active starter culture. Behavior of other pathogens during Cheddar manufacturing and ripening showed similar results. With normal starter activity, inoculated *Staphylococcus aureus* died rapidly (Ryser, 1987). Norholt (1984),illustrated die-off of *Salmonella* spp. after 2 weeks. Wood *et al*, (1984) found that, of 11 vats of *Salmonella*-contaminated Cheddar cheese curd, only 2 remained positive in the finished cheese immediately after manufacture. In 1 and 4 months, these 2 vats were clear of the inoculated *Salmonella*. This result is supported by studies of Goepfert *et al*. (1968) and Hargrove *et al,* (1969) in artificially inoculated Cheddar. Both groups found a 75-80% reduction in *Salmonella* after hooping and pressing during manufacture.

Numerous researchers have reported kill of pathogens at higher ripening and storage temperatures. *Salmonella spp*. survived longer when Cheddar cheese was stored at 4.5°C rather than 10°C (White, 1996). In general, a low pH and a high ripening temperature result in a higher inactivation rate for pathogenic organisms (Ryser, 1987). Using stirred-curd cheddar cheese, Goepfert et al (1968) showed that the number of *S.Typhimurium* decreased by a factor of 10 000 during 10-12 weeks of ripening at 13°C, whereas a similar decrease required 14-16 weeks at 7.5°C. Park et al. (1970) reported that salmonellae survived during

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ripening of Cheddar cheese for up to 7 months at 13°C and 10 months at 7°C. Ryser and Math (1987) reported an inactivation rate of L. monocytogenes 0.9logs less at 6°C than at 13°C. International Dairy Federation researchers demonstrated that the decrease in numbers of staphylococci in Cheddar was greater at higher temperatures (10°C and 13°C) than at 7°C (IDF, 1980).

d) Factors influencing growth of microorganisms in cheese

i. *Moisture*

Since microorganisms require water for growth, they can be controlled by reducing this water availability. This can be achieved by dehydration or through the addition of some water soluble component for example salt. Therefore in a cheese sample, high moisture content will lead to increased susceptibility to spoilage. During the initial stages of the cheese manufacture, the moisture content is high which is able to promote growth of starter cultures. As the process proceeds, there is whey drainages and salting which cause a significant decrease in water activity which then suppresses the growth of bacteria (Cogan, 2000).

ii. *Salt*

Salt and water activity are interrelated and thus addition of salt will result in the decrease of water activity which has an inhibition effect on starter and spoilage bacteria. However, the required concentration then depends on the nature of the food, pH and moisture content. Salt concentration in cheese usually ranges between 0.7 to 7g/100g which corresponds to a water activity of 0.99-0.95 to which many microorganisms are able to grow. This implies that growth inhibition of microorganisms is also contributed by other factors ((Ruegg & Blanc, 1981).

iii. *pH and organic acids*

Most bacteria commonly have their optimum growth pH around neutral with poor growth observed at pH around 5.0. During the post-manufacture stage, cheese curd has pH ranging from 4.5 and 5.3 which comes as result of increase in organic acid levels. The low pH values will cause an inhibition to growth of microorganisms especially those that are acid-sensitive (Steffen *et al*, 1993).

iv. *Redox potential*

As defined by Crow et al, (1995), redox potential (Eh) is a measure of the ability of a chemical/biochemical system to oxidise (lose electrons) or reduce (gain electrons). The Eh of cheese may be related to the fermentation of lactic acid by the starter during growth where it is thought that small amounts of oxygen are reduced from the milk to water. This creates an anaerobic system in the cheese interior which can only support growth of obligatory or facultative anaerobic microbes. Therefore the type of microorganisms which can grow in cheese are determined by the cheese's Eh thus obligate aerobes for example Pseudomonas cannot survive these conditions but can however develop on the cheese surfaces (Brown and Emberger, 1980).

v. *Ripening temperature*

Mesophilic and thermophilic microorganisms are usually involved during the manufacturing and ripening of cheese. These have their optimum temperatures approximately 30°C or 42°C respectively. The temperatures become a compromise to the ripened cheese due to the need to promote ripening reactions and control growth of the desirable secondary flora at the same time trying to prevent the propagation of potential spoilage and pathogenic bacteria. Therefore higher temperatures will result in accelerated ripening but detrimental effects are obtained due to changes in body and flavour of the cheese (Folkertsma *et al*, 1996).

vi. *Nitrate*

Nitrate is added to the cheese milk in the form of saltpetre (KNO_3) or ($NaNO_3$) in the production of other cheeses including Cheddar. Nitrate has a growth inhibition effect on *Clostridium tyrobutyricium* which ferments lactate to butyrate, hydrogen and carbon dioxide. The formation of butyrate leads to the formation of off-flavours in cheese. Therefore, growth inhibition of such microorganisms by nitrate is important since the moisture content in cheese is high and the equalisation of salt concentration takes long thus failing to control *Clostridium tyrobutyricium* (Nieuwenhuff, 1977).

e) Hygienic control in dairy plant

A dairy industry may comply with the pure food and public health law by making the hygiene control of dairy equipment one of their primary objectives. This also helps to ensure that there is consistent daily turn-out of safe and clean products. A secondary objective may then be to preserve the keeping quality of dairy products and enhance consumer confidence in them which important commercially. To accomplish these objectives, the bacteriological standards of the raw products should be of good quality and should be protected against contamination from equipment with which it comes into contact with during stages of production. There are therefore various factors which influence the maintenance of the dairy equipment and these include buildings, the materials and construction of equipment as well as personnel, water supply, cleaning procedures and laboratory control (Rice, 1981).

Therefore, the building facilities should provide floors which are constructed with material impervious to water, smooth and effectively sloped along drains to allow quick movement of water thus preventing water floods in the plant. The walls should have and adequate height, smooth and cleanable so that they do not harbour any microorganisms. The smoothness and light-coloured factors should also be considered for the ceilings as well. Doors should be self-closing and

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windows and other external openings should have screens to prevent entrance of insect and other pests. Sufficient lighting either from natural or artificial light should be provided for though direct sunlight is not recommended because it causes spoilage of dairy products by oxidative effect of the ultraviolet rays. Air circulation should be maintained by adequately ventilating all rooms to prevent moisture condensation and accumulation of odours (McDowall, 1987).

Cleaning practices should be done to prevent odour accumulation in the dairy plant and prevent product contamination by equipment from any remnants of milk soil. Compliance to the hygiene standards may also be achieved by monitoring personnel hygiene behaviours. Each employee should have a role in the factory sanitary programme and should be given responsibility when no compliance is observed since carelessness at any stage may result in quality deterioration or endangering consumer health. Therefore legal warnings should be advised for negligence of hygiene practices. It is then recommended to train employees on hygiene practices which may seem to be an effective way to encourage good plant hygiene. Therefore education should be provided at all levels. Training will make staff appreciate and understand the scientific principles upon which the sanitary laws are based. In addition to training, printed instructions may serve as reminders for any desired cleaning or other hygiene procedures thus promoting the enforcement of good hygiene practices. Personnel are also required to wear clean washable clothing and they themselves should be of clean personal appearance and habits. They also should be diligent to wash their hands after visiting the toilet or whenever they become otherwise soiled (Rice, 1981; McDowall, 1987).

Chapter Three

III. Research Methodology

a) *Research Design*

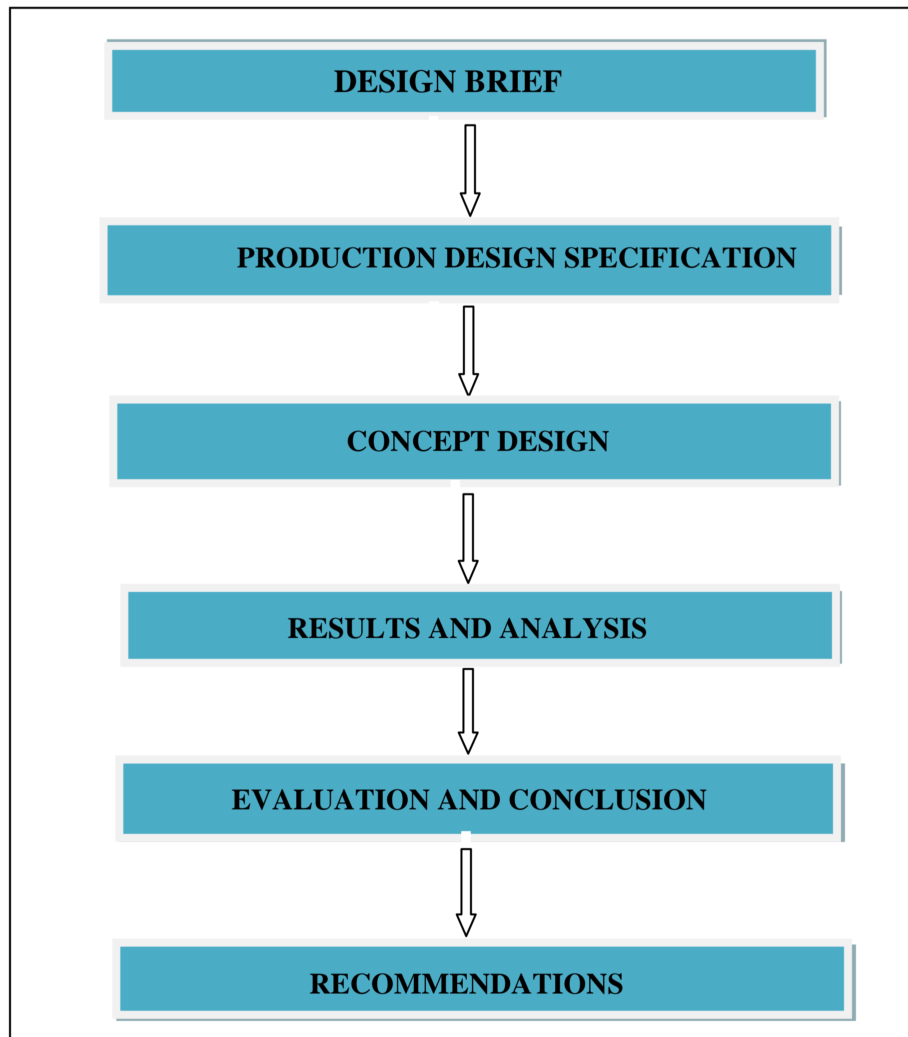

Figure 1 : The Design Model Diagram (Hertz, 2007)

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b) Design Brief/ Problem Statement

An investigation on the causes of *Escherichia coli* and coliform contamination on Cheddar cheese and how to reduce the problem.

c) Scope of Specifications

The Product Design Specifications for the Cheddar manufacturing was drawn from the following requirements: specific standards of the raw milk, the pasteurization and inoculation temperatures, and the desired product specifications after the pressing stage, cultures used and overall sensory characteristics expected. General cleanliness of the machine, materials, personnel and quality of water, quantity-scale of production, the way to manufacture and cleaning processes were also some of the requirements.

i. *Raw milk compositional analysis and requirements*

Cheese milk standards were collected from secondary sources. The information was tabulated in appendix 3.

Fat, protein, pH and lactose : Compositional test results were used to determine the quality of milk supplied by the producer. The tests for these parameters were done using a lactoscan. The determination of fat was also done using the Gerber test which is described in appendix 5.

Somatic cell count (SCC): A somatic cell counter was used to determine the results. This was done by the milk producers themselves.

Bacteria count : Traditional method of colony forming units (CFU) was used to determine total bacteria count. It took 2-3 days to determine bacteria count. The bacteria are an indication of milk hygiene.

Coliform and E.coli : For the microbiological tests of the sample, each sample was serially diluted using sterile maximum recovery diluent (MRD) as diluents and 1ml of 10^{-3} sample was plated in duplicate using the pour plate method on MacConkey agar and Plate count agar, the plates were incubated at 37°C for 24hrs. After incubation, the colonies that developed on the plates were counted and used to determine the total bacterial count and coliform count of the milk and cheese samples (CFU/ml) multiplied by the dilution factor. MRD was also used to determine Coliform and *E.coli* on swabs taken on production personnel as well as the equipment used.

Antibiotics : The antibiotic detection test was done on milk using the ßeta s.t.a.r test which is described in appendix 5.

Added water : A lactometer was used to determine if the milk contained any added water.

d) Process Audit for the Manufacture of Cheddar Cheese

Process audit for the manufacture of Cheddar cheese was evaluated to make sure all the parameters were being met.

i. *Prerequisite plan*

1) *Plant construction and equipment programme :* this component of the prerequisite plan addressed physical aspects of the primary production or food processing facility and their maintenance. It began with the facility's surroundings, as they have a bearing on such environmental issues as air quality and presence of pests and the general layout. Then it proceeded to design and materials of equipment and utensils- aspects that influence ease of cleaning and sanitation.
2) *Standard Operating Procedures (SOPs):* Standard Operating Procedures (SOPs) described how critical processing operations such as pasteurisation or cooling, instituted with the purpose of addressing a food-borne disease risk factor.
3) *Sanitary Standard Operating Procedures (SSOPs):* Sanitary Standard Operating Procedures (SSOPs) described how, with what and how frequently the facility and equipment must be cleaned and sanitized, and how the effectiveness of cleaning and sanitizing were ascertained.
4) *Personnel hygiene:* This aspect included the facility's policy regarding sick workers, the employees' general appearance of cleanliness, the clothing they wore and the policy regarding hand washing. It also included employees'facilities (toilets, hand washing stations, showers, locker rooms and eating areas)
5) *Supplier specifications and control :* Supplier specifications for all raw materials received by the primary operation or processing plant had to be available. The specifications addressed food-borne disease risk factors inherent to such materials. When relevant, evidence of supplier compliance with specifications, such as copies of certification and laboratory analyses, had to be at hand.

ii. *Microbiological analysis*

Samples were examined for coliform counts and *E.Coli*. MacConkey`s agar was used to determine coliform counts. The media was obtained in dehydrated forms and prepared according to the manufacturer's instructions. Glassware such as Petri-dishes, test tubes, pipettes, flasks and bottles were sterilized in a hot oven at 170°C for two hours, whereas all media was autoclaved for 15 min at 121°C.

Surface (spread) plate technique was done for yeast and mould counts. One millilitre from a homogenous sample was serially diluted into 9 ml ringer solution to prepare three fold dilutions from 10^{-1} to 10^{-3}.

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For coliform counts, one ml of each sample was transferred into a sterile duplicate plate and 15-20 ml of the selected media was added. Then the cultured plates were incubated at 37°C for 24 h and 25°C for 5 days for coliform.

iii. *Chemical analysis*

Total solids content was determined using a moisture analyser and verified using the Lactoscan. Protein content was also determined using a Lactoscan. Fat content was determined by Gerber method and the solids not fat were obtained by subtracting fat from total solids. The pH of cheese samples was determined using electronic pH meter.

iv. *Process validation*

The validation process focused on the collection and evaluation of scientific, technical and observational information to determine whether control measures were capable of achieving their specified purpose in hazard control.

a. *The process stages which were audited were as follows*:

- Pasteurisation (72-75°C/15sec) and efficiency
- Inoculation temperature (32°C)
- Inoculation
- Curd heating (39°C)
- Whey draining (pH6.4)
- Cheddaring (pH 5.2)

b. *Determination of pasteurization and inoculation temperatures, time and efficiency*

Pasteurization and inoculation temperatures and time were collected from secondary sources. This information was essential to evaluate the temperature and time combination which are essential for cheese milk in order to produce a quality product.

Phosphatase test: This test was used as a device to determine the efficiency of pasteurization. The test is also outlined in appendix 5.

Preparation of all the media used and procedures used in the analysis of raw milk are described in appendix 5.

v. *Monitoring of control measures*

Monitoring of control measures was an on-going collection of information at the steps the control measures were applied. The information established that the measures were functioning as intended i.e. within established limits.

vi. *Verification*

Verification was used to determine that the control measures had been implemented as intended.

vii. *Statistical analysis*

The results of the average coliform and *E.coli* counts on the cheddar cheese after production at validation and verification after the implementation of the corrective measures were analyzed using the T-test. Statistical analysis was performed using the SPSS version9.

Chapter Four

IV. Reults and Analysis

a) *Raw Milk*

i. *Compositional analysis*

Three milk samples from different producers were taken, these were analysed for compositional analysis using the Lactoscan. Tested were lactose, protein and fat. The samples were warmed to 30°C in a vial before being tested. The milk sample that met the required standards (see in appendix 3, table 5) was selected.

Table 1(a) : Compositional analysis of milk from producers

	Producer A	Producer B	Producer C
Fat%	3.60	3.06	2.68
Protein%	3.09	3.12	3.07
Lactose%	4.10	3.91	4.00

ii. *Somatic cell count (SCC)*

Somatic cell count was done by the producers due to the unavailability of the somatic cell counter or other means to test for somatic cell. But the ones with high somatic cell count are clearly labeled by the milk producers before they are accepted. Thus the milk received from the three producers was of low somatic cell count.

iii. *Bacteria count*

Bacterial count results were taken from previous results of the milk producers of past month and averaged to obtain an estimate since the process take long but a samples were taken for analysis for comparison and correlation with the resultant product.

Table 1(b) : Mean bacterial count of the milk samples

	Bacteria count (cfu $\times 10^3$/ml)
Producer A	5
Producer B	15
Producer C	12

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iv. *Coliform and E.coli*

This was used as an indicator of the sanitary quality of milk produced as well as contamination in the system. Testing for these organisms was therefore vital in determining the whether the milk was contaminated or not.

Table 1(c) : Coliform/*E.coli* results of milk samples

	Coliform count/*E.coli*
Producer A	-
Producer B	+
Producer C	-

v. *Antibiotics and Added water*

The antibiotic test and determination of added water were an important test since the presence of antibiotics means no fermentation and added water results in poor yields and very low total solids.

Table 1(d) : Other chemical tests

Producer	Antibiotics	Added water (%)
A	Negative	-
B	Negative	-
C	negative	0.002

Milk platform tests were carried out in order to use milk with the required standards which are tabulated in table 5 in appendix 3.

b) *Process audits*

i. *Process validation results*

Table 2(a) : First process audit results for process parameters at validation stage

Process parameter	Result
Pasteurisation (72^{0}C/15sec)	x
Inoculation temperature (32^{0}C)	✓
Inoculation	✓
Curd heating (39^{0}C)	✓
Whey draining (pH 6.4)	✓
Cheddaring (pH 5.2)	✓

Key: x- parameter not met

✓ - *parameter met*

Table 2(b) : Process audit results for coliform and E.coli test on cheese production personnel at validation stage

Process stage of operation	Personnel	Coliform and *E.coli* test
Pasteurisation	Vat attendant Pasteurizer operator	-ve -ve
Addition of colour	Vat attendant	-ve
Addition of $CaCl_2$ and KNO_3	Vat attendant	-ve
Temperature check	Vat attendant	-ve
Inoculation	Vat attendant	-ve
Temperature check	Vat attendant	++ve
Rennet addition	Vat attendant	+ve
Cutting	Vat attendant Production assistant	-ve +ve
Stirring after 15mins from cutting	Vat attendant	-ve
Stirring after 45mins from cutting	Vat attendant	-ve
Stirring after 75mins from cutting	Vat attendant	-ve
Wheying off	Vat attendant	+ve
Cheddaring 1st turn	Vat attendant	+ve
4th turn	Vat attendant	++ve
7thturn	Vat attendant	-ve
Salting and cutting	Vat attendant 1	+ve

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	Vat attendant 2	+ve
Shovelling and pressing	Production assistant 1 2 3 4	-ve +ve +ve ++ve

*Key: **-ve** negative for coliform and E.coli tests, **+ve** positive for coliform tests only, **++ve** positive for both E.coli and coliform test*

Table 2(c) : Process audit results for coliform and E.coli test on equipment at validation stage

Process stage of operation	Product	Coliform and *E.coli* test
Pasteurisation	Vat interior Agitator blade Speed knob 3 bucket system chlorinated water	-ve -ve -ve -ve
Addition of colour	Measuring cylinder Dip stick	-ve -ve
Addition of $CaCl_2$ and KNO_3	20l bucket	-ve
Temperature check	Thermometer	-ve
Inoculation	Culture sachet Knife	-ve -ve
Temperature check	Thermometer	++ve
Rennet addition	Measuring cylinder	-ve
Cutting	Cheese cutter	+ve
Stirring after 15mins from cutting	Thermometer	-ve
Stirring after 45mins from cutting	Thermometer Vial	-ve +ve
Stirring after 75mins from cutting	Thermometer Vial	+ve +ve
Wheying off	Shovel Strainer Clamps	++ve +ve -ve
Cheddaring 1st turn	Vat interior Vial Knife blade	-ve -ve ++ve
4th turn	Vial	-ve
7th turn	Vial	-ve
Salting and cutting	Cheese knife 5l salt container	++ve -ve
Shovelling and pressing	Shovel Working table Cheese cloth Cheddar forms	++ve -ve -ve ++ve

*Key: **-ve** negative for coliform and E.coli tests, **+ve** positive for coliform tests only, **++ve** positive for both E.coli and coliform test*

Table 2(d) : Coliform and E.coli counts in cheddar cheese during the validation stage

Sample	Coliform (cfu x 10^3/ml)	*E.coli* (cfu x 10^3/ml)
1	90	23
2	200	37
3	433	42
4	109	29
5	117	31
6	78	11
7	44	3

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8	150	34
9	97	20
10	630	44

Table 2(e) : observational results on plant personnel behaviours during production

Stage /Activity	Observation	Corrective measure
Milk pasteurisation	- The pasteuriser operator would set the pasteuriser on forward flow so that in cases when steam would drop the milk continued to flow in the vats without being diverted to the holding tube of the pasteuriser. - The pasteuriser operator would also give assistance to the vat attendant thus getting in contact with the cheese curd in process.	-Pasteuriser operators were trained on how to properly operate the pasteuriser -Pasteuriser operators were relieved of duties concerning vat attending
Vat cleaning	- Plant personnel used 20l buckets of hot water to sterilize the vat but in fear to self-injure themselves ineffective vat cleaning occurred - No standard procedure for vat cleaning was followed.	-Hose pipe which provided water at around 80°C was installed -A proper vat cleaning procedure was developed with appropriate quantities of detergent to be used.
Equipment cleaning and sanitisation	- Before using equipment during the cheese production, the vat attendant would dip the desired equipment in a vat of boiling water but because the water would be hot and the equipment is metallic the workers would partially deep the piece of equipment so that he/she would not be burnt. - Equipment like thermometers and vials were supposed to be sanitized in chlorinated water, however at times this sanitisation procedure was by-passed.	-Clamps to hold equipment when dipping in hot water were made available -A chlorinated water bath was introduced in the cheese plant -A policy was made that all equipment should be put in a vat of boiling water after every production cycle to make sure that they are adequately cleaned and ready for the next production.
Hand washing	- Plant personnel frequently neglected the three bucket system during production and at times would just rinse their hands in chlorinated water. - Personnel from other departments would pass through the cheese processing area without washing their hands and would actual greet cheese personnel by handshakes. - After taking some breaks, improper hand washing procedure was done by personnel - In fear to cause some bruises on their hands, workers would lightly brush their hands during washing.	-training sessions were provided for plant personnel -Hand swabs were taken at a frequent basis to make sure that people effectively washing their hands -printed signage were hanged on the walls which showed proper hand washing procedures -personnel trafficking through the cheese production area was restricted for cheese personnel only -Smooth hand scrubs were provided to substitute the hard brushes

ii. *Process verification results*

Table 3(a) : Process audit results for process parameters at verification stage

Process parameter	Result
Pasteurisation (72^{0}C/15sec)	✓
Inoculation temperature (32^{0}C)	✓
Inoculation	✓
Curd heating (39^{0}C)	✓
Whey draining (pH 6.4)	✓
Cheddaring (pH 5.2)	✓

Key: x- parameter not met

✓ *- parameter met*

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Table 3(b) : Process audit results for coliform and *E.coli* test on cheese production personnel at verification stage

Process stage of operation	Personnel	Coliform and *E.coli* test
Pasteurisation	Vat attendant Pasteurizer operator	-ve -ve
Addition of colour	Vat attendant	-ve
Addition of $CaCl_2$ and KNO_3	Vat attendant	-ve
Temperature check	Vat attendant	-ve
Inoculation	Vat attendant	-ve
Temperature check	Vat attendant	++ve
Rennet addition	Vat attendant	+ve
Cutting	Vat attendant Production assistant	-ve +ve
Stirring after 15mins from cutting	Vat attendant	-ve
Stirring after 45mins from cutting	Vat attendant	-ve
Stirring after 75mins from cutting	Vat attendant	-ve
Wheying off	Vat attendant	-ve
Cheddaring 1st turn	Vat attendant	-ve
4th turn	Vat attendant	++ve
7thturn	Vat attendant	-ve
Salting and cutting	Vat attendant 1 Vat attendant 2	-ve +ve
Shovelling and pressing	Production assistant 1 2 3 4	-ve -ve -ve -ve

*Key: **-ve** negative for coliform and E.coli test, **+ve** positive for coliform tests only, **++ve** positive for both E.coli and coliform test*

Table 3(c) : Process audit results for coliform and *E.coli* test on equipment at verification stage

Process stage of operation	Product	Coliform and *E.coli* test
Pasteurisation	Vat interior Agitator blade Speed knob 3 bucket system chlorinated water	-ve -ve -ve -ve
Addition of colour	Measuring cylinder Dip stick	-ve -ve
Addition of $CaCl_2$ and KNO_3	20l bucket	-ve
Temperature check	Thermometer	-ve
Inoculation	Culture sachet Knife	-ve -ve
Temperature check	Thermometer	-ve
Rennet addition	Measuring cylinder	-ve
Cutting	Cheese cutter	+ve
Stirring after 15mins from cutting	Thermometer	-ve
Stirring after 45mins from cutting	Thermometer Vial	-ve -ve
Stirring after 75mins from cutting	Thermometer Vial	-ve +ve
Wheying off	Shovel Strainer Clamps	+ve -ve ++ ve
Cheddaring 1st turn	Vat interior Vial Knife blade	-ve -ve +ve

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4th turn	Vial	-ve
7thturn	Vial	-ve
Salting and cutting	Cheese knife 5l salt container	++ve -ve
Shovelling and pressing	Shovel Working table Cheese cloth Cheddar forms	+ve -ve -ve -ve

*Key: **-ve** negative for coliform and E.coli tests, **+ve** positive for coliform tests only, **++ve** positive for both E.coli and coliform test*

Table 3(d) : Coliform and *E.coli* counts in cheddar cheese during the verification stage

Sample	Coliform (cfu x 10^3/ml)	*E.coli* (cfu x 10^3/ml)
1	44	20
2	1	0
3	15	4
4	9	3
5	22	44
6	2	0
7	8	2
8	2	0
9	18	9
10	13	7

c) *Statistical analysis*

The test statistics was done using the t-test at 5% significance level. The calculation in appendix 7 showed that the null hypothesis was rejected since the T cal > 2.23 implying that the alternative hypothesis was accepted which stated:

H_1 : There is a difference in the average *E.coli* count measured after the cheddar cheese process improvement.

Chapter Five

V. Discussion

At the milk reception point, samples of milk were taken from each producer's cans as well as the tanker truck and were tested for *E.coli*. Norholt (1984) reported that cattle can harbour this bacterium, *E.coli*, without any ill effects, shredding them in their faeces from which they gain entrance into raw milk. Therefore, this gave a reason why raw milk received from various producers tested *E.coli* positive. However, when proper heat treatment 'pasteurization' is applied to the milk, then the bacteria would be destroyed thus leading to the production of cheddar cheese with milk which is free from *E.coli* as a result *E.coli* counts on Cheddar cheese were low when effective heat treatment was practiced.

The level of hygiene during milking and the cleanliness of the vessels used for storing and transporting the milk are factors which determined the number of spoilage bacteria in raw milk. Raw milk is however, protected from spoilage by inherent natural antibacterial for the first 2-3 hours after milking and if not cooled these antibacterial substances break down causing bacteria to multiply rapidly(Sutherland *et al*, 1994). Thus upon arrival, milk received was transferred to the storage tanks where it is stored at 4^0C.

From the results of three milk samples from the three producers, it was observed that producer A had the best milk which meets all the standards. As milk was an important raw material in this research there was indeed the need to select milk with the highest microbiological quality and also to reduce the effects of contamination in the product. Since the scope of the study was to eliminate microbial contamination it was important to use milk free from microbiological, chemical and physical contamination. Therefore proper hygiene was practiced as evident by the coliform test results.

According to the results of this study, the pasteurization temperature had effect on the microbiological quality of the milk which was to be used for Cheddar cheese production. The phosphatase test was done to measure the efficiency of the pasteurizer. According to Oliver *et al,* (2009), there is a natural enzyme in milk called phosphatase. Phosphatase is heat sensitive and when proper pasteurization occurs, the enzyme is destroyed which will result in a negative result when tested. Going back to our test results, the pasteurized milk tested positive for the phosphatase test implying that pasteurization was insufficient to destroy the enzyme. The validating the process systems, it came to light that the pasteurizer was malfunctioning having the milk continuing to flow forward into the vats at times when the pasteurizer steam had dropped which made the pasteurizer's temperature to drop as well. This forward flow of milk would continue until the temperatures had dropped to around 65^0C instead of diverting back the milk immediately when the

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temperatures had dropped. At times the pasteurizer operator would set the pasteurizer at forward flow allowing raw milk into the vats when the steam was low. Because pasteurization is credited with dramatically reducing pathogens in milk and improving the shelf life and safety of processed milk (Leedom, 2006), this would have meant that some of the *E.coli* and coliform strains had survived this heat treatment resulting in processing Cheddar cheese with milk containing E.coli and coliform. Since there is little heat treatment during the Cheddar process which can reliably destroy these pathogens, it possibly might be the reason why Cheddar cheese tested positive for *E.coli* and coliform after production thus having high cfu/g of the Cheddar samples which however decreased after the fluctuation of the cut off temperatures of the pasteurizer went through maintenance. The pasteurizer efficiency was then verified using the same phosphatase test which the tested negative for the phosphatase enzymes thus implying that the milk was then being sufficiently pasteurized. The pasteurizer operators also received thorough training on how to properly operate the pasteurizer at correct temperatures and were also informed on the dangers of putting the pasteurizer on forward flow. Therefore *E.coli* and coliform occurrence in decreased in the Cheddar cheese after this corrective measure was taken.

Reddy (2013) reported that no *E.coli* should be present in milk after pasteurization. The handling of the raw milk before pasteurization is also important to reduce the probability of contamination of different processing facilities because when this organism is introduced to equipment and preparation surfaces; it may survive and multiply (Reddy, 2013). Therefore in order to avoid contamination of equipment from milk residues, Rice (1981) suggested that all floors of milk reception and processing rooms should be cleaned after a daily operation. The procedure has to be to first hosing down the floor with water to remove the milk remnants or the cheese curd if it would be in a cheese plant, then sweeping with a detergent solution using a stiff broom and finally hosing with hot water.

However, the validation results showed that equipment produced E.coli and coliform positive results due to poor sanitation. Assessment on the vat cleaning showed that the vats were poorly sterilized before the beginning of production since personnel used 20l buckets of hot water to sterilize the vats. As a result not all parts of the vat would receive that water for sterilization leaving a high possibility of having surviving coliform bacteria including *E.coli*. Due to the cautiousness of the worker trying not to hurt him/herself by the hot water when pouring or scooping onto the vat, insufficient cleaning resulted. However, as a corrective measure, a hot water pipe with water at 80^0C was then installed and used instead of pouring hot water using buckets. This method proved to be more effective and less risky. In addition a vat cleaning procedure was developed and the procedure was put on the walls in the cheese processing area so that every cheese maker is continually reminded on the steps to follow.

Not only was the sterilization of the vat a problem but also of the rest of the equipment. Therefore, a chlorinated water bath for sanitizing equipment during cheese production was also introduced into the cheese plant so that the equipment would be sanitized at frequent intervals. After every production, it was made a policy that all the equipment used during the cheese production should be put in a vat for boiling to make sure that the equipment will be thoroughly cleaned and stay ready for the next production cycle.

It was made possible that the production of Cheddar cheese be separated from Gouda and Mozzarella cheese to allow closer monitoring of hygiene systems and to avoid cross contamination of the cheeses with cultures and bacteria. Therefore the implementation of these corrective measures on the cheese production showed a significant improvement in terms of coliform and *E.coli* contamination. The verification stage of the process audit showed a decrease in the *E.coli* and coliform counts implying that the corrective measures implemented were effective to greater extent.

Reddy (2013) also suggested that the best way to control *E.coli* is through employee education and strict sanitation measures. From the validation results, milk after pasteurization tested negative for *E.coli* and coliform in some instances and during the process the curd turned out to be *E.coli* and coliform positive. This therefore indicated that the frequent handling of the curd by personnel might have led to *E.coli* and coliform contamination. The assessment of personnel behaviour at the validation stage gave some insight as to how personnel led to the contamination of the curd up to the final cheddar cheese. They neglected the three bucket system which is a hand washing procedure which was to be observed frequently (at an average after every 10 minutes). It started by washing hands with water containing a chemical disinfectant, followed by rinsing with clean water then finally sanitizing with chlorinated water. Some habits like hand shaking with one another or even touch some other surfaces and their skin and continuing with the handling of the in-process curd before they wash their hands. These habits just went on without being given much attention. Therefore these might have been the reasons why Cheddar cheese was highly contaminated with *E.coli* and coliform bacteria. In addition, workers used some brushes to wash their hands. Two possibilities which might have led to contamination at this stage that, either the previous person using the brush would leave the brush with his/her dirty on it so that the next person to use the brush would thus get that dirt onto him/herself and without effective washing the person will have hands

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contaminated with this dirt or that in fear to cause some bruises on hands due to the use of these brushes, personnel would lightly brush their hands thus leaving some hidden dirt on their hands. After evaluating the behavior of plant personnel and carrying some one-on-one interviews, it was shown that little knowledge was available in terms of personal hygiene and poor training was also received on hygiene issues.

Therefore the hazard analysis identified points which required correction and at this stage attention was to be given to personnel hygiene. The most effective way to encourage good hygiene practices was by education of employees, at the appropriate levels. Thus, employees were trained to appreciate and understand the scientific principles upon which such laws are based. Hand washing procedures were clearly demonstrated and signage with recommended chlorine and soap dilutions were displayed in all departments to ensure effective washing and sanitizing of hands.

The pasteurizer operators were relieved of all vat attending duties and these were left to the cheese maker to avoid contamination of the pasteurized milk in the vat just because the pasteurizer operators handle raw milk and unpasteurized cream thus creating a high possibility of cross contamination of microorganisms from raw milk to the in-coming pasteurized milk in the vats.

Therefore with the continual assessment on hygiene practices in the cheese production, these corrective measures showed decrease on *E.coli* and coliform contamination since plant personnel were now following sanitary procedures whilst knowing the importance of doing so. Each employee took it as a responsibility to become ones keeper in terms of observing hygiene practices. In addition, signage to stop plant personnel from other departments were also put at every entrance of the cheese production area since a lot of traffic was taking place and from the validation results, these people were also the possible causes of *E.coli* and coliform contamination in the cheese plant.

From the statistical analysis in appendix 6 using the t-test it was shown that there was a decrease on the average E.coli and coliform counts after the implementation of corrective measures on the Cheddar cheese process as compared to the E.coli and coliform counts obtained during the validation audits. Results then showed that poor hygiene practices had played a major role to the contamination of the cheddar cheese with E.coli and coliform bacteria though some other factors like pasteurization efficiencies also came into consideration.

Chapter Six

VI. Conclusion and Recommendations

a) *Conclusion*

The investigation on possible causes of *E.coli* and coliform contamination on Cheddar cheese successfully highlighted what corrective actions which were to be taken which then resulted in a significant decrease in the occurrence of the bacteria in the product thus increasing the confidence of the company by producing microbiologically safe products.

b) *Recommendations*

The scope of the project design could not manage to implement all the possible corrective measures to maintain the product safety from *E.coli* and coliform contamination. However, some general recommendations were made which had to be given attention by the authority in charge of the cheese production area. Therefore the following recommendations were developed:

- The cheese supervisors should make sure that they are always present in the cheese factory to enforce the hygiene systems which were put in place to ensure consistent improvement of results,
- The foot baths in cheese production should be sealed off to avoid collection of whey as well as avoiding the multiplication of bacteria,
- The doors are to be changed with self-closing doors to avoid entry of flies and other pests as well as preventing plant personnel from frequent contact to the surfaces thus reducing the probabilities of contamination,
- Flies are supposed to be managed in the factory as some flies were found in cheese pieces and flies are also considered as the major carriers of bacteria,
- The cheese makers should be given a different grade from the rest as they have a high level of responsibility. This will also help in the reduction of under graded cheese as they will be having a sense of ownership for the process, and also
- A hygiene related bonus system will help in enforcing hygiene in the factory.

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Appendices

a) *Appendix 1*

Table 4(a) : Specifications for Cheddar cheese

Name of product	Cheddar cheese
Date of issue of specifications	
Produced by	KEFALOS QUALITY CONTROL DEPT.
Ingredients	Whole milk, Approved Culture, Natamax (preservative), salt, calcium chloride and potassium nitrate.
HACCP	-Pasteurization temperature: 75^0C 15minutes. -Inoculation temperature: 32^0C -Antibiotic detection in whole milk.
Analytical quality standard of finished product	Physical -colour: orange -texture: smooth. Chemical -pH: 5.2 -B/fat: 3.5% Microbiological -Coliform: Absent -E.coli: absent
Shelf life and storage temperatures	3-24 months at 10^0C
Intended use	-for consumption.
Legislation	-Not for baby feeding

b) *Appendix 2*

Table 4(b) : Cheddar Cheese Production Process

STEP PROCESS STEP	CONTROL/CHECK PARAMETERS
1. Milk input	Fresh raw milk/ reconstituted milk. Butterfat of milk should be <2.90%. Total solids of milk <8.4% (pasteurised at 72-75^0C for 15-20sec.). pH raw milk/reconstituted milk >6.65.
2. Standardisation	Fat to 3.5%.
3. Pasteurisation	Pasteurise milk at 72^0C for 15secs.
4. Cooling to inoculation temperature	Cool down to 32^0C.
5. Inoculation	Confirm with thermometer incubation temperature 32^0C before inoculation. Sampling for solids, fat and pH after inoculation.

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6. Incubation	Incubate for 30-40mins at 29-32^{0}C
7. Curd cutting and heating	Curd was cut after milk coagulation and cooked at 39^{0}C for 20-60mins until pH is around 6.1-6.4
8. Whey draining	Whey was drained at pH 6.1-6.4
9. Cheddaring	Curd was cut into loaves at pH 6.4 which were stacked and turned after every ten minutes until pH is 5.1-5.4
10. Salting	Curd was milled and added salt at 1-3%/weight
11. Pressing	Milled curd was moulded in the cheddar cheese forms and put on a cheese press for 48hrs.
12. Aging	Pressed cheese was undressed from the cheese clothes, strayed with Natamax (a preservative) and stored in aging room at 10^{0}C.

c) *Appendix 3*

Table 5 : Raw milk intake standards

Attribute	Requirement	
pH	6.60 - 6.90	
Butterfat	Min. 3.0%	
Total solids	Min. 12.2%	
SNF	Min. 8.5%	
Clot-on-boiling	Must not clot on boiling	
Colour	Typical milk white colour free from visible matter	
TBC	Special <100 000cfu/ml	Normal <200 000cfu/ml
SCC	Winter : <600 Summer : <600	Winter : <750 Summer : <850
Coliform	Nil	
Antibiotics	Negative	

d) *Appendix 4*

i. *Media Preparation And Autoclaving*

a. *Procedure For Preparation Of Mackonkey Agar*

MacKonkey agar is a selective media for presumptive test for E.coli and coliform bacteria in foodstuffs and water. Their presence is indicated by acid and gas production.

- *Method*

We measured 1000ml of distilled water into a measuring cylinder and transferred about 500ml into a conical flask. We weighed 40g of MBP into the flask and thoroughly mixed until a homogenous suspension is obtained. We added the rest of the water from the measuring cylinder in the flask making sure to rinse down any material adhering on the walls of the flask.
6ml of this MBP were dispensed into test tubes with Durham tubes and then stick an indicator tape to a tube for every 30 tubes. We loaded the tubes into baskets and sterilized for 15 minutes at 121^{0}C.

- *Quality control*

After cooling all the tubes, they were placed in an incubator at 37^{0}C for 24 hours. Then checked for any bacterial growth in all tubes discarding all tubes showing growth of bacteria. The same procedure is performed for other media with only the amount of dosage being the difference.

1. Maximum Recovery Diluent (Mrd)

It was used as a diluent for serial dilution of cheese and milk samples as well as equipment and personnel hand swabs for TVCs, and other microbiological analysis. This medium was used to enrich and develop the inoculums that were used to inoculate the agar plates. It was also used to maintain the cultures for some biochemical tests.

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- *Preparation*

It was a combination of 8.5g Sodium chloride and 1g Peptone powder in 1L distilled water and was autoclaved for 15 minutes at 121^{0}C.

2. Plate Count Agar (Pca)

The medium was used for performing total bacteria counts by pour plate or surface methods in milk and cheese samples.

- *Preparation*

We suspended 23g in 1l distilled water. Boil whilst stirring until completely dissolved. This was autoclaved at 121°C for 15 minutes.

e) Appendix 5

i. Analysis Of Raw Milk Tests And Procedures

1. Total Bacteria Count

- *Requirements*
  - Plate count agar and Petri dishes
  - Dilution tubes with 9ml maximum recovery diluent (MRD) 1ml sterile pipettes
  - Test tubes -3 dilutions
  - Incubator maintained at 30^{0}C
  - Water bath maintained at 45^{0}C.

Total viable/plate count is used to get a general estimation of colony forming units per 100ml of sample (either liquid or dissolved solid).This method enumerates most chemoheterotrophic bacteria in an all-purpose plate count agar media in the presence of oxygen at 30°C.

- *Procedure*

1. Melt the PCA and leave it to cool in a water bath at 45^{0}C for at least 15 minutes.
2. Make first dilution (1/10: 10) of the solid sample by weighing 10g of the sample into a stomacher bag and adding 90ml of MRD.
3. Blend the sample for 120 seconds.
4. Make a 1/100 (10-2) serial dilution of the homogenate sample by transferring 1ml of the sample into 9ml MRD. For milk, ice cream mix and yoghurt you just your serial dilutions from the sterile vials and mix using vortex mixer.
5. Mix the dilution tube and use a fresh pipette to transfer 1ml of the sample into next dilution (1/1000: 10-3) .
6. Remove bottle of PCA from the water bath and wipe excess moisture with a disposable paper towel.
7. Add 1ml of the diluted sample into a sterile Petri dish the pour 12- 15ml PCA (45°C) into the Petri dish.
8. Mix well and leave to solidify on a flat cool surface.
9. When the medium has set, turn the plate upside down and place into the incubator.
10. Incubate for 72 hours at 30^{0}C.

- *Interpretation of results*

Count all the forming colonies including those with pinpoint sizes. The results are recorded as the counted number of cfu × the dilution factor.

2. Determination Of Fat Content In Milk

For this we used the Gerber test. Milk fat is separated from proteins by adding sulphuric acid. The separation is facilitated by using amyl alcohol and centrifugation which allows the separation of the oil from the aqueous phase. The fat content is read directly via a butyrometer (there are butyrometers used for cream, ice cream mix and raw milk).

- *Method*

1. Measure 10ml 92% sulphuric acid into a butyrometer
2. Add 5ml of the sample (well mixed) into the butyrometer without wetting the neck.
3. Add 1ml of amyl alcohol and then about 5ml of distilled water. Insert the stopper/locker.
4. Shake vigorously to mix the sample and acid until no white particles are seen.
5. Centrifuge the butyrometer for 4- 5 minutes at 1100 rpm.

- *Interpretation and reporting of results*

Insert the key into the lock stopper and apply sufficient pressure to bring the lower end of the fat column on to a graduation mark. Record the scale reading corresponding to the lowest point of the meniscus.

3. Inhibitory Substances/Antibiotic Test

ßeta s.t.a.r is used to determine the presence of antibiotics. ßeta s.t.a.r is a receptor assay for rapid determination of ß lactam antibiotics such as penicillin and ampicillin extensively used in the prevention and treatment of dairy cattle diseases, particularly mastitis. The test involves specific ß lactam receptor linked to gold particles. The preliminary incubation of the receptor with milk containing antibiotics will result in interaction of the antibiotics with the receptor. In the second stage, the solution is transferred onto an immunochromatographic medium. The first band of this medium will capture all the receptors which have not interacted with any antibiotic during the first incubation. The second band on the immunochromatographic medium serves as a reference (control) band.

- *Test procedure*

1. Add 25μLof the solution to a conical polypropylene Eppendorf type vial.
2. Add 100μL of the milk to be tested.
3. Mix and incubate at 47.5^{0}C.
4. After 3 minute incubation, with clean and dry hands you take a dipstick and put it in the incubation vial still in the incubator.
5. After additional 2 minutes, take out the dipstick out of the vial and interpret immediately.

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- *Incubation temperature*

The correct ßeta s.t.a.r incubation temperature is 47.5°C±0.5°C. Incubation temperatures below 46°C will slow down the ßeta s.t.a.r reaction while incubation temperatures above 49°C will result in the destruction of the ßeta s.t.a.r reagents. Both of these extreme situations will impair the proper performance of the test.

4. The Lactometer test

Milk has a specific gravity. When it is adulterated with water or other materials are added or both misdeeds are committed, the density of milk changes from its normal value to abnormal. The lactometer test is designed to detect the change in density of such adulterated milk. Carried out together with the Gerber butterfat test, it enables the milk processor to calculate the milk total solids (% TS) and solids not fat (SNF). In normal milk SNF should not be below 8.5%.

- *Procedure*

Mix the milk sample gently and pour it gently into a measuring cylinder (300-500). Let the Lactometer sink slowly into the milk. Read and record the last Lactometer degree (°L) just above the surface of the milk. If the temperature of the milk is different from the calibration temperature (Calibration temperature may be=20°C) of the lactometer, calculate the temperature correction. For each °C above the calibration temperature add 0.2°L; for each °C below calibration temperature subtract 0.2 °L from the recorded lactometer reading.

f) Appendix 6

1. Determination Of Pasteurisation Efficiency For Milk

When milk is pasteurized at 63°C for 30 min in batch pasteurizer or 72°C for 15 seconds in heat exchanger, continuous flow pasteurizers, ALL PATHOGENIC BACTERIA ARE DESTROYED, there by rendering milk safe for human consumption. Simultaneously various enzymes present in milk, and which might affect its flavour, are destroyed.

In order to determine whether or not milk has been adequately pasteurized, one of the enzymes normally present in milk phosphatase is measured. A negative phosphatase result indicates that the enzyme and any pathogenic bacteria have been destroyed during pasteurization. If it is positive, it means the pasteurization process was inadequate and the milk may not be safe for human consumption and will have a short shelf life.

- Test tubes
- 5 ml pipettes
- 1 ml pipettes
- l00 ml volumetric flask
- 500 ml volumetric flask
- water bath at 37°C

Note: All glassware must be rinsed, cleaned, rinsed in chromic acid solution and boiled in water for 30 min.

Reagent:

Buffer solution: Was mixed by 0.75g anhydrous sodium carbonate and l.75g Sodium bicarbonate in 500 ml distilled water.

Buffer-substrate solution: Place 0.l5 g of di-sodium paranitrophenylphosphate (the substrate) into a clean 100ml measuring cylinder.

Add the buffer solution to make to 100 ml mark.

Store this buffer-substrate solution in a refrigerator and protected against light. It should not be used after one week. Prepare a fresh stock.

- *Procedure:*

Pipette 5mls buffer-substrate solution into a test tube, stopper and warm the solution in the water bath at 37°C. Add to the test tube 1ml of the milk to be tested, stopper and mix well and place in water bath at 37°C. Prepare a blank sample from boiled milk of the same type as that undergoing the test. Incubate both the test samples and the blank sample at 37°C for 2hrs. After incubation, remove the tubes and mix them thoroughly.

Place one sample against the blank in a Lovibond comparator" ALL PURPOSES" using A.P.T.W. disc and rotate the disc until the colour of the test sample is matched and read the disc number.

Interpretation :

Disc Reading after 2 hrs incubation at 37°C	Remarks
0-10	Properly pasteurized
10-18	Slightly under pasteurized
18-42	UNDER PASTEURIZED
> 42	NOT PASTEURIZED

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g) *Appendix 7*

i. *Statistical analysis*

H_0: There is no difference in the average *E.coli* count measured before and after cheese process improvement.

H_1: There is a difference in the average *E.coli* count measured after the cheese process improvement.

ii. *Calculating T-cal*

a. *Paired Sample Statistics*

$$H_0: \mu_1 = \mu_2$$

$$H_1: \mu_1 \neq \mu_2$$

- This was tested using the t-test at 5% significant level
- Reject the null hypothesis if

$$|T\ cal| > t\ \alpha/2(n1+n2-2)$$

$$t\alpha/2(n1+n2-2) = 2.23$$

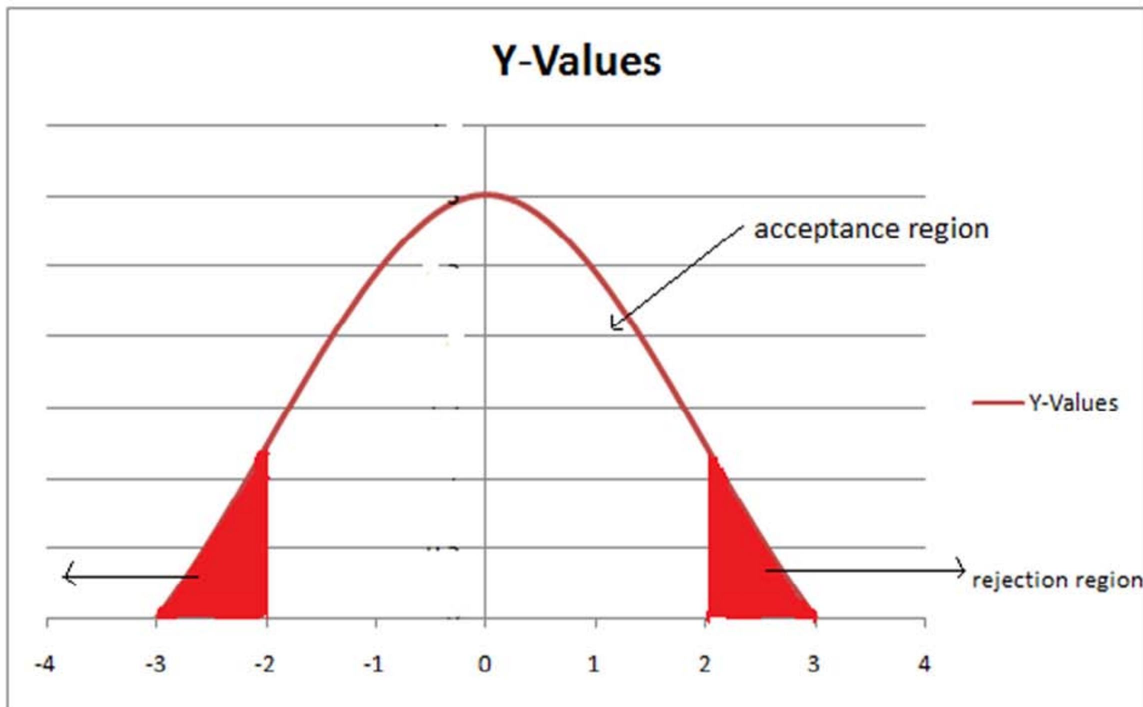
Statistics	Sample 1	Sample 2
Sample size	1	2
Mean	27.4	8.9
Standard deviation	13.24	13.75

b. *Test Statistics*

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s_p\sqrt{(\frac{1}{n_1} + \frac{1}{n_2})}}$$

$$S_p^2 = \frac{27.4\ (13.24)^2 - 8.9\ (13.79)^2}{10+10-2}$$

$$S_p = 13.5$$



From the calculation T cal = 13.5

Since T cal > 2.23, therefore we reject H_0 and conclude that there a significant difference between the *E.coli* count tested before and after cheese process improvement.

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Genetic Variation of Salt Tolerance Character and Organic Components Studies in Selected Salt Tolerant Genotype and Sensetive Cultivar of Wheat

By Ibrahim I. H. Al-Mishhadani
Al-Nahrain University, Iraq

Abstract- Genetic evaluation was estimated for salt tolerance between the selected wheat genotype through plant breeding program and local cultivar by using random amplified polymorphic DNA (RAPD-PCR reaction) with two selected primers (OP1-01 and GB8). According to the amplification results, the genetic distance, Dendrogram illustrated genetic fingerprint and relationships between the selected genotype (Dijila) and local cultivar (Tamooze-2) were determent under non and saline condition. Also the chlorophyll content and organic compounds of the upper leaves were measured.

Keywords: *wheat, salt tolerance, RAPD-RCR reaction, chlorophyll, organic compounds.*

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Genetic Variation of Salt Tolerance Character and Organic Components Studies in Selected Salt Tolerant Genotype and Sensetive Cultivar of Wheat

Ibrahim I. H. Al-Mishhadani

Abstract- Genetic evaluation was estimated for salt tolerance between the selected wheat genotype through plant breeding program and local cultivar by using random amplified polymorphic DNA (RAPD-PCR reaction) with two selected primers (OP1-01 and GB8). According to the amplification results, the genetic distance, Dendrogram illustrated genetic fingerprint and relationships between the selected genotype (Dijila) and local cultivar (Tamooze-2) were determent under non and saline condition. Also the chlorophyll content and organic compounds of the upper leaves were measured.

The amplification results using two primers (OP1-01 and GB8) showed that there are no differences in banding patterns between the selected genotype and local cultivar under non-saline condition, this reflecting that there isn't genetic variation between them under such condition with the two primers which used. Whilst the result showed that there are differences between the selected genotype and local cultivar in their banding patterns with the two primers when grown under saline conditions. Also these results reflecting that there are large genetic variation and distance between the selected genotype and local cultivar in their salt tolerance only under saline condition. The chlorophyll content in the upper leaves of selected genotype increased under saline condition while it decreased in the upper leaves of the local cultivar under saline condition as compared with those under non-saline condition. Also the results indicated that the organic compound in upper leaves increased under saline condition in both genotype and cultivar as compared with those organic were much higher in the selected genotype than local cultivar.

Keywords: wheat, salt tolerance, RAPD-RCR reaction, chlorophyll, organic compounds.

I. Introduction

Salinity is a major factor limiting plant growth and production in the middle and south of Iraq, which caused reduction in agricultural land production. Hence induced salt tolerant cultivars or genotypes of crops are needed to sustain in increase in yield production in agricultural land under salinity condition, and also reducing the spread of secondary salinity (Munns, 2005). Increased salt tolerance in plant requires new genetic sources of this character. Powerful new molecular techniques for determining the genetic variation are useful for increasing this character in plants. But we need to the applications of the new technologies to introduce new genes for salt tolerance in to current local cultivars. Genetic diversity is very important factor for the development of many crop plants including wheat plants, because the breeders depend on the availability of genetic diversity during the selection to the developed cultivars. Genetic distance estimation among genotypes is useful to select the parents, which be used in a breeding program (Van Becelaere *et. al.,* 2005). The random amplified polymorphic DNA (RAPD) technique has been used for the identification of genetic diversity within a population of breeding materials, which important for genotypes improvement (Williams et. al., 1990; Welsh & McClell and, 1990; Mainifes to *et.al.,* 2001). Also they reported that the most distinct genotypes or cultivars will be used to increase the genetic diversity in wheat, which will be used in the plant breeding program. RAPDs have been used for identification of genotypes in crop plant, for determining the genetic variability within species and for showing the relationships among populations (Freitas *et. al*., 2000). Using RAPD for variability estimation canguide plant breeders to select genotypes with diverse genetic base, which can be used in their breeding programs. On the their hand, RAPD analysis also has been used to determine phylogenetic relationships among species, subspecies and cultivars (Landry *et.al*., 1994) and to measure genetic variation in populations and species (Nesbitt *et.al*., 1995), as well as identification of cultivars, breeding line and clones (Nybon, 1994).

Organic compounds would be essential to balance the osmotic pressure of the cell cytoplasm and to allow turgor maintenance of cells that would otherwise dehydrate (Wyn Jones *et.al.,* 1977). In addition, these compounds could stabilize membrane proteins and so maintain growth at high salinity levels. The benefit of these compounds can be measured only their effect on leaf injury and plant growth rate, so an increasing in any one of these compounds may not make a plant grow more quickly, but make it grow more slowly under a biotic stress (Manns, *et. al.,*1983).

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The aims of the present study are investigation of genetic diversity and identification between selected genotypes and local cultivar of wheat for salt tolerance by using RAPD technique and genetic distance analysis. Also some organic compounds in upper leaves of these genotypes were measured.

II. Materials and Methods

a) Salt tolerance test

The growth of the wheat genotypes which were selected for salt tolerance through plant breeding programs after 6 cycles of exposure and selection, as compared with the local cultivar (Tamooze-2) were examined in salinized soils at two levels (0, 16, ds/m). The experiment was carried out in pots, set up in glasshouse. 7 seeds were sown in each pot. Seeds and plants were watered with tap water (200 mill/ pot) according to the filed capacity. Leaves samples were taken after 6 weeks from the sowing date, which will be used for the molecular studies and for organic compounds measurement. Also the chlorophyll content in the upper leaves was measured by using chlorophyll meter (Japan).

b) RNA isolation and cDNA synthesis

Total RNA were isolated from the upper leaves according to the manufacturer's instructions, by using Gene aid total RNA purification mini kit (Taiwan). Isolated RNA was treated with RNase-free DNase-I (Biobasic, Canada) for 20 min at 37°C, DNase-I was in activated at 65°C for10min. The RNA integrity was verifiedafter separation by electrophoresis on a 1.5% agarose gelcontaining 0.5% (v/v) ethidium bromide. First-strand cDNA wassynthesized from 500ng of total RNA using Reverse Transcriptionsystem (Bioneer, Korea) with an oligo-dT_{15} primer. The reaction solutionwas used as templates for reverse transcriptase polymerase chainreaction (RT-PCR).

c) cDNA-RAPD

Two primers (table1) were used for amplification of cDNA using optimized PCR protocols and master mixes. Polymerase chain reaction was initiated with hot start method using the single strand cDNA template on LabnetThermocycler (USA). The PCR reaction was carried out according to the program of 35 amplification cycles (94°C for 30 s, 61°C for 45 s and 72°C for 90 s). Ethidium bromide agarose gel electrophoresis (1%) used for analysis of PCR products. The generated bands were compared, the differential amplified bands were recorded and the sequences of these bands aligned to related sequences in NCBI blast database (Chen *et.al.*, 2003).

Table (1) : RAPD primers used

Primers	Sequence
OPI-01	5'-AACCTGGCA-3'
GB8	5'-GTCCACACGG -3'

d) Estimation of genetic distance

Data generated from the detection of polymorphic fragment were analyzed. The amplification profile of all the used isolates for any given were compared with each other, the presence scored as (1) and the absence of the same band of the same size in other isolate scored as (0). Only clear and reproducible amplified fragments were considered for genetic relationship analysis. Estimates of genetic distance (G.D) were calculated between the selected genotype and local cultivar according to (Neiand Li.,1979) Based on following formula: G.D=1-{2Nab/ (Na + Nb)}.

Where Na= the total number of fragments detected in individual (a); Nb= the total number of fragments shown by individual (b) and Nab= the number of fragments shared by individuals (a) and (b). Cluster analysis was performed to construct genetic relationship tree diagrams among studied. Among Triticum *aestivum L.*cultivars using an Unweighted Pair-Group Method with Arithmetic Average (UPGMA). All computations were carried out using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc), Version 1.7 package (Rohlf., 1993). The percentage of polymorphic bands was defined as ratio of the number of polymorphic bands amplified by a single primer to that of the total number of bands produced by the same primer.

e) Estimation of organic compounds

i. *Ash (carbon) percentage*

Ash percentage was estimated according to the (Aoac, 1980) 5g of the plant were taken and burn in the oven (Muffle Furance) at 500 C^0 until the plant simple changed to the white Ash, then the sample was weighted and the percentage of the ash was calculated.

ii. *Total lipids percentage*

This percentage was estimated according to the (AACC, 1984) by using soxhlet. 200ml of the pertroleum ether were added the soxhlet and the thumble which contained 10g the dried plant material was put in the soxhlet. After 8h from the extraction, the solvent was evaporated by using Rotary evaporator at $45C^0$. Then the total lipids were weighted and the total lipids percentage was calculated.

iii. *Crude protein percentage*

Nitrogen percentage in the plant sample was measured by using microkjeldahl (Heilenz *et.al.,* 1972). The percentage of crude protein was calculated as following:-

% crude protein = % Nitrogen X 6.25

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f) Chlorophyll content

The Chlorophyll content in the upper leaves was measured by using Chlorophyll meter.

III. Result

a) Genetic variation and Distances

The genetic variations between the selected wheat genotype and local cultivar were evaluated using RAPD markers amplified from two primers, each primer varied greatly in their ability to resolve variability between the selected genotype and cultivar. Fig.1 shows the amplification results using two primer OPI-01 and GB8) of the two cultivars grown under non-saline condition. The results showed there are no differences between the selected genotype and local cultivar in their banding patterns with the two primers, this reflecting that there are not genetic differences between the selected genotype and cultivar under non-saline condition with the two primers which used to identify genetic variation in their salt tolerance. Whilst the result in Fig.2 showed that there are differences between the selected genotype and local cultivar in one – bands (300 bp) with OPI-01 that found only in selected genotype (Dijila) when grown in saline conditions (16 ds/m). Also the results showed that the selected genotype and cultivar differed in four bands with GB8 which only found in selected genotype (Dijila) under the same saline conditions (16 ds/m), two of them with 300 bp and the other two, one with 200 bp and the other with 150 bp (fig.2). These results reflecting that there are large genetic differences between the selected genotype and cultivarin their salt tolerance with GB8 primer under salinity condition as compared with the OPI-01 primer.

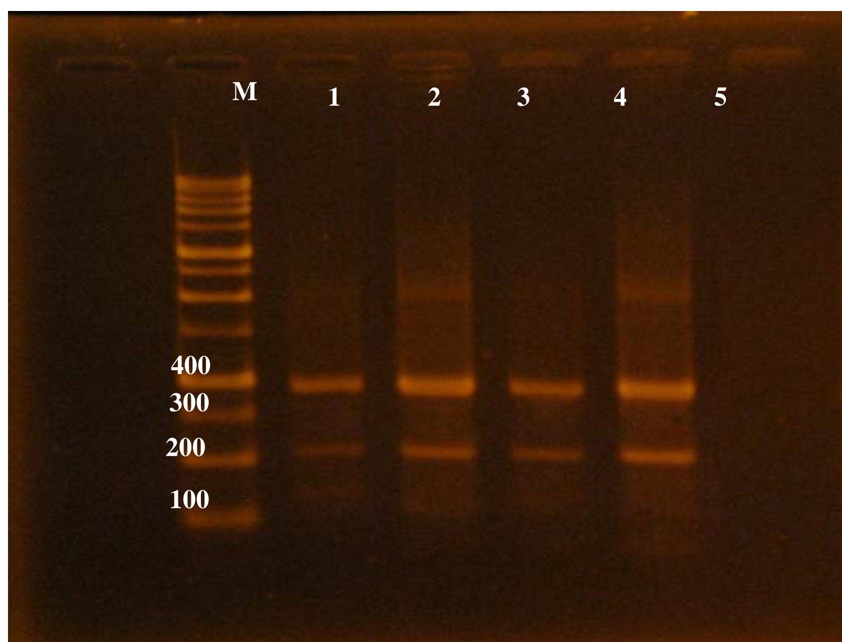

Figure 1 : Agarose gel electrophoresis of RAPD-PCR reaction two primers, cDNA samples of the selected genotype and cultivar(Dijila & Tomooze-2) under non-saline condition. Bands were fractionated by electrophoresis on a 1.2% agarose gel (2hr, 5V/cm, 1X Tris-borate buffer) and visualized under U.V. light after staining with ethidium bromide. M: 100bp DNA ladder. Lane 1: Dijila, Lane 2: Tomooze-2 (primer OPI-01). Lane 3: Dijila, Lane 4: Tomooze-2 (primer GB8). Lane 5: negative control.

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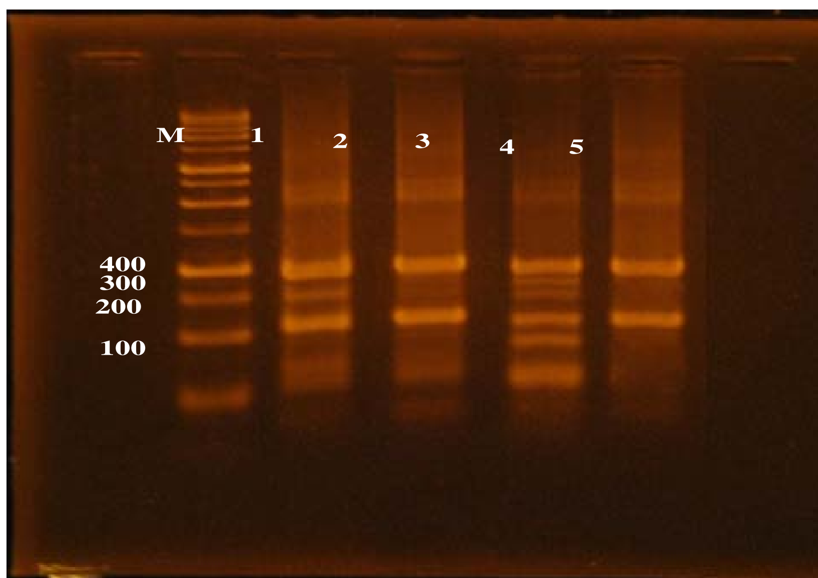

Figure 2 : Agarose gel electrophoresis of RAPD-PCR reaction two primers, cDNA samples of the selected genotype and cultivar(Dijila & Tomooze-2) under saline condition. Bands were fractionated by electrophoresis on a 1.2% agarose gel (2hr, 5V/cm, 1X Tris-borate buffer) and visualized under U.V. light after staining with ethidium bromide. M: 100bp DNA ladder. Lane 1: Dijila, Lane 2: Tomooze-2 (primer OPI-01). Lane 3: Dijila, Lane 4: Tomooze-2 (primer GB8). Lane 5: negative control.

Genetic distance value and dendrogram illustrated genetic distanced were summarized in fig.3. The value of the genetic distance was 0.64404, this reflecting there are large genetic variations between the selected genotype and local cultivar in their salt tolerance. The genetic distance between the selected genotype and cultivar depends on this value;therefore,this value (0.64404) revealed there is high distance between the selected genotype and cultivar in their genetic variation for salt tolerance. Also the result of the dendrogram appeared there are two groups 1 & 2 and the distance between them reflecting the genetic distance between the selected genotype (Dijila) and local cultivar (Tomooze-2) in their salt tolerance (Fig.3).

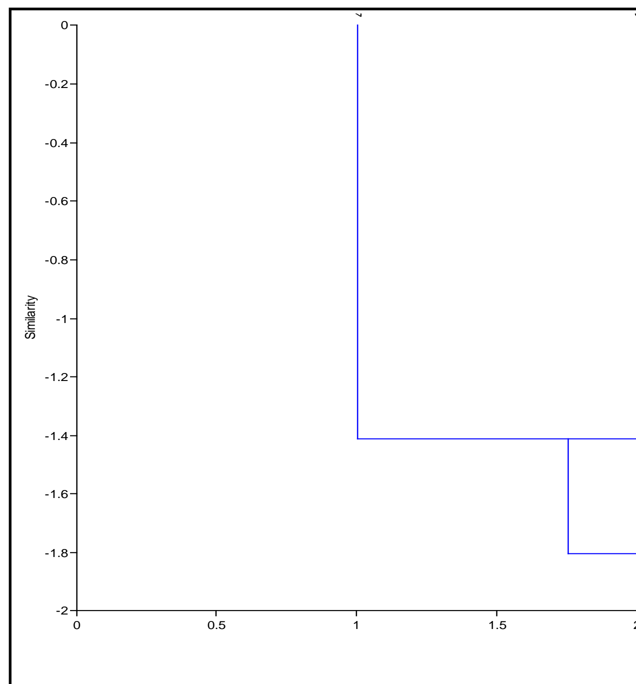

Figure 3 : Dendrogram illustrated genetic fingerprint and relationships between selected genotype and cultivar(Dijila & Tomooze-2) developed from RAPD data

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b) Chlorophyll content

The results in Fig.4 showed the Chlorophyll contentrelation in the upper leaves of the selected genotype and local cultivars grown under saline condition (16 ds/m) and non saline conditions (0 ds/m). These results indicated that the Chlorophyll concentration in the upper leaves was similar in the both the selected genotype and cultivar under non saline condition, but this concentration was differed between the selected genotype and cultivar under saline condition (16 ds/m). By contrast, under saline condition the Chlorophyll concentration was increased in the upper leaves of the selected genotype, while it decrease in the leaves of the local cultivar (fig.4) as compared with those when grown under non-saline condition.At 16 ds/m the difference between the selected genotype and cultivar in their Chlorophyll content washighly significant ($p \leq 0.01$).

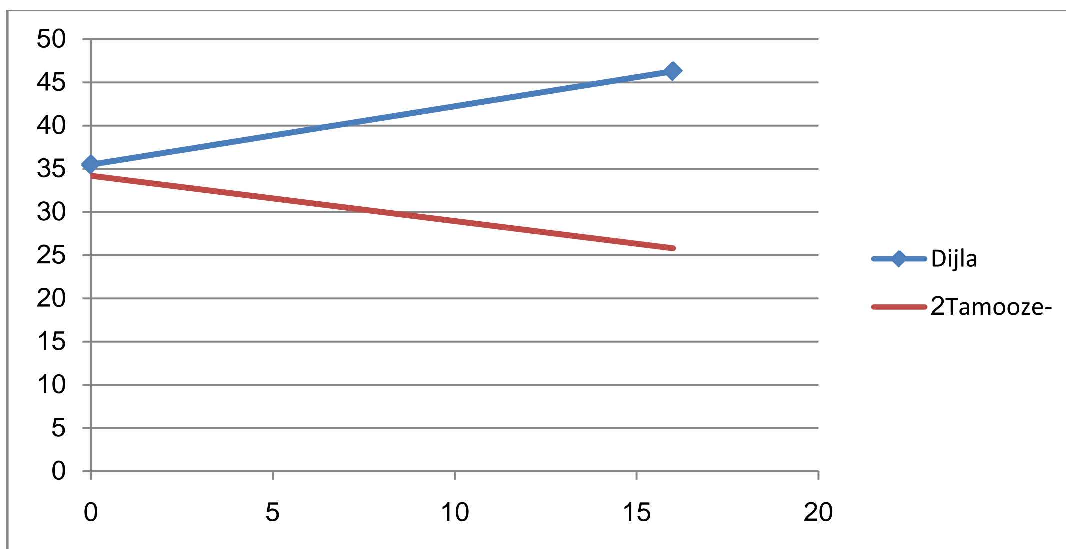

Fig 4 : Chlorophyll concentration in the upper leaves of the selected genotype and local cultivar under salinity conditions

c) Organic compounds

Carbon (Ash), total lipids, and crude protein contents in the upper leaves of the selected genotype and cultivarwere grown under saline and non-saline conditions were summarized in table 2. It showed that the Carbone content in the leaves of selected genotype was higher than this of local cultivar at both salt levels, but it was much higher under saline condition (16 ds/m). At 16 ds/m, the carbon content increased in both the selected genotype and cultivaras compared with this at non-saline condition, but was higher in the selected genotype than local cultivar. Also the result showed that the total lipids content increased in both the selected genotype and cultivarwere grown in the saline condition as compared with those grown under normal condition, but increasing was much higher in the Dilila genotype than Tamooze-2cultivar (Table-2). However, the leaves of selected genotype (Dijila) had total lipid much higher than those of the local cultivar (Tamooze -2). The results of crude protein showed that the leaves of selected genotype also had much higher crude protein percentage than the leaves of the local cultivar at both salinity levels. At both the selected genotype and cultivar, the crude protein percentage was higher at salinity level than the normal condition (non-saline condition) (table.2)

Table 2

	0 ds/m			16 ds/m		
genotypes	Organic compounds			Organic compounds		
	Carbone	Lipid	protein	Carbone	Lipid	protein
Dijila	9.33%	0.98 %	1.6 %	11.5 %	5.75 %	2.2 %
Tomooze-2	8.1 %	0.22 %	1.1 %	8.6 %	1.2 %	1.53 %

IV. Discussion

The selected genotype (Dijilla) was induced through plant breeding programs for salt tolerance; this selected genotype was derived from F2-F7 generations after exposure to the 30 ds/m drainage water. Some experiments were done to exam the salt tolerance of the selected genotype (Dijilla) at different techniques and salinity level, they reported that the selected wheat genotype (Dijilla) is high salt tolerance genotypes and

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grow and product very well under high salinity condition (AL- Mishhadani, 2012: AL- Mishhadani *et. al*., 2014). Also ismail, 2013 showed that Dijilla genotype has salt tolerant gene with high expression under salinity condition (20 ds/m). The improve of salt tolerance in this genotype may due to that this genotype was selected from F2-F7 generation generally contain high genetic variation in salt tolerance and also the seeds and seedling plants were exposed to high salinity concentration (30 ds/m) which allow only high salt tolerant genes segregated in very few plants still survived after this exposure. These genes exhibited high gene expression when grown in saline conditions. In contrast, these genes may not exist in the local cultivar which was sensitive to salinity. Munns (2005) reported that salt tolerance in plant is correlated with the salt tolerance mechanisms which controlled by segregated salt tolerant genes. Unless salt tolerance is controlled by major genes which seem unlikely to such a complex character (Ashraf and McNeilly, 1988). It is clear that salt tolerance is inherited and the degree of inheritance depends on the genetic variation range and salinity level (Azhar and McNeilly, 1989; AL- Mishhadani *et.al*., 2003). The difference in the salt tolerance between the selected genotype (Dijilla) and local cultivar may due to large genetic distance between them under high salinity condition as shown in fig.3. This genetic distance value (o.64404) reflects the large difference between them in their salt tolerance this conclusion have been supported by Van Becelaere *et.al*., 2005 which they reported that genetic distance estimation among genotypes is used to selected the parents, which be used in a breeding program to create high genetic variation in F2 materials.

One the other hand that reported by ismail 2013, showed that selected genotype (Dijilla) has salt tolerant gene (*TaGSK1*) with high gene expression under salinity condition, while this gene absence in the local cultivar. Also she reported that the salt tolerance degree in genotype was associated to this result, she concluded that Dijilla genotype more salt tolerance that local cultivar therefore, the results that reported by AL-Mishhadani, 2012: AL- Mishhadani *et.al.,* 2014 confirmed this conclusion. The results of RAPD marker amplified (figs. 1, 2) showed that there are not difference between the selected genotype and local cultivar in specific bands, but the difference between them in these bands appeared in salinity condition only. These bands reflect the genetic variation between them in their salt tolerance, because these bands appeared in salinity condition and in salt tolerant genotype only. The same genetic variation in wheat by using RAPD marker was reported by (sajida BiBi *et.al.,* 2009). Also the same RAPD technique has been used for the identification of improved genotypes (Manifeston *et.al.,* 2001) to screen the genetic similarity and difference between wheat germplasm. The superiority of selected genotype (Dijilla) in salt tolerance of the local cultivar may associated with high chlorophyll content in the upper leaves (Fig .4), that is important factor in photosynthetic and then increase the dry matter (Ashraf, 1994: munns ,1993). Increasing dry matter is very important in plant growth, tissues extantion, and in yield and its components production under salinity conditions (Al-mishhadani, 2010). Increasing chlorophyll content in upper leaves of the selected genotype may due to the modification in morphological characters as physiological mechanisms conferring salinity tolerance character. Also the difference between the selected genotypes and local cultivar in their salt tolerance may refer to their difference in organic compounds of the upper leaves (table 2). These organic compounds that much higher in the upper leaves of the selected genotypes (Dijilla) ascompared with those of local cultivar one very important in osmo regulation in cells plant, which is one the salt tolerance mechanisms to overcome the osmotic pressure of the soil affected salt (Al- mishhadani *et.al*., 2003: munns, 1993).

Generally, from the above results, the conclusions are there significant improvement in salt tolerance of the selected genotype (Dijilla) through plant breeding program and there is large genetic variation and genetic distance between selected genotype and local cultivar under salinity condition only. Also the high salt tolerance in selected genotype more correlated with high contents of chlorophyll and organic compounds in their upper leaves under high salinity level.

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Antmicrobial Packaging, a Step towards Safe Food: A Review

By Muhammad Afzaal, Urooj Khan, Hafiz Arbab Sakandar & Muhammad Nadeem Akhtar

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Abstract- Food is the primary concern of living organisms, provision of diet for maintenance of good physical and mental health is a basic right of an individual and the outcome of factors related to diet on health has been matter of apprehension since ancient times. Healthy and fresh food always demanded by the consumers. Modern research has find out many alternatives of traditional packaging. Now the consumer knows that good packaging system is that which protects the food from the contaminants and increases shelf life of food product. While in Pakistan about 40% of fruits and vegetables lost due to spoilage caused by poor handling, transportation, and poor packaging interaction with other environmental conditions. So it is crucial for developing countries like Pakistan to pay attention to these exacerbating situations for economy losses by considering food packaging an ultimate solution to the problem.

Keywords: packaging, food safety, antimicrobial, food losses.

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Antmicrobial Packaging, a Step towards Safe Food: A Review

Muhammad Afzaal [α], Urooj Khan [σ], Hafiz Arbab Sakandar [ρ] & Muhammad Nadeem Akhtar [ω]

Abstract- Food is the primary concern of living organisms, provision of diet for maintenance of good physical and mental health is a basic right of an individual and the outcome of factors related to diet on health has been matter of apprehension since ancient times. Healthy and fresh food always demanded by the consumers. Modern research has find out many alternatives of traditional packaging. Now the consumer knows that good packaging system is that which protects the food from the contaminants and increases shelf life of food product. While in Pakistan about 40% of fruits and vegetables lost due to spoilage caused by poor handling, transportation, and poor packaging interaction with other environmental conditions. So it is crucial for developing countries like Pakistan to pay attention to these exacerbating situations for economy losses by considering food packaging an ultimate solution to the problem.

Keywords: packaging, food safety, antimicrobial, food losses.

I. Introduction

Food contrasting to resilient goods such as furniture, electronics and metals are usually perishable products and heterogeneous mixtures. It will be the most difficult task to prepare such mathematical models which are representative of physical and chemical properties of food to forecast their behavior during processing. In addition, food has safety aspects and fairly very short shelf life. Therefore, the packaging of food is relatively different from that of other durable products (Han, 2013).

In early history, widely using and the first active packaging material was wine. Packaging was done with wine skin, but it impart some unwanted conditions like interaction of wine with food, increasing quantity of oxygen inside the package which cause oxidation of some foods after this the most commonly used active packaging technique was named canning.

Tin plates were used for the manufacturing of cans. Tin was sacrificially corroded, thus protecting food from big amount of iron by protecting iron support of can. Then it was indicated that this increase auto-oxidation when residual oxygen is present. As well as tin also act as reducing agent for the food such as pigment degradation. It also impart its flavor as in traditionally canned orange juice which effect the food quality and hence consumer acceptability. So, Aluminum cans were introduced which are economically beneficial and do not generate any additional flavor to food(Rooney, 2005).

Good packaging system is that which protects the food from the contaminants and shelf life limiting factors. As well as packaging must be inert, prevent microorganisms, non-toxic, stable and also protect food from the aspects which deteriorate food quality during transporting and handling (Sung, 2013).

Mostly the food products prepared at a place must come to the consumer and for that they have to travel. So, Packing give benefit to the consumer, as packaging protects the food and let it travel to come to us in safe manner, doesn't matter how long distance has to be covered. A well designed packaging is eye-catching and alluring to the consumer, and assures the demand of Product Safety. In Addition,

a) Packaging is something to provide with the inert barrier to the packaged food and to protect it from the external environment thereby reducing spoilage and assure the food to be completely hygiene and safe, thus also reducing the rate of food wastage through contamination.
b) Some types of packaging also extend the shelf life of food.
c) Some packaging systems are also used for safe and hygiene transportation of the food product.
d) While packaging is also used to provide the customers with the information about food they are going to purchase, it fulfills some legal requirements as well (Suet-Yen et al. 2013).

The public demand of extended shelf life of food products, food safety has become the major element of interest especially in last decade. Public is on track to concern with food and water requirements in the figure of bioterrorism (P. Appendini, & Hotchkiss, J. H. , 2002).

Traditional methods for the preservation of food include, dehydrating, freezing, irradiation, modified atmosphere, refrigeration, these were applied efficiently long times ago. They are now being used but in relatively lesser extent. All these techniques have some restrictions. In relation to the demand of today these are not very much efficient. Thus for the anticipation of the attack of microorganisms, fungal spores, environmental conditions and other food destroying means we have to

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use the latest technique of packaging, named "Antimicrobial Packaging" which is a promising application to achieve this goal(Quintavalla, 2002).

About 90 million tons of the food is annually wasted in the Europe in which 12% are fruits and vegetables, 9.5% are seafood, 4% meat is wasted in distribution and retail, according to European commission. Round about 1.3 billion tons of food regarded as one third for human consumption is wasted globally every year. In industrialized countries the ratio of food wastage is high as compared to the developing countries, about 40% of food loss in developing countries at harvest and processing conditions while in industrialized countries about 40% of food loss occur at marketing and consumer level (Robertson, 2002).

While in Pakistan about 40% of fruits and vegetables lost due to spoilage caused by poor handling, transportation, and poor packaging interaction with other environmental conditions.

Antimicrobial packaging is the result of the demands of progressively more complex society, which is more advance and innovative. For this to give extra function to the packaging different varieties of active substances are now incorporated into the packaging material.

When antimicrobial agents are integrated with polymer, then the resultant microbial growth stops for further. This application is used for food not only in the form of films but also in containers or utensils. Antimicrobial property of system is the blessing of antimicrobial substances. The new methods assisting antimicrobial packaging includes, Addition of sachets/pads containing volatile antimicrobial agents into packages, Incorporation of volatile and non-volatile antimicrobial agents directly into polymers, Coating or adsorbing antimicrobials onto polymer surfaces, Immobilization of antimicrobials to polymers by ion or covalent linkages, Use of polymers that are inherently antimicrobial(P. Appendini & Hotchkiss, 2002).

The antimicrobial packaging reflects the concept of protection against harmful environment in addition to the harmful or pathogenic microorganisms which are the main cause for limiting the shelf life of food. Antimicrobial property can be achieved by the addition of antimicrobial agent into the packaging system, and to go well with conventional packaging requirements. We can also use antimicrobial polymers. The mechanism of action of antimicrobial packaging is that, agents used for this purpose have to prolong the log phase and reduce the growth rate of microorganisms and to prolong the shelf life of the products (Han, 2013)

To add antimicrobial action into the packaging material, antimicrobial agents are incorporated. For example, antimicrobial activity of the food preservative-releasing films is due to the release of preservatives as antimicrobial agents at controlled rate. Oxygen absorbents work by reducing the head space oxygen and preventing the aerobic spoilage of food like mold growth (Duncan, 2011).

Some preservatives are used as antimicrobial agents for the food products such as organic acid and their salts, nitrates, alcohols, sulfites and antibiotics. For Example, for the packaging of food product, sorbic acid and potassium sorbet are used. They are used by incorporation with wax layer which is applied to the packaging material (RD, 2007).

Antimicrobial packaging has been divided into two main groups, one is biodegradable and the second is non-biodegradable packaging. Most extensively used and the most synthetic polymers are non-biodegradable. Their plus points are the cause of their advantages, like low cast, inert, low density, fine barrier property, excellent mechanical strength, high transparency as well as they have the capacity to be heat-sealed which is made the printing easier(Gemili, Yemenicioğlu, & Altınkaya, 2009).

Most widely used packaging plastics such as, low density polyethylene (LDPE),propylene (PP), linear low density polyethylene (LLDPE), ethylene vinyl acetate (EVA), high density polyethylene, polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and nylone/polyethylene bags are used. While on the other side, biodegradable films are produced by the agents having natural antimicrobial property or by the incorporation of antimicrobial agents into natural polymer. Chitosan is the natural polymer with inherited antimicrobial property and is one of the ideal biodegradable film (Sung, 2013).

On the other hand, garlic oil is also beneficial in suppressing the growth of decomposing bacteria when used into the packaging system. The use of Natural and synthetic antimicrobial polymers is also common in antimicrobial food packaging world. Another promising application is the use of Lysozymes. Additionally, the mono and multi layer packaging is the technique which is widely used in the packaging material. Ethyl Loryl Arginate (LAE) is also used against microorganisms in the food packaging system. A newly launched technique in antimicrobial packaging system is known as nanotechnology (Ferrari et al., 2009).

Other numerous natural substance are being used including grape fruit extract to bacterially synthesized antimicrobial peptides named as bacteriocins Nisin is a bacteriocin which is used for the antimicrobial purpose in packaging system. Other newly introduced substances for antimicrobial purpose include Millard reaction products. Carbon dioxide is also used for preventing the growth of microorganisms, sometimes it become more efficient with oxygen while sometimes the presence of oxygen is not good for its antimicrobial activity (Trinetta, Cutter, & Floros, 2011).

Titanium dioxide is also rarely used in selective food products for its antimicrobial activity. Irradiation is

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also employed for the sterilization purpose. Antimicrobial aspect of food preservation also takes the account of self safety of food, that is the food should be free from microorganisms and also decreases the chances for further microbial growth. So, juice Pasteurization is one of the processes which follow this aspect (S.F. Aguilar-Rosas et al. 2007).

Future aspects of antimicrobial food packaging provide the promise of safety, quality protection and shelf life extension of food. Industries can effectively reduce their spoilage, minimize waste, and can grow their economic status as well by following the intention of introducing antimicrobials into the food packaging materials, which will comply with the consumer demands for food as antimicrobial packaging is determined to prevent the spoilage and put a stop to food-borne illness caused by microorganisms which vigorously contaminate the food products.

II. Characteristics of Packaging Material

a) Physical Characteristics of the Packaging Materials

When the antimicrobial character is supplemented to the packaging material, consequence comes with the change in its physical/mechanical properties in some aspects. General material properties of the packaging materials take account of mechanical characters like stiffness, tensile strength, burst strength, elongation, tearing conflict, and physical characters including transparency, oxygen permeability, gloss wettability, water vapors permeability, water absorptiveness, wettability, vividness, wettability, haze with others.

The action of packaging material can be sustained by the adding antimicrobial agents, even throughout the substance having heterogeneous composition. In case of plastics are added in very small amount and are generally lesser molecular weight chemicals in contrast to the dimension of polymeric structures. In an appropriately premeditated antimicrobial packaging system, chemicals amend their positions in shapeless structural states of the polymeric structure and are might ineffective for the mechanical potency of polymeric packaging system(P. Appendini & Hotchkiss, 2002).

III. Effects of the Antimicrobial Substances on Mechanical and Barrier Properties

Integration of antimicrobial agents into the polymer can badly affect the mechanical integrity, physical properties and thermal constancy of packaging when the antimicrobial agents used are not attuned with the polymer. Whereas, antimicrobial agents that are compatible with packaging materials can saturate well into spaces among the polymer chains. In other words, it does not manipulate the film properties when appropriate amount is added. Hence, the study of polymer structure and its chemistry are important in forecasting the influence of specific antimicrobial agents on the packaging. Consequently, the selection of AM agents, incorporation methods and packaging polymers can be more effectual (Han, 2013).

IV. Edible Antimicrobial Films and Coatings

Edible antimicrobial films and coatings are prepared from the proteins, polysaccharides and lipids. These antimicrobial agents are being applied to the food exteriors with direct contact. These are also used for a variety of muscle foods and fruits. Example: The alginate coating which contain organic acids is applied on beef carcassesis slightly effective, suppressing the level of *Salmonella Typhimurium, Listeria monocytogenes, and Escherichia coli (Han, 2013).*

a) Organic Acid

Propionic acid, benzoic acid, Sorbic acid, propionic acid, with their salts are being extensively employed to avoid the spoilage of food by microorganisms.

b) Sorbic Acid Film

It is a conventionally used preservative, having no harm to food and consumer health. It is permitted according to the law of various kinds of foods. Sorbic acid films impart no allergenic or health hazard. Lacquer contains antimicrobial agents embedded in it which is at the surface of packaging material. The lacquer follows a consecutive discharge of sorbic acid in small amount to the surface of the food packed in system. In this manner, the safeguard of the packed food from microbial contamination can be offered at contact spot.(Stewart et al., 2012).

c) Film preparation

Polyvinyl acetate is stirred in the ethyl acetate and dissolved properly at room temperature. Sorbic acid is introduced into the lacquer when the polyvinyl acetate is completely dissolved. After this, the lacquer is applied on an ordinary polyethylene packaging film by a lab bench coater and then it is applied at polyethylene polyamide composite film respectively. Finally the lacquer is dried out by air(Hauser, Müller, Sauer, Augner, & Pischetsrieder, 2014).

d) Potassium Sorbate

A potassium salt of the sorbic acid which play significant role to inhibit fungal action, is known as Potassium sorbet .It is employed for the preservation purpose to the dairy products, cheese and kinds of dough (Realini & Marcos, 2014).

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e) Plant essential oils

There are some natural plant extracts which have antimicrobial property. These agents are safe and are proved to improve food safety. Plant essential oils are rich in volatile phenolic particles and terpenoids, they are greatly probable to hold back a wide range of microorganisms. Usually, the active components of plant essential oils retard microorganisms by disturbing their cytoplasmic membrane, disrupting the electron flow, proton motive force, inhibition of protein synthesis and active transport and inhibition.

Those natural agents which do not pocess any note worthy environmental ormedicinal impact can possibly be served as an efficient substitutes to the conventional antifungal or antibacterial comounds.

Examples of plant extracts and essential oils which are most widely incorporated into the food packaging are thymol, carvacrol, cinnamaldehyde, clove oil, linalool and basil essential oils.(J. P. Smith, Ramaswamy, & Simpson, 1990).

By using paraffin coatings the potential utility of active paper manufacturing was tested in assay a number of common bacterial and fungal deteriorates of food. In these tests, the fortified cinnamon essential oil paraffin varnish totally inhibit *Eurotium repens, Aspergillus flavus* and *Candida albicans*, and provide important role against *Penicillium nalgiovense* as well as *Penicillium roqueforti*. Mostly the foods with this type of packaging are often fruits and vegetables which are more vulnerable to spoil by fungi as compared to other agents. On the other hand, no suppressing was observed in the case of tested Gram-positive bacteria *(Bacillus cereus, Staphylococcusaureus, Enterococcus faecalis and Listeria monocytogenes)* and the active coating which reflects suppressing action against Gram-negative bacteria was supplemented with cinnamon essential oil, while oregano essential oil to a lesser extent. The first analysis of active coating generated atmospheres was through single drop microextraction (SDME) which had been trailed by the technique gas chromatography–mass spectrometry (GC–MS), it showed major dissimilarities among them. The essential oil percentage added to the coatings had considerable effect on their potency to hold microorganisms (Stewart et al., 2012).

f) Garlic Oil

It is declared by research that the incorporation or contact of garlic oil reduce the number of gram-negative and gram-positive bacteria when used in a little amount in edible films. An experiment was shown that 0.2% w/w garlic oil-incorporated alginate film retarded the growth of Bacillus cereus and *Staphylococcusaureus*. Blown film extruder (40-50 micro meter) was used to prepare these films.

g) Film preparation

LDPE (low density polyethylene) film were incorporated with different amounts of garlic oil ranging from 0, 2, 4 6 and 8 weight percent (% w/w). A master batch preparation was prepared by grinding the EVA (ethylene vinyl acetate copolymer) to powder form by using ball mill grinding machine and 2% w/w of garlic oil was also added in it.It was then mixed carefully by using tumbler, LDPE pellets were supplemented into it and mixed to get 5 batches. The procedure was repeated with 4, 6 and 8% w/w of garlic oil respectively. These films were fashioned by blown film extruder. The temperature of 170 oCwas maintained throughout all the regions from barrel to die, and the thickness of target film was 40e50 mm. To prevent the loss of evaporation of antimicrobiual agents, the extruded films were wraped into aluminium foil(Ferrari et al., 2009).

V. Antimicrobial Polymer Ackaging

a) Antimicrobial Polymers

Polyanhydrides are used in food, medical and personal care controlled release systems. Depending upon the antimicrobial agents, the polymers degrade accordingly. The polymer can be incorporated with other antibiotics or antioxidants and can be used in many ways i.e. films (Anastasiou et al. 2003), (Whitaker-Brothers et al. 2004). Recent studies revealed the effects of polyanhydride polymers on biofilm formation and noticed that the release of salicylic acid from polymer films can retard *Salmonella typhimurium*. These results were very effective, especially biofilms are highly resistant on the other hand, eradicating persistent biofilms is difficult because they require action of antimicrobials and physical removal (P. Appendini, & Hotchkiss, J. H.,2002).

b) Synthetic Polymers

Plastics are considered of being polymer of choice for food packaging with high performance in low cost. The cost of plastic is reduced by viewing its vast use in packaging field with the inherent possessions of barrier material Another incredible role playing material, known as Biodegradable polymer which is created from the marine source or agricultural raw material and can be broken down by chemical or biological reactions. For over 3 decades these are used for biomedical purposes and are achieving popularity among plastics Their limitations include the mixing of various polymers to achieve a particular packaging goal. The underlying principle for using combination of different polymers is to achieve the variations in the polymers' chemical and mechanical properties (Dill, 2010).

c) Polymer films (LDPE)

Films of low density polyethylene (LDPE) are used for packaging systems which directly contact the food. Therefore, among the synthetic polymers LDPE

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films are extensively used in food packaging materials for purpose oftheir antimicrobial action.

d) Polyethylene/Nylon Bags

Commercially existing polyethylene/nylon bags of 95 μm thickness were used for sample packaging purpose. These were facilitated with 1.64 g/m2 transmission rate of water vapor day1 and the transmission rate of oxygen 50.65 cc/m2 day1.Nylon 6,6 films which were irradiated by the laser of 193 nmin air showed the antimicrobial activity, it appears as it was due to the transformation of amide groups by 10% on the nylon surface to the polymer chain bound amines(P. Appendini & Hotchkiss, 2002).

VI. Mono and Multilayer Antimicrobial Food Packaging

Mono and Multilayer packaging having antimicrobial potential as well is used for the successive discharge of antimicrobial material. Monolayer is a single layered film structure while multilayer is tri-layered film system in which the primary layer called outer barrier, which protect the food from environmental contaminants, secondary layer is known as matrix layer which contain active agents, while tertiary layer gradually discharge the antimicrobials into the product(Dill, 2010).

VII. Addition of Antimicrobial Sachets to the Packages

Some specific antimicrobial agents are often enfolded in individual sachets/pads which are fixed inside the packaging system. The antimicrobial agents thus released in vapor form to the packaging-headspace and be in contact with foodstuff. Three forms of which are preponderated moisture absorbers, oxygen absorbers and ethanol vapor generators.

a) Moisture Absorbers

Moisture absorbers, also known as desiccants are generally used for suppressing moisture in food products. In soild foodstuffs, some amount of moisture is trapped, penetrated or generated inside the package, which may cause spoilage of food or deteriorate its quality. Most of the events cause increased moisture such as moisture due to horticulture product respiration, temperature changes inside the package including high relative humidity, melting of ice, or drip loss from cut meat and its products. This can be minimized by using humidity buffering or liquid water absorption technique in packaging systems (Quintavalla, 2002).

b) Oxygen Absorbers

The key factor of reducing shelf life of the product is the presence of oxygen in it, which causes change in color, flavor and odor with the wastage of important nutrients also promoting the growth of aerobic bacteria, insects and molds. Therefore, it is basic challenge for the present scientists to remove oxygen from the package headspace. Oxygen scavenging system is promising for the inhibition of aerobic bacteria as well as molds, employed mainly for bakery and dairy products. Generally, an oxygen concentration of about 0.1% by volume or low is required in the headspace for successful antimicrobial packaging. Oxygen absorbing agents which are used for commercial purpose are enzymes, ascorbic acid, iron powder, unsaturated fatty, photosensitive dyes and immobilized yeast at solid substrate. A substitute of sachets engages with the integration of the oxygen scavenger in the system itself. This results in minimizing of pessimistic consumer retort while proposes good economic benefit by amplified outcomes. This eradicates the threat of unintended consumption of the components by rupture of sachets as well (J. P. Smith, Ramaswamy, H.S., and Simpson, B.K, 2003).

c) Liquid water absorption

Chief intention to employ liquid water absorption technique is to subordinate water activity (aw) in food product, thus the growth of microorganisms automatically decreases in applied food product. It works by having a superabsorbent polymer which is situated among two layers of nonwoven or micro-porous polymer. These sheets are being utilized for the absorption of drip loss in the form of absorption pads which are introduced inside the chickens cuts or the whole chicken. Sheets of large size are also introduced in air transportation process of packed seafood for the purpose to absorb melted ice. Graft copolymers of starch and polyacrylate salts are the most favored polymers employed for this principle.

d) Humidity buffering

The system is engaged with the interception of water vapors through falling down the relative humidity range in the pack and consequently the surface water contents of the product. It could be attained by a moisture-absorbing sachet or by placing humectants among two coats of a plastic film which is well permeable to the water vapor (Duncan, 2011).

e) Ethanol-Vapor Generator

Packages including antimicrobial agents like ethanol, chlorine dioxide and sulfur dioxide are known as volatile gas formers, and are used at commercial scale for food preservation. Package which contains ethanol is commonly known as ehanol-vapour generator. Food grade ethanol is enclosed in sachet, the fine fabric of that sachet gradually release ethanol vapors, which prevent microbial spoilage(Han, 2013).

f) Ethicap (ethanol-vapour generator)

Ethicap, an ethanol vapor emitter, is a commertially used product. It is a paper wafer, consists of ethanol-silicon dioxide in acetic acid which is sandwiched between plastic films layers which are

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permeable to ethanol. The ethanol vapors are thus released into packaging headspace and result in suppressing the mold growth (J. P. Smith et al., 1990).

VIII. Other Antimicrobial Food Packaging Techniques

a) Ethyl Lauroyl Arginate (LAE)

Ethyl lauroyl Arginate (LAE) is synthesized by reacting tionyl chloride, l-arginine•HCl, sodium hydroxide, lauryl chloride ethanol and deionized water (Y. Kawamura 2008). Ethyl lauroyl Arginate is food grade, soluble in water, having strong antimicrobial action against an extensive variety of food spoilage microorganisms and pathogens having cationic surfactant property It is introduced as an antimicrobial agent in the packaging materials It is commercialized in solution form or as a white powder. It is mainly used as an antimicrobial agent against moulds, Gram-negative and Gram-positive bacterial species. It retards microbial growth by influencing microbial proteins which are negatively charged, as they are present in the enzymatic system or in the cell membrane of bacteria, the cationic surfactant character of Ethyl lauroyl Arginate cause their denaturation, hence results in the death of microorganism. It does not effects the properties of packed food as it does not impart any taste or smell. Maximum permit tedamount of Ethyl lauroyl Arginate is 115–225 mg kg‑1, depending upon the category of food packed. It has high chemical and thermal stability. It is experimentally proved that it is effective in inhibition of the population of microorganisms such as *Listeria monocytogenes* Moreover, the antimicrobial action of LAE can be improved by making its interaction with other charged substances in specifically intricate environment (Walsh & Kerry, 2012).

IX. Nano Technology

This term deals with the fabrication or the manipulation of structures, strategies, organizations or material which have leastwise one dimension of around 1-100 nanometer by length. This application doesn't follows the method of directly adding nano particles into the food product, that's why this would be preferred in food packaging systems, thus are more anticipated to be introduce to the public in quick-fix.

a) Lysozyme (enzymes)

Lysozyme is the enzyme obtained from hen egg white. It is categorized as the natural antimicrobial agent and is generally incorporated into the food packaging materials. It is efficient in opposition to pathogenic and spoilage microorganisms as it destroy the structure of their cell wall.

b) Chitosan

Chitosan is a natural polymer which acquires inherent antimicrobial activity. It is a model degradable film but is expensive to create. Two major characteristics of chitosan made it prominent in antimicrobial world are its inherent antimicrobial property and its biodegradability. It can be used in many ways as an antimicrobial purpose, either by its own or by mixing it with some other polymer as it reduces cost and give structural support as well.

c) Film production

Chitosan films were produced by mixing dried chitosan powder with acetic acid, and then the solution was being spread gently on glass plates. These plates were then dried overnight at room temperature. Chitosan retards a broad spectra of bacteria, yeast and moulds by causing their intracellular material to leaked out by damaging the cell membrane of bacteria which directed to bacterial cell death (Trinetta et al., 2011).

d) Triclosan

Triclosan is an inorganic antimicrobial agent. Afascinatingviable technique is Triclosan antimicrobial packaging including agents like Sanitized®, Microban® and Ultra-Fresh®. Triclosan incorporated plastic (TIP) is a good application used against populations of food borne pathogenic bacteria. Vermeiren and others in 2002documented that Low density polyethylene films which contain triclosan of about 0.5 and 1.0% by weight in the in agar diffusion testshow antimicrobial activity in opposition to *Brocothrix thermosphacta,L. monocytogenes, Brocothrix thermosphacta, Salmonella enteritidis, Escherichiacoli* O157:H7and *S. aureus*. Film having triclosan 1.0% by weight has a powerful antimicrobialaction in in-vitrosimulated vacuum systemin opposition to the psychrotrophic pathogenL. *Monocytogenes* (Realini & Marcos, 2014).

e) Nisin (bacteriocin)

Nisin is a bacterioc in which is generated by the bacteria named "*Lactococcus lactis*", and has been used as natural preservative, while bacteriocin is defined as the compound having antimicrobial property and is produced by microorganisms. It is known to use for the preservation of cheese. It is predominantly useful against the growth of Clostridium botulinum. Nisin is a natural antimicrobial agent and is permitted by U.S Food and Drug Administration (FDA).

Other than natural antimicrobial substances obtained from animals, plants, and essential oils, AM agents which are produced by bacteria (bacteriocin) are progressively gaining recognition to meet their capability to endure with acidic environment as well as high temperatures. Bacteria produce it as their metabolic byproduct (antimicrobial peptide) by their defense system from nearly all kinds of bacteria. This natural process permits the bacteria of one stain to retard the growth of other one of adjacent stain. Bacterioc in which is generated from lactic acid bacteria (LAB) is greatly accepted by public, because Lactic acid bacteria is

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being has been used from centuries for fermentation purpose. Traditional fermented foods such asmiso, bean paste, cheese, soy sauce, win, kimchi etc. are consumed from centuries. Nisin, generated by *Lactococcus lactis*bacteria is usually present in milk and is certified as "Generally Recognized as Safe" (GRAS) antimicrobial agent by the U.S Food and Drug Administration (FDA), hence utilized commercially for the purpose of food preservation. Other bacteriocins such as Enterocin 416K1, enterocins A and B, pediocin AcH and Sakacin have been proved to hold the growth of *L. monocytogenes* in synthetically contaminated foods. (Suet-Yen Sung & Han, 2000)

f) Maillard reaction products (MRPs)

In addition, Maillard reaction products (MRPs) are also being used for the purpose of antimicrobial food packaging. Millared reaction can be defined as the reaction in which carbonyl compounds (reducing sugars) react with amino acids either in free or bound form, while the resultant product formed are called Maillard reaction products, for example the products formed at some stage in baking bread, heat processing of milk, or roasting of cocoa beans (Hall, 1995).

In current years, antimicrobial activity of the Maillard reaction products produced by representative mixtures (heated solutions of reducing sugar with amino acid) of food extracts were studied. Therefore, the antimicrobial action of Maillard reaction products by model mixtures was identified against various strains of bacteria like *Typhimurium Salmonella, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Aeromonas hydrophila,* or *Helicobacter pyloriand* also against different types of yeasts (Einarsson et al. 1983).The present study illustrated the use of Maillard reaction products as successful antimicrobial components to be used in active food packaging systems (packaging films).

g) Film Preparation

Aliquots lyophilized MRM85EtOH and polyvinyl acetate of 2.5 g by each were homogenized in 20.0 g 85% ethanol with the help of vortex mixer. This mixture was then applied on LDPE film which was previously treated with corona (Wipack, Walsrode, Germany) through a laboratory bench top coating system (K303 Control Coater, Erichsen, Hemer, Germany). The obtained filmswere then dried and stored for 24 hours at the temperature of 23ºC with relative humidity of 55% (MRM85EtOH-film) (Robertson, 2002).

h) Carbon Dioxide

Carbon dioxide (CO_2) is also utilized against microbial growth. Relatively high intensity of carbon dioxide about 60 to 80% inhibits the microbial growth on surfaces, hence prolong the storage time for food product. Consequently, packaging structures are impregnated with carbon dioxide generating system or added later in the form of sachets, this approach is complementary to the oxygen scavenging system. Combination of carbon dioxide generators with oxygen scavengers is also being used in the packaging systems in which the appearance and volume of package is critical, this is done for the prevention of package to collapse by oxygen absorption. CO_2 generator as its own is employed only in specific requests such as poultry, cheese, fish and fresh meat packaging. So, they recommended a combined treatment which consists of oxygen scavenging with refrigeration or thermal processing system, or using the atmosphere with added carbon dioxide for the absolute retardation of these microorganisms form food products. They concluded that the combination of carbon dioxide and oxygen absorber retarded the growth of *Clostridium sporogenes* while the combination of oxygen absorber and carbon dioxide generator promoted its growth. So, it shows that the selection of proper combination for specific microorganism is most important (Gemili et al., 2009).

i) Titanium dioxide

A U.S FDA approved, non toxic compound Titanium dioxide (TiO_2) has been utilized in foods and in its contact packaging materials. In titanium dioxide packaging titanium ions are used against the growth of microorganisms.

j) Incorporation of Antimicrobial Agents directly into Polymers

Chemical preservatives are also added in packaging materials to introduce antimicrobial activity in them. For example, films which release preservatives at controlled rate offer antimicrobial activity as they gradually diffuse the preservatives which suppress the conditions for food spoilage by microorganisms. Among all the antimicrobials, the extensively utilized in food packaging systems as polymer additives is silver substituted zeolite, especially in Japan (P. Appendini & Hotchkiss, 2002).

k) Coating or Adsorbing of Antimicrobials to Polymers

Fungicides are added into waxes and are coated to fruits and vegetables to meet the requirement of antimicrobial food packaging. Such as, LDPE (Low density polyethylene) film was incorporated with Nisin by means of methylcellulose / hydroxypropyl methylcellulose (MC/HPMC) which acted as a transporter (Gemili et al., 2009).

l) Immobilization of Antimicrobial agents by Covalent or Ionic linkagewith the Polymers

Besides sorption and diffusion, some antimicrobial packaging systems uses covalently immobilized antimicrobial agents against microorganisms. This immobilization scheme works on the principle of having functional groups on

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antimicrobial as well as polymer, such as organic acids, enzymes, peptides and polyamines(RD, 2007).

m) Irradiation

In food packaging systems irradiation is used to sterilize packaging material and food to some extent. Radiation technique may includelaser-excited, radioactive materials, ultraviolet-exposed films, radioactive materials, or far-infrared-emitting ceramic powders.

Gamma-radiation is used against microorganisms to the packaging systems which aids in the aseptic packaging of acidic foodstuffs. The amount of about 1.5 Mega-radian (Mrad) is used for decontaminating acidic food containing equipments Packages are sterilized in massive amount at commercial irradiators. Recently, irradiation for aseptic packaging of low acid foods is also approved. Radiation for decontamination is being used in more amounts for low acid foodstuff than that of acidic ones. Aseptic packaging system only uses gamma-radiations while others are not being utilized extensively in aseptic packaging. The ultraviolet light (UV-C) is also employed for the decontamination of food containers but it also has some drawbacks, including low penetration and troubles related to 'shadowing' which restrict the employment of Ultraviolet light (UV-C) for aseptic packaging of the products with low acid contents.Whereas cost, size and speed of equipments have not allowed the employing of electron beam irradiators till yet. (Stewart et al., 2012)

n) Aseptic packaging System

The commercially sterilized foodstuff packed into a sterilized container which is then hermetically sealed beneath aseptic conditions to prevent reinfection. Basically there are two fields of aseptic packaging process to apply.

Packaging of sterile and pre-sterilized products. For examples sauces, puddings, soups, desserts, fruit and vegetable juices, milk and dairy products.

Packaging of the non-sterilized food for the purpose to prevent contamination from harmful microorganisms. For example fermented dairy products like cheese and yoghurt.

o) Scheme for Aseptic Packaging

Aseptic processing embraces following steps:
Sterilization of foodstuffs prior to filling.

Sterilization of equipment or containers used for packaging and finishes prior to filling.

Sterilization of the aseptic installation prior to procedure (UHT unit, sterile gases, lines for products, filler and related machine parts)
Preserving sterility all over the system throughout the process include sterilization of the entirestuff ingoing the system as gases, water, air.

Manufacture of sealed packaging systems.

i. *Conventional Food Packaging*

Conventional methods of food packaging are also being used for the intention of antimicrobial food packaging, in which canning is the most universal and broadly used method.

ii. *Canning*

Canning is a conventional food processing technique in which the food is treated before filling. Preliminary procedures inactivate the enzymes so that these will not be able to decompose or spoil the food in processing operations. Package has cleaned firstthen foodstuff (usually hot) is filled in it. Usually air is being removed from inside as a measure to prevent oxidative damage. The package is sealed hermetically and heat is applied. The final requirements of package include it to resist high temperature to about 100°C recommended for high acid foodstuffs while for low acid foods it is up to 127°C s, with the heat applied to destroy all the microbial spores which are heat-resistant as well. Packages which contain food of low-acid with pH above 4.5 should be endured with pressure too.

iii. *Bulk Aseptic Bags*

A bulk aseptic bag can be described by a structure with multi-layer which consists of an external blockade cover and an internal bag whichis in contact with the foodstuff. Bags are pre-sterilized from inside by means of gamma radiations and are supplied flat. Gamma irradiation intensity is exclusively chosen for packaging consists of low or high acid foodstuffs.

iv. *Aseptic Bulk Packaging*

The method 'Aseptic Bag-In-Box' provides packaging of foodstuff with high as well as low acid contents, while their capacity of product containing ranges from 25 liters to 1140 liters. Some packaging applications using this system are milk and cream, purees, fruit Juices, tomato products, concentrates, coconut products and jam.

v. *Bulk Filling Machine Mechanism*

The packaging systems are developed from a wide range of laminates compatible with food which is to be packed and prevent spoilage to the required extent. The packaging systems considered for aseptic filling purpose are granted with the patented spouts. Before delivery the packages are sterilized from inside and are supplied flat avoiding the entrance of gas or air. These are offered in capacity ranging from the size of catering to the sizes planned to transport the foodstuff by the producer/ farmer to processor, packager and dispenser. When all of the packages are filled then is projected to sustain the external container, for example heavy-duty box or a drum.(Realini & Marcos, 2014).

X. Conclusion

There are numerous ways of food packaging among them antibacterial packaging is most updated

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and important. Antibacterial food packaging is playing a vital role in the regime of food packaging and is also influencing developing countries after fascinating the developed world.

It has positive effects on food and has tackled the 30 to 40 percent losses of food commodities worldwide.

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Child Malnutrition and Low Access to Health Care Facilities in Mumbai Metropolitan Region

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Abstract- The child malnutrition is widely viewed in slums of Mumbai Metropolitan Region. The incidence of underweight and stunting is higher among female as compare to male. The undernourishment among children is related to parent's education and health status. Media exposure and health care access is low among women. Most of the women are working as unskilled labors in suburbs. Child care does not exist across the slums. The women have to keep children at home, relatives or neighbors. Most of the undernourished children have cough, cold, fever and diarrhea. But few children are treated in public health care facilities. The logistic regression shows that underweight among children is negatively co-related with age, mothers normal BMI, ANC and anganwadi food. Stunting is negatively co-related to age, mother's secondary school, per capita income, ANC, normal delivery and anganwadi food.

Keywords: *health care, sanitation, water supply.*

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Child Malnutrition and Low Access to Health Care Facilities in Mumbai Metropolitan Region

Sanjay Rode

Abstract- The child malnutrition is widely viewed in slums of Mumbai Metropolitan Region. The incidence of underweight and stunting is higher among female as compare to male. The undernourishment among children is related to parent's education and health status. Media exposure and health care access is low among women. Most of the women are working as unskilled labors in suburbs. Child care does not exist across the slums. The women have to keep children at home, relatives or neighbors. Most of the undernourished children have cough, cold, fever and diarrhea. But few children are treated in public health care facilities. The logistic regression shows that underweight among children is negatively co-related with age, mothers normal BMI, ANC and anganwadi food. Stunting is negatively co-related to age, mother's secondary school, per capita income, ANC, normal delivery and anganwadi food. Stunting is positively co-related with father's moderate BMI. Therefore the policies are required to provide basic amenities such as health care, water supply, sanitation and electricity in slums. Government and NGO's must prepare the maternal child health related programs and run on television and radio. The state government must provide vocational training to youths. Such policies will reduce the malnutrition among children at some extent in slums of Mumbai Metropolitan Region.

Keywords: health care, sanitation, water supply.

I. Introduction

Malnutrition among children is the world's most serious health problem. It is the single biggest contributor to child mortality. Nearly one-third of the children in the developing world are either underweight or stunted and more than thirty per cent of the developing world's population suffers from micronutrient deficiencies. Asia continues to have both the highest rates and the largest numbers of malnourished children in the world (Khan A.A. et.al 2006). In India, half of the children are malnourished. Poor growth is attributable to a range of factors closely linked to overall standards of living and the ability of populations to meet their basic needs (Joshi H. S.et.al 2011). The malnutrition among children has short and long term effects.

Malnutrition affects physical growth, morbidity, mortality, cognitive development, reproduction, and physical work capacity, and it consequently impacts on human performance, health and survival. It is an underlying factor in many diseases for both children and adults (Salah E.O. et.al. 2013). The malnutrition not only affects individual but its effects are passed from one generation to the next generation. The malnourished mothers give birth to infants who struggle to survive and develop. If these children are girls then they often grow up to become malnourished mothers themselves. Therefore malnutrition among children is a long term issue (Gillespie and Haddad 2003).

Maharashtra is one of the highly industrialized and economically developed states of India. Mumbai Metropolitan Region is the centre of commercial and economic activities in Maharashtra. The higher incidence of malnutrition among children is not expected in this region. But there is rapid migration of rural people into the metropolitan region. It has put extensive pressure on existing civic amenities. The demand for housing is not equal to supply of housing. It has resulted in higher prices of houses. The poor people cannot afford the good housing at lower price in region. It has resulted into the development of slums in a large number of places within the region. The density of population is always high in the slums (Haque et al. 2014). It has resulted into overcrowding, unhygienic and poor sanitary conditions, along with economic insolvency. Therefore it leads to malnutrition and poor health conditions of children. The health care facilities, water supply, transport are spread unevenly in suburbs. Such amenities have effects on the nutritional outcome of children. At early age, children are affected adversely due to unhygienic environment in slums. The physical growth of the children has affected due to lower socio-economic status of families and infrastructural facilities. Low educational status of parent does not support children to grow with good nutritional status. Educated mothers understand the importance of medicines and access various community level services. Similarly, educated mothers are more conscious about their children's health; they tend to look after their children in a better way. Health status of the father is important to earn higher income. Usually father is the main earner and decision maker of a family and so their higher level of education plays an important role to ensure better nutritional status of children. Healthy mothers take good care of their children. They can breastfeed for longer duration and provide adequate supplementary food. For smaller children, continuous emotional and physical care is required at early stage. The malnourished

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mothers cannot provide sufficient breastfeeding because of their nutritional deficiency. Mass media provides knowledge of breastfeeding, antenatal, postnatal care and immunization. Women are expected to get knowledge through newspaper, radio and television. There are number of programs shown for women and children on television and radio. The pregnant women should get at least four antenatal check up and medicines such as folic acid tablets and injections. But the women with low status tend to have weaker control over household resources, tighter time constraints, less access to information and health services, poorer mental health, and lower self-esteem. These factors are thought to be closely tied to women's own nutritional status and the quality of care they receive and in turn to children's birth weights and the quality of care they receive (Smith et. al.2006).

Child care does not exist across slums. Women are required to carry their children to work. There are no separate arrangements for child care at slums or at work. At work, they need to provide care and breastfeed to children. If it is not possible at work then children are required to keep with relatives or neighbors. They remain without care and supplementary food. It is further deteriorates their health status and condition. Most of the children in slums are suffering from cough and cold, fever and diarrhea. Children do not get proper treatment from health specialists. The public health care facilities are overcrowded and inefficient staff exists in it. In order to provide health treatment to children, more time and physical energy of mothers is required. The private health care facilities are expensive and beyond the reach of poor people. The women and children are carrying drinking water from long distance. It affects on income generating activities of women. The study of children also gets affected in slums. Most of slums are facing various infrastructural problems in region.

II. Data

For this study, we have collected primary data of slum households in Mumbai Metropolitan Region. We have collected 767 households data from eight slums such as Mankhurd East and West, Govandi East and West, Kalwa, Koparkhairne , Rabale, Turbhe, Vashi and Ghatkopar. The household heads and women are interviewed during survey. The questionnaire comprises as different questions related to household members, fertility, immunization, child care and illness. The primary data is collected in May-June 2014. We have analyzed data in SPSS@20 and STATA@12 software. The children's weigh for age, height for age and weight for height is calculated in the excel sheet.

III. Methodology

The nutritional status of children is mainly expressed in terms of height for age and weight for age. We compared the child's weight and height with reference population. It is given by the World Health Organization (WHO). Children who are too short for their age are called stunted. The height for age reflects a child's past or chronic nutritional status. The stunting is also called as the cumulative indicator of slow physical growth. The children who become stunted typically remain so throughout their lives and thus never catch up. The child, whose weight is too low for their age is called as underweight.

IV. The Z Score

The weight for age and height for age indicators are commonly expressed in the form of Z score (Harold 2000; Kostermans 1994; Galloway 1991; Giliespie and Lawrence 2003). This score compares a child's weight and height with the weight and height of a similar age and sex of child from a reference healthy population. More precisely weight and height of children of a certain age group follow more or less the normal distribution. The stunting Z score of a child is the difference between the height of that child Hi and the median height of a group of healthy children of the same age and sex from the reference population Hr divided by the standard deviation of the height of those same group of children (same age and sex) from the reference population SDr. The value of the Z score can be conceived as the number of standard deviations that the child is away from the median of the concern indicator of the children of that age/sex group from the standard population.

Mathematically,

$$\text{Z score} = \frac{C_{av} - M_{rf}}{SD_{rf}} \qquad (1)$$

Where

C_{av}: Child's anthropometric value

M_{rp}: Median of reference population

SD_{rf}: Standard deviation of reference population

It is assumed that the given child comes from a healthy population. Under this null hypothesis, the Z score should follow the standard normal distribution. If the value of the Z score is sufficiently low that it has a very small probability of occurring, we reject the null hypothesis and classify the child as malnourished. Relatively short children have negative height for age, Z score and thus moderately stunted children are classified as those that have Z score –2. The severely stunted children are classified as those that have Z score –3.

The Z score for low weight for age underweight is calculated in the same way using the weight of the child (instead of height) and the median weight (and standard deviation) of the child of the same age and sex from a healthy reference population. The international

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reference population advocated by the US, Centers for Disease Control (CDC) is based on data from the National Center for Health Statistics (NCHS). The stunting measures in the long run social condition because it is reflecting past nutritional status. Thus the WHO recommends it as a reliable measure of overall social deprivation (Glewwe et al., 2002) and it is proxy for multifaceted deprivation. By consequences, being wasted is a better indicator for the determination of short-term survival, whereas sensitivity and specificity of survival in a one or two year period is highest for weight for age (Kostermans, K. 1994). The weight for age indicator is intended to capture both long term (stunting) and short term (wasting) under nutrition. It has been the indicator used most frequently by World Health Organization (WHO), United Nations International Children's Emergency Fund (UNICEF) and other international organizations concerned with the health status of children.

V. Economic Model

The child malnutrition is classified as stunting, underweight and wasting. In this study, the malnutrition is classified as follows.

$$M=(U,S) \quad (2)$$

Malnutrition among children is classified as stunting and underweight in Mumbai Metropolitan Region.

$$M=(Pe,Pn,Ha,Cu,Hc,Cc,Ci) \quad (3)$$

Malnutrition among children is a function of household characteristics. It is related to parent's education (Pe), nutritional status (Pn), household assets (Ha), contraceptive use (Cu), health care (Hc), child care (Cc) and child illness (Ci). Such factors are further divided into number of sub factors as follows.

$$Pe=(P,S,Hs,C) \quad (4)$$

Parent's education is considered as mothers and fathers educational attainment and it is considered as primary, secondary school, high school and college education.

$$Pn=(S,Mo,Mi,N) \quad (5)$$

Parent's nutritional status is classified as normal, mild, moderate and severe malnourished. It is measured in terms BMI. The BMI of parents is defined as weight divided by height (m) square.

$$Ha=(Ph,E,V) \quad (6)$$

Household assets are classified as physical assets, electronic goods and vehicles.

$$Cu=(M,T) \quad (7)$$

Contraceptive use by parents is classified as modern and traditional contraceptives. The modern contraceptives comprises as pills, condoms, IUD and family planning operation. The traditional contraceptives comprises as periodic absentee and withdrawal method. Parents use suitable contraceptive method for spacing among children.

$$Hc= (A,D,I) \quad (8)$$

Health care access comprises as the ANC to pregnant women, delivery conducted in hospital and care during illness.

$$Cc= (B,F,C) \quad (9)$$

Child care comprises as the breastfeeding, supplementary food and care.

$$Ci=(C,F,D) \quad (10)$$

Child illness comprises as the cough and cold, fever and diarrhea.

All the factors are interlinked with each other but finally they decide the nutritional status of children.

VI. Incidence of Child Malnutrition in Mumbai Metropolitan Region

We have classified children according to the current nutritional status and the suburbs. The number of wasted children was very low therefore they are not reported in the table. The incidence is shown for eight suburbs in metropolitan region.

Table 1 : Incidence of malnutrition among children (percent)

Suburb	Underweight		Stunting	
	Boys	Girls	Boys	Girls
Mankhurd(E)	14.29	0.00	21.43	7.14
Mankhurd (W)	0.00	11.54	4.17	11.54
Govandi (E)	6.25	20.00	12.50	20.00
Govandi (W)	0.00	3.33	6.45	6.67
Kalwa	2.42	10.58	5.65	14.42
Koparkhairn	0.00	16.00	8.00	32.00

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Rabale	0.00	0.00	0.00	7.69
Turbe	11.76	4.00	20.59	16.00
Vashi	0.00	14.29	0.00	28.57
Ghatkopar	28.57	0.00	0.00	0.00
Total	4.17	8.76	8.33	14.74

Source: primary data collected

In Mankhurd (E) 14.29 percent boys are underweight. In the same suburb, 21.43 percent boys are stunted. In Mankhurd East and West, 11.54 percent girls are underweight and stunted. In Govandi (E), 20 percent girls are underweight and stunted. In Govandi (W), total 6.67 percent girls are stunted. In Kalwa, 14.42 percent girls are stunted. In Koparkhairne, total 32 percent girls are stunted. It is the highest incidence of stunting among girls in this suburb. In the same suburb, only 16 percent girls are underweight. In Rabale, only 7.69 percent girls are stunted. In Turbhe, 20.59 percent boys are stunted. In Vashi, 28.57 percent girls are stunted. In Ghatkopar, 28.57 percent boys are underweight. It is the highest incidence of underweight among boys as compare to other suburbs. We found that total 8.76 percent girls are underweight whereas 4.17 percent boys are underweight. Nearly 8.33 boys and 14.74 percent girls are stunted in metropolitan region. It is clear that the incidence of stunting among boys and girls is higher in slums. It means children are not provided health care during different kinds of illness. Therefore it has affected on their physical growth. Stunting is a cumulative and long term indicator of child malnutrition.

a) Parent's education and nutritional status of undernourished children

The parent's education and nutritional status are the key determinants of the child nutrition. Low education and nutritional status is the prime cause of child malnutrition. Such factors cannot be ignored in this study.

Table 2 : Parents education and malnutrition among children (percent)

Parents education	Underweight	Stunted
Mother		
Illiterate	17.65	28.33
Primary	2.94	6.67
Secondary	8.82	3.33
High school	0.00	0.00
College	0.00	0.00
Father		
Illiterate	17.65	26.67
Primary	0.00	0.00
Secondary	14.71	15.00
High school	2.94	1.67
College	0.00	0.00

Source: As per table one

The literate mothers can understand the nutritional requirement of children. But illiterate mothers may responsible for malnutrition among children. Among illiterate mothers, the incidence of underweight is 17.65 percent and incidence of stunting is 28.33 percent. The primary studied mothers, the incidence of underweight among children is only 2.94 but the incidence of stunting is 6.67 percent. Those mothers are secondary studied the incidence of underweight is 8.82 percent whereas the incidence of stunting is 3.33 percent. We have not found any incidence of child malnutrition with high school and college education of mothers. If the father is illiterate then the incidence of underweight among children is 17.65 percent. The incidence of stunting is 26.67 percent. Those fathers are secondary studied then the incidence of underweight is 14.71 percent. The incidence of stunting is 15 percent. Those fathers are high school studied, the incidence of underweight is 2.94 percent. The incidence of stunting is 1.67 percent. It is clear that the illiteracy among parents is the prime cause of malnutrition among children. As the education of parents increases, the incidence of malnutrition among children declines fast.

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Table 3 : Parents nutritional status and malnutrition among children (percent)

BMI category	Underweight	Stunted
Mothers		
Severe	5.88	5.00
Moderate	2.94	1.67
Mild	5.88	5.00
Normal	2.94	15.00
Fathers		
Severe	0.00	1.67
Moderate	0.00	1.67
Mild	0.00	0.00
Normal	2.94	13.33

Source: As per table one.

Those mothers are severely malnourished the incidence of underweight is 5.88 percent but the incidence of stunting is only 5 percent. The moderate malnourished mothers have 2.94 percent incidence of underweight. Those mothers have mild BMI, the incidence of underweight is 5.88 percent whereas the incidence of stunting is 5 percent. It means mothers with severe and mild BMI have similar underweight and stunting incidence among children. Those mothers have normal BMI, the incidence of underweight is 2.94 percent whereas the incidence of stunting is 15 percent. Those fathers have mild and moderate BMI, the incidence of stunting is observed as very low. The incidence of stunting is 13.33 percent if the father has normal BMI. We found that mothers and fathers normal BMI has no relationship with stunting among children. Stunting is related to health care access and not BMI of mother and father. Above table shows that children are not treated in health care facilities. Most of the public health care facilities are overcrowded and visiting such facilities required more time. Poor families have high opportunity cost of time. Therefore they avoid visiting health care facilities.

b) Household characteristics and under nutrition among children

We have classified the household characteristics of underweight and stunted children. They are determinants of malnutrition among children.

Table 4 : Water requirement to family of undernourished children (percent)

Drinking water	Underweight	Stunting
Daily per capita water requirement (Liters)	113.73	126.63
Per capita drinking and cooking water (liters)	7.74	8.43
Trips required(No)	4.82	4.47
Trips by women(No)	4.03	3.82
Trips by children (No)	0.32	0.31
Trips by others(No)	0.29	0.23

Source: As per table one

The daily per capita norm of drinking water is 135 liters. But all the slum households get less drinking water. Among the underweight children, the daily water availability is 113.73 liters for drinking and cooking. Among stunted category, it is 126.63 liters. Total 4.82 trips are required to carry water among underweight children. For stunted, it is 4.47 trips. The women carry 4.82 trips in undernourished children category. For stunted children, it is 3.82 trips. The young children carry water and it is 0.32 trips in undernourished category whereas in stunting category, it is 0.31 trips. The other family members also carry water but the numbers of trip are very low.

Table 5 : Household assets of undernourished children (percent)

Household assets	Underweight	Stunting
Cooker	47.06	51.67

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Chair and table	17.65	15.00
Watch	23.53	23.33
Electricity	73.53	68.33
Bicycle	2.94	6.67
Swing machine	0.00	0.00
Radio	2.94	3.33
Telephone	29.41	33.33
Refrigerator	0.00	1.67
Television	52.94	48.33
Bike	0.00	1.67
Car	0.00	1.67

Source: As per table one

Household assets are important to improve nutritional status of children. Households have cooker but 47.06 percent children are underweight and 51.67 percent are stunted. Households have chairs and table but 17.65 percent are underweight and 15 percent are stunted. Households have watch but 23.53 percent are underweight and 23.33 percent are stunted. Total 73.53 percent households have electricity but children are stunted. In case of stunting category, it is 68.33 percent. Only 2.94 percent households with underweight children have bicycle. Among stunting category, it is 3.33percent. Household with malnourished children do not have swing machine. Only 2.94 percent households with underweight children have radio. In case of stunted children, it is 3.33 percent. Total 29.41 percent households with underweight children have telephone. In stunting category, it is 33.33 percent. In our sample only 1.67 percent households with stunted children have refrigerator at home. Refrigerator is important in house because it is useful to preserve food for longer time and provide nutritious food to children on demand. Total 52.94 percent households have television but children are underweight. In case of stunting category, it is 48.33 percent.

The contraceptive methods are important to plan number of children. Modern contraceptives are reliable because failure rate is low. But knowledge of modern contraceptive among poor parents is low.

Table 6 : Contraceptive use and nutritional status of children (percent)

Contraceptive use	Underweight	Stunted
Pills	17.65	8.33
Condom	11.76	11.76
IUD	0.00	0.00
Sterilization	23.53	25.00
Vasectomy	0.00	1.67
Periodic absentee	27.59	27.78
Withdrawal method	20.59	23.33

Source: As per table one

Women are taking pills but 17.65 underweight and 8.33 percent are stunted. Women's use condom as family planning method but 11.76 percent children are underweight and stunted. Women do not use IUD in slums. Such method required to visit health care facility and take precautions. Women do not time for repetitive visits to health care facility. Women had sterilization but total 23.53 percent are underweight and 25 percent are stunted. Only 1.67 percent fathers had vasectomy but children were underweight. Families use periodic absentee method but 27.59 percent are underweight and 27.78 percent children are stunted. The withdrawal methods are used by parents but 20.59 percent children are underweight and 23.33 percent are stunted. It is clear that most of the parents rely on traditional method of contraceptives. For use of modern contraceptives, knowledge, money and consultation with heath staff is required. But it is not possible with poor parents.

The health care access to pregnant women is most crucial to reduce malnutrition among children in slums. But there are number of factors responsible for lower access of ante natal care and delivery in health care facility.

Table 7 : Health care access for undernourished children (percent)

Health care access	Underweight	Stunted
Ante natal checkups received	17.65	21.67

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Average ANC trips	1.94	2.26
Injections	52.94	65.00
Tablets	17.65	28.33
Delivery in public hospital	67.65	56.67
Normal delivery	94.12	86.67
Caesarian	2.94	6.67

Source: As per table one

Women had antenatal check up but the 17.65 percent children are underweight and 21.67 percent children are stunted. Among underweight children, women had less than two ante natal check ups whereas among stunted category, it is more than 2 ante natal visits. Total 52.94 percent women of underweight children category had received injections. For stunting, it is 65 percent. Only 17.65 percent women received tablets but children are underweight. In the stunting category, it is 21.67 percent. Total 67.65 percent women had delivery in public hospital but the children are underweight. For stunting, it is 56.67 percent. Total 94.12 percent women had normal delivery but children were underweight. For stunting, it is 86.67 percent. Total 6.67 percent children are stunted but women had caesarian.

Table 8 : Breastfeeding, care and under nutrition (percent)

Breastfeeding and care	Underweight	Stunting
Immediate breastfeeding	73.53	81.67
Still breast feeding	76.47	70.00
Breastfeeding and supplementary food	20.59	25.00
Anganwadi food	2.94	5.00
Care		
At home	67.65	65.00
On work	2.94	1.67

Source : As per table one

Children must be provided breastfeeding immediately after delivery. They must be exclusively breastfeed up to six month. After six month, they must be provided supplementary food along with breastfeeding. In our study, 73.53 percent women have immediately breastfeed their baby after delivery. In stunted category, it is 81.67 percent. Total 76.47 percent underweight children are still breastfeed by mothers. For stunting, it is 70 percent. Only 21 percent underweight children have given the breastfeeding and supplementary food. For 25 percent stunted children have given breastfeeding and supplementary food. Only 2.94 percent underweight children have given anganwadi food. The 5 percent stunted children have received anganwadi food. It means very few children have access to anganwadi food. Total 67.65 percent underweight children are kept at home. This is because there is no care exists at work place. Total 2.94 percent underweight children are on work with their mothers. In stunting category, it is 1.67 percent.

Table 9 : Child illness and treatment to malnourished children (percent)

Child illness	Underweight	Stunting
Cough	20.59	21.67
Fever	20.59	20.00
Diarrhea	8.82	11.67
Blood in stool	2.94	5.00
Treatment in public health care	5.88	3.33

Source : As per table one

Children must be health and they should not suffer from any illness. Illness among small children affects physical growth and weight gain. During illness if the health care is not provided then they become malnourished. In this sample, we found that total 20.59 percent underweight and 21.67 percent stunted children had cough. Around 20 percent underweight and stunted children had fever. Around 8.82 percent underweight children had diarrhea. In stunting category, it is 11.67 percent. Total 5 percent stunted children had blood in stool. Only 5.88 percent underweight children received treatment from public hospitals. For stunting, it is only 3.33 percent. It means very few malnourished children get treatment from the health care facility.

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VII. Regression Result

In order to examine the socio-economic and demographic co-relation with the malnourished children, we have used the logistic regression (Greene W. 2003). The logistic regression gives the odd ratio for malnourished children with compare to non malnourished children. We have used this model to underweight and stunted children in Mumbai Metropolitan Region. The model is explained as follows

$$f(Z) = e^z/e^{z+1}$$

$$=1/1+e^z$$

$$Z=\beta_0+\beta_1x_1+\beta_2x_2+\beta_3x_3+\dots\dots\dots+\beta_kx_k, \quad (11)$$

Where

Z = Dependent variable

β_0= Intercept

The β_1, β_2, β_3 are "regression co-efficient" of x_1, x_2, x_3 respectively. The variables x_1, x_2, x_3 are considered as the independent variables. Such independent variables are of socio-economic and demographic category. The results are presented in following table.

Table 10 : Regression results for underweight children

Variables	Co-efficient	S.E	Wald
Age	-1.75*	0.33	27.63
Mothers normal BMI	-2.28*	1.04	4.8
ANC	-0.19*	0.11	3.13
Anganwadi food	-1.76**	1.08	2.81
Constant	0.51***	0.4	1.61
	- 2log likelihood=158.86	Cox and Snell R^2 square=0.16	Nagelkerke R^2= 0.43

- **Significant at 1 percent , ** significant at 5 percent , *** significant at 10 percent*

Age of the child is negatively co-related with underweight. At lower age, children in slums are more vulnerable. The odd is 75 percent negatively related with underweight children. The socio-economic conditions are not favorable in slums for child growth. At lower age or after birth, the child is exposed to unclean environment and unsafe drinking water, sanitation. Such factors adversely affecting on weight gain of small children in slums. Most of the mothers in slums do not get adequate food and rest. It is basic requirement of mothers. They breastfeed their children, but they need to work at home and on daily wage. Their body does not support the physical hardship. Therefore the BMI of women is always lower. It is negatively co related to underweight among children and the odd ratio is 2 times negatively co-related. Women in slums do not get all the antenatal checkups. It is negatively co-related and the odd is 19 percent less because they do not get the antenatal care. After conception, women are expected to visit health care facility. They must get the iron, folic acid tablets and injections as well as physical checkups. Such ANCs and checkups are completed in three visits. For complications, number of visits can be more. But women in slums do not have time to visit health care facilities. Families are often depending upon home remedies. Government of India has established Anganwadi centers in all states in India. Such centers are expected to provide food, measure weight, height and provide medical care to small children. The underweight children have not received food and medical care. The odd is one and it is negatively co-related. We have used logistic regression for stunting among children to understand co-relation with household factors. The results are reported in the table.

Table 11 : Regression results for stunted children

Variables	Co-efficient	S.E	Wald
Age	-1.66*	0.23	51.19
Mothers secondary school	-2.21*	0.81	7.6
Mothers moderate BMI	-2.18***	1.31	2.78
Fathers moderate BMI	3.22*	1.36	5.59
Per capita income	-0.01*	0.00	5.33
ANC	-0.426*	0.19	4.9
Normal delivery	-0.89**	0.52	2.94

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Anganwadi food	-1.57*	0.65	5.82
Constant	2.66*	0.75	12.54
	- 2log likelihood= 232.08	Cox and Snell R^2= 0.24	Nagelkerke R^2= 0.47

- *Significant at 1 percent , ** significant at 5 percent , *** significant at 10 percent

Lower age of the child has more chance of stunting. The odd ratio is 1.66 and negatively co-related with age of children. The parents do not health care treatment to children. The health care facilities are overcrowded. The parents are poor therefore they have high opportunity costs to visits such health care facilities. Most of the mothers are less educated in slums. The odd is 2 times less of secondary school of mother's education. Educated mothers can provide health care and nutrition to children. It helps children to achieve adequate growth at lower age. But lower education of women is responsible for stunting among children. Most of the women do not have normal BMI is slums. Normal BMI is essential for good health of child. The odd ratio is 2 and it is negatively co-related with moderate BMI of mothers. Such low and negative relationship of mothers BMI do not help for adequate growth of children. Fathers BMI is co-related with stunting of children. The odd ratio is 3 and it is positively co-related with stunting among children. Fathers normal BMI helps to earn more income and provide nutrition to small children. But low BMI has effect on child health. Per capita income of family is sole determinant of child malnutrition. The odd is negatively co-related with stunting among children. Higher per capita income helps families to spend more on food, care, medicine and household assets. But lower per capita income does not support various basic necessities required for families. Therefore parents cannot spend money on child health care and supplementary food. Therefore PCI negatively co-related to stunting among children. Those women have not received the ANC, the stunting among children is 42 percent more. During ANC, the folic acid tablets and injection are given to pregnant women. Similarly the women are treated with complications and any deficiency of nutrition. But no ANC means children are exposed to malnutrition. Most of the women in slums do not have adequate body growth. They get marry early and get pregnant. It affects on the health of children. Most of the times complications occur when the girls get marry early. The cesarean type of delivery affects on mother and child health. The women do not have normal delivery and stunting is 89 percent more with stunting among children. Children do not get anganwadi food. The stunting is two times more with no anganwadi food. Anganwadi food is most important for child health. Physical checkups, supplementary food are most important for good child growth. But women in slums do not have time for children and send regularly them to anganwadi. They are busy with household chores and daily wage earning.

VIII. Policy Implications and Conclusion

This study shows alarming results of malnutrition among children in region. The incidence of stunting is higher among females. The incidence of stunting is double of underweight among male in suburbs. The literacy among mothers and fathers are lower. The lower BMI of parents is related to under nutrition among children. Only few women read newspaper and watch television in slums. Most of the women delivered baby in public health care facilities. Women in slums do not have access to child care. While working, they keep their children at home. Most of the children are suffering from cough, cold and fever. They are treated in public health care facilities. The high levels of malnutrition in the present study underline the great need for nutritional intervention. Therefore timely introduction of appropriate complementary feeding is a key factor in child growth. The complementary foods should be given to the children as from six months old. Nutrition education is the most sensitive factor that is needed by all mothers in slums. It is because this will keep them informed about the right food for them and their children at different stages of life and from there better living can be assured that will give the assurance of a better nutritional status for mothers and their children. All the households in slums must have access to health care, sanitation and electricity. The maternal child health care must be given priority in slums of Mumbai Metropolitan Region. Government must sanction more anganwadis in slums of Mumbai Metropolitan Region. Government and NGO's must prepare maternal child health related program and run on television and radio. It will help to spread information and awareness of child and maternal health among poor people. Public health care facilities must provide modern contraceptives in slums. It will help couples to keep proper space between the children. It will help to reduce the malnutrition among children. Government and NGO's must work to increase the age of marriage of girls in slums. Mother's body must supply her baby with everything it demands. Thus the mother's micronutrients level directly affects her baby's development. When it comes to eating and drinking, what is good for a mother also benefits her child. This

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natural fact therefore helps mothers to positively influence their baby's growth and development as well as baby's long term health and well being (Opara et.al 2011). Adolescent girl's education is most important aspect in this direction. The poor urban slum girls must be given scholarship to continue education. There are number of alternative policies such as better strategic interventions for nutritionally vulnerable population, adequate investment in terms of technology and implementation of evidence based strategies and comprehensive evaluation of programs by the government and development partners. It will no doubt achieve improvements in nutrition of mothers and children (De Silva A. et.al 2009). Similarly women need to strengthen their nutrition care knowledge, attitude and practice, especially breastfeeding and weaning practice. It is the key to achieve better nutrition status of their children. The development and implementation of health education intervention should include the participation of targeted mothers in order to understand their needs. Health communications should be in a format that attract community and appropriate with the local culture and language. Art-based communication activities such as singing, drama contest, and roll-play are important. The municipal corporations must be give the priority to improve standard of living of people. The illegal status of the slum has hindered the expansion of municipal services to serve them. This has resulted to the poor being denied access to safe drinking water and proper sanitation. The results suggest that water from the tap is safe for human consumption according to WHO guidelines (Wambui E et.al 2007). But most of the slums are neglected the safe drinking water supply, sanitation, solid waste, roads and electricity. The nutrition programs should be established in slums to tackle the problem of malnutrition at community level. Nutrition education by health extension works should be strengthening to improving the feeding practice of parents on appropriate children feeding. Households should treat drinking water by boiling, bleaching and strained through cloth (Kebede et.al. 2013). Government must focus on income generation activities at household level. Government must train youth and women with basic skills. It will help to improve family income and reduce incidence of malnutrition. All the policies will certainly reduce the child malnutrition at some extent in Mumbai Metropolitan Region.

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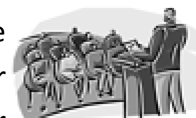

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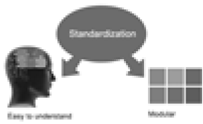

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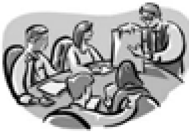

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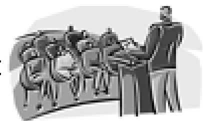

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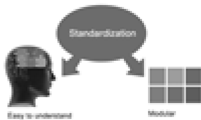

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Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
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- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

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- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
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- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

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- Submit to generally acknowledged facts and main beliefs in present tense.

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References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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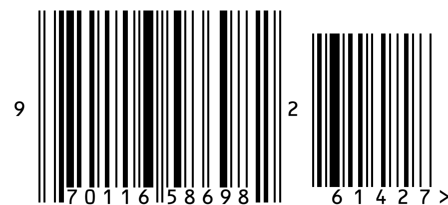

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