IDENTIFICATION OF CAUSAL ORGANISM(S) FOR GHERKIN (*Cucumis sativas*) SOFTENING DURING BRINING

By

Millehena N.P.

(03/AS/041)

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Department of Food Science & Technology,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Belihuloya,
Sri Lanka.

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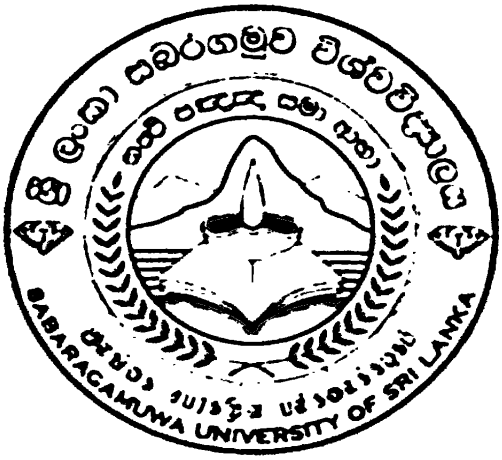
Sabaragamuwa University of Sri Lanka



DECLARATION

The work described in this thesis was carried out by me at the Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya under the supervision of Ms. T.C. Kananke, Lecturer, Department of Food Science & Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka & Dr R.G.A.S. Rajapaksha, Research Officer, Division of Plant Pathology, Horticultural Crops Research & Development Institute, Gannoruwa, Peradeniya & Mr. H.M.C.K.H. Marage, Assistant Manager (Quality), Sunfrost limited, Nelumdeniyawatta, Alawwa. A report on this has not been submitted to any other university for another degree.

Millehena N.P.

(03/AS/041)

.....
Signature

29.05.2009
Date

Certified by,
External Supervisors,
Dr .R.G.A.S. Rajapaksha,
Research officer,
Division of Plant Pathology,
Horticultural Crops Research & Development Institute,
Gannoruwa,
Peradeniya,
Sri Lanka.
Tel +94 0812388011

.....
Signature

29.05.2009
Date

Mr H.M C K H Marage,
Assist Manager - Quality,
Sunfrost limited,
Nelumdeniyawatta,
Alawwa
Sri Lanka
Tel, +94 0372279181

.....
Signature

30.05.2009
Date

Internal Supervisor,
Ms. T.C Kananke,
Lecturer,
Department of Food Science & Technology,
Faculty of Applied Sciences,
Sabaragamuwa University of Sri Lanka,
Belihuloya,
Sri Lanka.
Tel: +94 452280298

Signature

01.06.2009
Date

Head of the Department,
Mr M.C.N. Jayasooriya,
Act. Head of the Department,
Department of Food Science & Technology,
Faculty of Applied Science,
Sabaragamuwa University of Sri Lanka,
Belihuloya,
Sri Lanka.
Tel: +94 452280298

Signature

01/06/2009
Date

Head/Dept. of Food Science & Technology
Faculty of Applied Sciences
Sabaragamuwa University of Sri Lanka
Belihuloya

Affectionately
Dedicated

To

My Parents

&

Teachers

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ABSTRACT

The Gherkin (*Cucumis sativus*) is a cucumber type vegetable, belongs to the family *Cucubitaceae* that use mostly for pickle products fermenting with the brine solution. Softening of Gherkin during fermentation is a problem that degrades middle lamella containing pectic substances of fruit tissues.

This study was conducted to identify the causal organism(s) responsible for the softening of gherkin during fermentation process, at the Division of Plant Pathology, Horticultural Crops Research & Development Institute, Gannoruwa, Peradeniya and Sunfrost limited, Alawwa from 3rd of November 2008 to 20th of March 2009. Initial Symptoms were identified as bubble formation on skin, mostly 25-44 mm diameter size fruits during first 5-7 days that is being brining. Ultimately the whole interior flesh would be destroyed remaining only the skin part. Microscopic observation shown that middle lamella of the tissues was destructed of the defected area of brined fruits while it remains undistracted in undefected areas.

According to the analysis of the brine solution, pH of the brine solution varies between 2.8-3.5, acidity 0.5-0.81% and salinity 5 -8.5% within first 30 days of fermentation. Dissolved oxygen concentration in brined solution is not distinctively varies between the top and bottom layer of the vats. During the first 14 days dissolved oxygen content varies between the ranges of 6-8mg/L.

The results of the presumptive microscopic observation and streak plate technique on PDA medium were clearly emphasized that any fungal mycelium growth was not present, in the fruits except *Yeast* and *Actinomycetes* of defected fruits kept in the humid chamber. Also according to the observation on MacConkey agar, brine solution doesn't contain any presumptive coliforms. The study was able to identify gram (-), cocci shaped, catalase (+) bacteria and gram (+), diplobacilli bacteria. Further biochemical tests are necessary to identify the exact types of microorganisms. Also it was found that the softening of the fruits was not caused by the obligatory parasites as the fruits remain undefected after injecting the defected fruit ooze It has recorded that the defect caused by the genera *Fusarium*, *Alternaria*, and *Mucor* But this study proved that no positive correlation between these genera in softening of gherkin A detailed investigation is needed to be carried out to find the reasons for the softening mainly during the Yala season and localized fruit softening

Key words: Gherkin fermentation, microbial softening

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LIST OF ABBREVIATIONS

°C	; Centigrade
DO	; Dissolved Oxygen
LAB	; Lactic Acid Bacteria
mg	; Milligrams
NA	; Nutrient Agar
PDA	; Potato Dextrose Agar
SLSI	; Sri Lanka Standard Institute

CHAPTER 1

INTRODUCTION

1.1. Background

Gherkin and cucumber (*Cucumis sativus* L) classified to one species but they are two deferent types. This was introduced in to Sri Lanka in 1985 as an export crop and cultivate as seasonal crop mainly in Hambanthota, Kurunegala, Mahiyangana, and Mahaweli C and H zone in the dry zone. Sri Lanka is a country to supply gherkin to the world with accepting characteristics like flavor, color, crispiness of product.

Brined Gherkin produced by the fermentation of fresh gherkin in a brine solution until the sugar content becomes zero of fruits, by lactic acid bacteria, in vats. Brine solution contains 10% Sodium Chloride, and 3.2% Calcium Chloride and Potassium Sobate. Under room temperature, fermentation initiates spontaneously which is completed within 17-21 days. This controls the undesirable microbial growth due to lactic acid production as a result of fermentation and preserves the food. It generates acceptable flavors. Processed Gherkin is packed in glass bottles with liquid medium known as cover brine which is prepared by adding salt, vinegar, flavor and water (Barnwart, 1998).

Gherkin softening in curing (fermentation, brining) result in large losses to the pickle industry. Disintegration of the tissue is due to the breakdown of pectic material in the middle lamellar. Pectic enzymes and cellulolytic enzymes catalyze the hydrolysis of pectic materials presence in middle lamella and cellulose in cell wall. However the possible sources of softening agent(s) are limited to these enzymes which are not inactivated by acid and salt content existing in the brine use in fermentation (Demain and Phaff, 1957).

This is progressive softening spoilage which occurs most frequently soon after the Gherkin are brined for the produce pickle. The skin of the cucumber attacked first, usually the blossom end. In the short time, the entire skin is affected, become slippery and be easily removed (Prescott *et al.*, 1987). Mushy pickle resulted when the softening progresses into deeper layer of the cells in pickles and more and more pectic materials, present in the middle lamella separating the individual cells of the cucumber are attacked(Barnwart, 1987).

The aerobic bacilli once considered to be impotent spoilage organism appear to play a less prominent role in softening that was thought formally. Softening of cucumbers occurs in fermentation due to purging at high air-flow rates is caused by molds growing in the brined cucumbers (Fleming *et al.*, 1982). Air purging brine solution create aerobic. Brine solution is very good nutrient medium for growth of microorganisms. The probable causes are fungi introduce via heavily contaminated gherkin Flowers and the gherkin themselves (Ralph *et al.*, 1980).

Variety of bacteria and molds, are known to produce pectolytic enzyme. Three kind of pectolytic enzymes are produce by the bacteria and fungi. Pectinomethylesterase split off methyl groups from the pectin molecules leaving pectin acid. Polygalactouronase degrade pectic acid leaving saturated digalactouronic acid. Polygalactouronase trans-eliminase split pectic acid leaving unsaturated digalactouronic acid (Prescott *et al.*, 1987). Cx cellulase degrades cellulose. All these enzymes cause to softening which highly loss Gherkin.

There are mainly two variety of Gherkin *Ajex, Vlassette* use for processing. H J S Condiments limited is the only processing plant in Sri Lanka which processes the Gherkin products, from brined gherkin receiving raw gherkin from suppliers through their collecting centers.

This research project is to Identification and Prevention of Causative Organism for Gherkin Softening to lowers the economic loss and getting good quality product.

1.2. Overall Objective:

- Identification of Causal organism(s) for gherkin softening during brining

1.3. Specific Objectives:

- Studying the Brined gherkin manufacturing process
- Identification microorganisms by culturing methods
- Studying the major causes for Gherkin softening and evaluating prevention methods

CHAPTER 2

LITERATURE REVIEW

2.1. Theory behind gherkin fermentation

The fermentation is oldest method of preserving foods by microbial action. This can be accomplished by the natural (traditional) method or by inoculation with pure cultures.

In fermentation process, cucumber are placed in a salt brine of about 25^0 Salometer and allowed to ferment. Salt concentration is high in cover brine and lower inside the Cucumber uses for fermentation. Cover brine water content is low and cucumber water content is high. Thus the solute defuse according to concentration gradient. Sugar and other nutrients diffuse from the cucumbers during the holding period in the brining. This is nutritious medium for growth of microorganisms (Barnwart, 1987).

The cucumbers are washed and sanitized with a chlorine solution that removes most of undesirable microorganisms. After brining the cover brine is acidified with acetic acid and buffered with sodium acetate or sodium hydroxide the brine is purging with nitrogen to remove dissolved CO_2.A pure culture of *Lactobacillus sp* is added for the fermentation (Barnwart, 1987).

2.2. Introduction to Gherkin

2.2.1. Origin and Distribution

Cucumbers originated in northern India which included 20 to 25 species found mostly in Asia. pH of flesh is 5.1 - 5.7. This belongs to

Family - *Cucurbitaceae* (gourd family)

Genus - *Cucumis* (Robinson and Decker, 1997).

2.2.2. Cultivars

Numerous cucumber cultivars have been developed in many part of the world differing in size and shape of the fruits. Gherkin is pickling type of cucumber use for pickle production (Robinson and Decker, 1997).

2.2.3. Ecology

Cucumber requires worm climate. But in temperate countries they are usually grown under glass. Optimum temperature is about 85^0F.optimum night temperature 60-75^0F. They need fair amount of water without a too high atmospheric humidity which facilitates disease. They can't stand water logging condition. But they can grow n on a variety of soil. They do best on well with a pH of 6.5-7.5 (Purseglove, 1968).

Cucumbers are planted on hills 3-4ft apart with several seeds per hill and thinned to 2-3 plants or in rows 4-5ft apart thinned to 1ft between plants. The crops responds to organic manures at the rate of 10-15 tons per acre and mixed fertilizer s can be applied at the rate of 600 lb per acre of a 4:16:4 N P K mixture (Purseglove, 1968).

2.2.4. Nutritional Composition of Gherkin

Vitamin A -13%

Vitamin C -27%

Calcium - 4%

Fe – 4%

Energy -10Kcal	Water -96g	Monosaccharide -1.8g	Sodium -13mg
Protein -0.6g	Fat -0.1g	Calcium -23mg	Thiamin -0.04g
Niacin -0.3mg	Riboflavin -0.04g	Vitamin C-8.0mg	

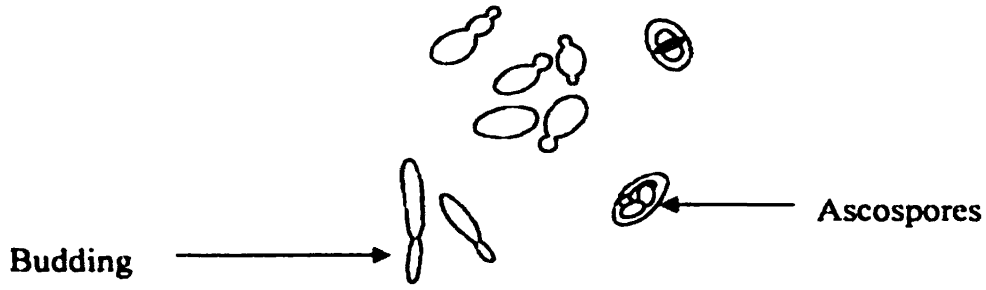
(For 100g raw edible weight)

Table2.1. Nutritional Composition of Gherkin (Homer *et al.*, 1957).

2.3. Natural Microflaura in Fermentation of Gherkin.

There are three groups of natural microflora which are often active during the fermentation of cucumbers for salt stock. These are lactic acid bacteria, *yeasts*, and gram-negative bacteria of the *Coliform* group (Barnwart, 1987)

2.3.1. *Yeast* Morphological Characteristics



(**Source:** Snowdon A.N., 1990)

Fig. 2.1. Budding of *Yeasts* and ascospores

Yeast included to fungi and single cell form "bud" directly to form new cells. The colonies are pasty in appearance. Some *Yeast* may form ascospores within their cells. *Yeasts* Common in moist habitats and often able to grow at reduced oxygen levels. The size range of cells 5-10 micrometers(Prescott *et al.*, 2005).

Yeasts themselves are nonfilamentous, and larger in size compared to bacteria (Hutkins, 2006; Prescott *et al.*, 2005). The majority of *Yeasts* are aerobic, however some *Yeasts*, such as those involved in fermentation, are classified as facultative anaerobic (Prescott *et al* 2005). *Yeasts* are able to grow in a broad range of pH, including highly acidic pH levels (Jay J.M et al, 2005). *Yeasts* also undergo an alcoholic (ethanol) fermentation to metabolize sugars. *Yeast* can be affected the fermentation by the utilizing sugars that would otherwise be metabolize to lactic acid (Michael *et al.*, 1993).

2.3.1.1. *Yeast* associated with cucumber brining

The dominant species of *Yeasts* associated with causing gaseous cucumber fermentation come from the genera *Brettanomyces*, *Torulopsis* (*Torulopsis holmii* ,*Torulaspora rosei*), *Hansenula* (*Hansenula subpelliculosa*), *Torulospora*, *Saccharomyces* and *Zygosaccharomyces*, *Brettanomyces versatilis*. It was reported that several species from each genera of the previously identified gas producing spoilage *yeasts*, excluding *Saccharomyces*, did not demonstrate the ability to hydrolyze the glycosidic bonds in pectin and were not responsible for cucumber softening during fermentation (Presscott and Dunn, 1987).

2.3.2. *Coliforms* Bacteria

During fermentation population of *Coliforms* increases were not large. Thus, it is believed that the *Coliforms* played no significant part in these fermentations. Rather, it is believed that the *Coliforms* entered the brines on the cucumbers, found conditions unfavorable for growth and died. They are defined as rod-shaped Gram-negative non-spore forming organisms. Some forms can ferment lactose with the production of acid and gas when incubated at 35-37°C (Garrity, 2001).

2.3.3 Biochemical characteristics of Lactic Acid Bacteria

Lactic acid bacteria (LAB) are defined as gram-positive, non-spore forming, rods and cocci that produce lactic acid. Gram-positive bacteria stain purple when performing a gram-stain, while gram-negative bacteria stain red. LAB includes members from the *Lactobacillus, Lactococcus, Leuconostoc,* groups these bacteria are generally facultatively anaerobic, meaning they prefer to live without the presence of oxygen, but is able to survive when oxygen is present(Salminen, 1998).

2.3.3.1. Homofermentative and Heterofermentative

LAB can survival in acidic, high salt environments and the end-products they produce through sugar metabolism. Organic acids, mainly lactic acid and acetic acid, are generated by LAB during fermentation (DeVuyst and Vandamme, 1994). In addition to producing organic acids during fermentation, LAB can generate other inhibitory compounds such as bacteriocins and hydrogen peroxide (Breidt, 2006).

LAB are classified as either homofermentative or heterofermentative based upon how they metabolize available carbohydrates.

Heterofermentative LAB includes *Leuconostoc mesenteroides, Lactobacillus plantarum* (Hutkins 2006). Lactic acid with the remaining products comprised of carbon dioxide, ethanol, and acetate (DeVuyst and Vandamme, 1994).

2.4. LAB Action in Gherkin fermentation

The sugar are diffuse from the cucumbers are fermented sequential by *Leuconostoc mescenteroids, Pediococcus ceriviceae, Lactobacillus bravis,* and *Lactobacillus plantarum*. Depending on the conditions of fermentation, about 0.6-1.2% lactic acid formed in about 7-14days .As the pH is lowered to 3.2 the metabolism of *Lactobacillus plantarum* is inhibited and in one study about 0.25% sugar remained after lactic acid formation had ceased (etchells *et al.*, 1968).

With the inhibitors, salt and, anaerobic environment, the lactic acid bacteria tend to dominate .The fermentation is started by *Leuconostoc mescenteroid*, it converts the sugar to lactic acids, alcohol, CO_2 and to the products that contribute to the flavor of pickle .the CO_2 help to maintain aerobic conditions in the fermenting pickle(Pederson, 1979).

As the acids accumulate *Leuconostoc mescenteroidis* inhibited but the fermentation continues with *Lactobacillus bravis, Pediococcus ceriviceae*, and finally *Lactobacillus plantarum*. The proper concentration of salt favors the growth of lactic acid bacteria in the correct sequence .too little salts results in poor flavors and pickle. Too many salts inhibit the lactic acid bacteria and may results in and acid flavors, darkening, and growth of *yeasts* (Pederson, 1979).

2.5. Stage of vegetable fermentation

The natural fermentation of the vegetables involves growth sequences of various types of microorganisms .this sequences may be categorized in to three stages .These are based on changes in the chemical and physical environment during fermentation and storage of the product (Barnwart, 1987).

2.5.1. Primary Stage

The initial stage may include growth by many of facultative and strictly anaerobic microorganisms originally present on the fresh materials. The quality of the final product depends on largely on the rapidly with which the lactic acid bacteria are established and the undesirable bacteria are excluded (Barnwart, 1987).Primary stage normally lasted 2-3 days. But exceptionally it can be as long as 7 or more. During this period lactic acid bacteria rapidly increases. Both fermenting and oxidizing *yeasts* increase significantly. And the extraneous and undesirable forms decrease rapidly and may disappear entirely

(grams negative and spore forming bacteria). At the same time, steady increase in total acidity and corresponding decreasing the pH of the brine is observed (Dunn, 1987).

2.5.2. Secondary stage

During the Secondary stage, lactic acid bacteria and the fermentative *yeasts* are predominate active micro flora .They grow in the brine until the fermentable carbohydrate are exhausted or until the lactic acid bacteria are inhibited by low pH value. Resulting from production of lactic acids and acetic acids .Buffering capacity and the fermentable carbohydrate content of the plant material are important factor which govern the extent of fermentation by lactic acids bacteria and extent of subsequent fermentation by *Yeast* (Barnwart, 1987).

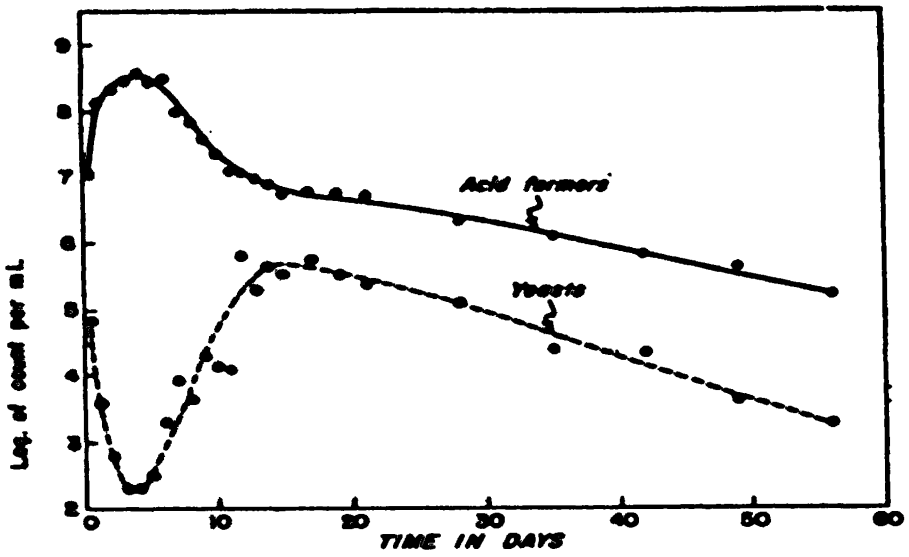
Secondary fermentation is essential due to fermentative *Yeasts*. these *Yeast* may become established during the primary fermentation are acid tolerant and ,if fermentable sugar remain after the lactic acids bacteria are inhibited by low pH values, continue to grow until the fermentable carbohydrate are exhaulated (Barnwart, 1987).

2.5.3. Post fermentation stage

During the post fermentation stage ,when fermentable carbohydrate are exhaulated ,microbial growth is restricted to the surface of brine expose to air .when the surface of the brine is exposed ,oxidative *Yeasts*, molds ,and ultimately spoilage bacteria may become established on the surface of improperly managed tanks. No surface growth occurs in anaerobic tanks (Barnwart, 1998).

2.6. Average population of Acid-forming organisms

The availability in cucumber brines of certain vitamins and amino acids which are essential for the growth of LAB



(Source: Costillow *et al.*, 1957)

Fig. 2.2. Average population fermentations by acid-forming bacteria and *Yeasts*

It was found to rise sharply within 1-3 days after brining reaching the peak within 5-6 days. The total number of organisms declined rapidly for the following 5-10 days decline at a slower rate (Costillow, 1957).

In contrast, the *Yeasts* were generally found to decline in number during the first few days after brining, started rapid growth after about 5 days, and reached their maximum population in from 10 to 20 days of fermentation. Thereafter, there was a steady decline in *Yeast* population (Costillow, 1957).

2.7. Changing population of acid formers with salt content

Average changes in the acid and salt concentrations for the 10 commercial fermentations are given in figure 2. No evident correlation was found between the total titratable acidity formed and the maximum populations of acid-formers observed in the various brines. However, in two tanks where the initial salt concentrations were relatively high (9 to 11 per cent), low populations of acid-forming bacteria were observed and very little acid was formed (Dunn's *et al.*, 1987).

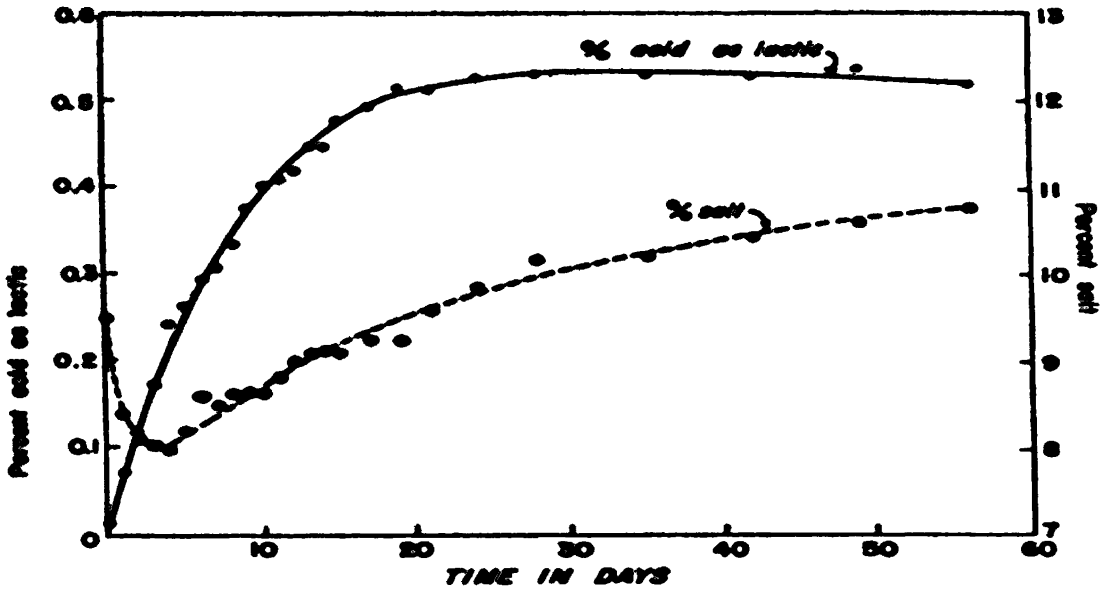


Fig. 2.3. Average changes in acid and salt concentrations in the brines during the fermentation of gherkin.

2.8.*Yeast* growth in gherkin fermentation

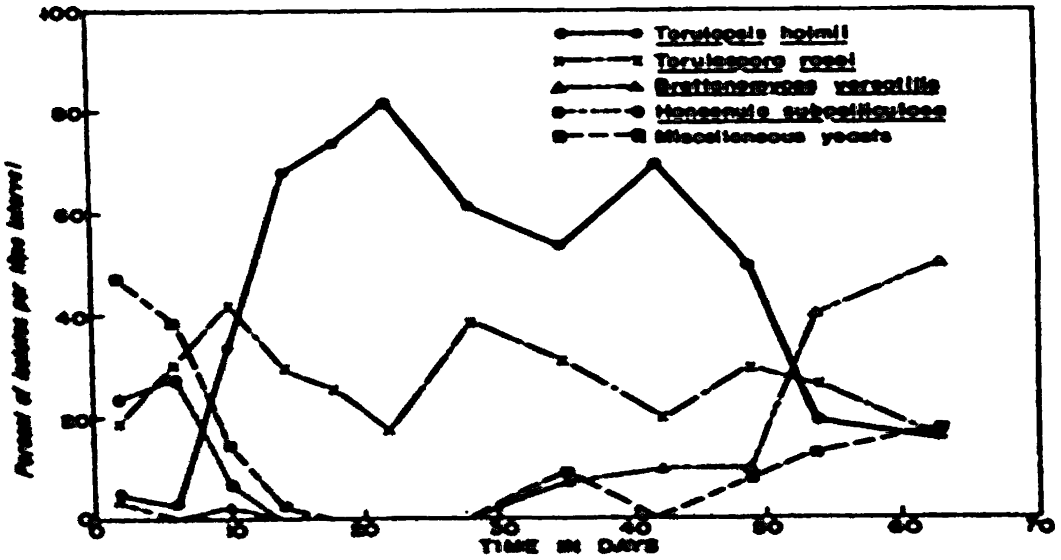


Fig. 2. 4. Estimated sequence of various *Yeast* species in the fermentation of cucumbers In the cucumber fermentation *Torulaspora rosei* was apparently responsible for the major *yeast* fermentation. This is illustrated in figure 3. The first few days after brining a miscellaneous group of *Yeasts* apparently predominated (Dunn's *et al*., 1987).

2.9. Inhibition of unfavorable microorganisms

The formation of sufficient lactic acid is an impotent factor in the quality and preservation of the fermented pickle. The rate of acid production and the total acid produced depend on the variety and size of the cucumber, initial salt concentration, the temperature and the natural microfloura of the cucumber. During the fermentation, the pH is lowered to about 3.5.During storage salt is gradually added to a *Salometer* level of 45- 60. These levels of salt with the low pH, enzymatic and bacterial activities are inhibited and help preserve pickle. An impotent aspect of the preservation is the removal of the fermentable sugars from the pickle (Barnwart, 1978).

2.10. Pectin

Pectic substances are polymers of axial axial (1-4) linked D-galactouronic acid units containing L-rhamnmose rich reagons with side chains composed mainly of arabinose, galactose, and xylose .The carboxyl groups are partially methylated .and the secondary hydroxyl groups may be acelated. The molecular weights range from 30000-120000(Mavin, 1976).

Calcium hydrates of pectin are a part of the middle lamella structure in the cell wall and provide rigidity to fruits and vegetables. Pectanolytic microorganisms can cause important changes (notably softening) in the properties of stored fruit and vegetables as well as processed products by alteration of the pectin molecule (Mavin, 1976).

2.10.1. Structure of Pectin Molecule

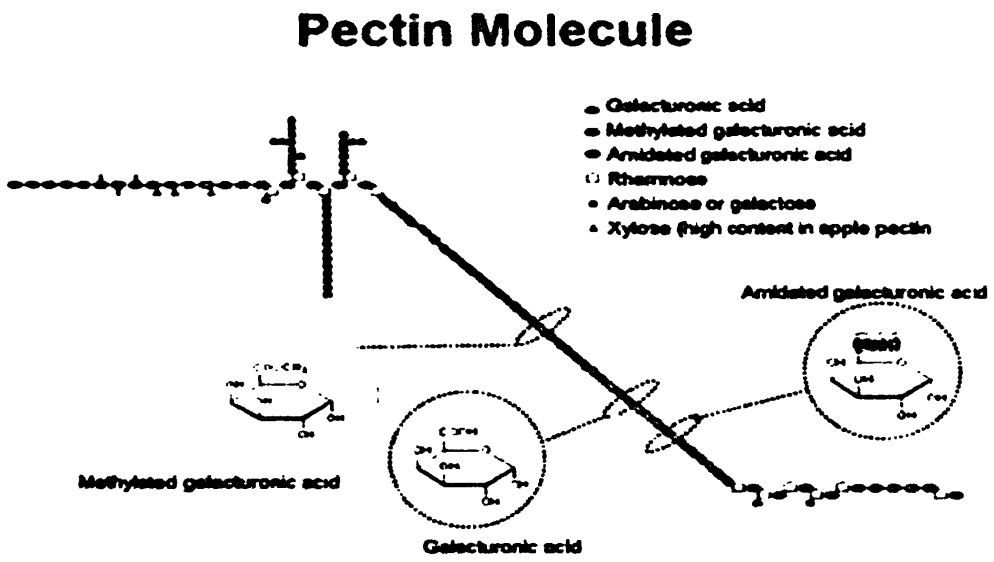


Fig. 2.5. Structure of pectin molecule, (**Source:** CatLab, 2007)

2.10.2. Pectin substances

- Pectin substances-anhydrous
- Propectin
- Pectinic acids-produces gel with water
- Pectin-water-soluble pectic acid with water
- Pectic acids

2.11. Gherkin softening

2.11.1. Softening Cause Pectolytic microbial enzyme

- Pectinomethylesterase- Split off methyl groups from the pectin molecules leaving pectin acid.
- Polygalactouronase- Degrades pectic acid leaving saturated digalactouronic acid.
- Polygalactouronase trans eliminase- Split pectic acid leaving unsaturated digalactouronic acids (Prescott *et al*., 1987)
- Cx cellulase- Degrades cellulose. All these enzymes cause to softening which highly loss Gherkin (Prescott and Dunn, 1987).

2.11.2. Gherkin Softening due to its' own enzymes

Pectanolytic activity in the seeds ,leaves, petioles, stems, flowers, and fruits(green whole fruits an ripe whole fruits)of cucumber leaves ,petioles, stems and unpolynated pestilate flower were negative for pectinase enzyme activity .but seeds, staminate flowers, polynated pestillate flowers and ripped fruits were strongly positive green whole fruits were weakly positive or negative. Pickling cucumber contain pectanolytic enzymes that are mostly active in large fruits. Therefore softening was associated with the activity of pectanolytic enzymes native to the fruits, particular in their seeds, flowers, and ripped fruits (Flaming *et al.*, 1978).

Softening of the salt stock pickle is attributed to pectolytic enzyme that degrades the cucumber tissue. one source of these enzymes is molds that enter the vat with cucumbers .specially with potion of flowers that may remain attach to the cucumber .pectin degrading enzymes are naturally present in the cucumber (fruit and seed) the purging of the brine at high rate of air flow results in increased softening suggested that softening is caused by microorganisms growing in or on the cucumber (Flaming *et al.*, 1978).

2.11.3 Pectolytic genera of microorganisms

Most pectin degrading organisms are associated with agricultural products and with soil. up to 10 % of microorganisms in the soil have been shown to be pectanolytic .These include but are not limited to species of *Achromobacter, Aromonas, Arthrobacter, Agrobacterium, Enterobaceter, Bacillus, Clostridium, Erwinia, Pseudomonas, Flavobacterium, Xanthomonas*, and many mold, *protozoa* and *nematodes* (Mavin, 1976).

Recently, pectonolytic activity was found in a strain of *Leuconostoc mescenteriodes*. This is the first reported pectonolytic activity in lactic acid bacteria.

2.12. Pectolytic *Bacillus* Characteristics

The pure cultures of pectolytic, salt-tolerant microorganisms such as bacteria, like members of the genus *Bacillus*, were catalase-positive, mesophilic, aerobic, sporulating, gram-positive, rods which acidified various carbohydrates without visible gas production (Inter science, 2006).

As already stressed, invariably, species of Bacillus were the predominating pectolytic organisms recovered from brines of the softened cucumbers encountered. This circumstantial evidence indicated that perhaps bacteria did produce the enzymes that caused the softening. Therefore, because pH is one factor known to have a marked effect on the activity of the pectinolytic enzymes produced by bacteria considerable attention given to the possible relationships of pH values and softening (Inter science, 2006).

It is apparent that the freshly harvested gherkin has pH values within the nearly neutral range where the pectinolytic enzymes of the bacilli described above would be active. Yet, since it is well known that the pH values of fermenting brines decrease rapidly 4. 5- 6. 9, it is unlikely that the bacilli would be able to elaborate softening enzymes under normal conditions of fermentation (Inter science, 2006).

If, on the other hand, the fermentation was slow to start, or arrested, one might expect the bacilli to predominate the fermentation and then soften the cucumbers, particularly if they could decompose lactic acid (Vaughn, 1954).

However, there is reasonable cause for conjecture concerning the ability of these bacteria to decompose lactic acid. It is known that the bacilli associated with the softening of cucumbers are not highly tolerant to lactic acid (Vaughn, 1954).

2.12.1. Bacillus predominating conditions in brining

Bacilli were predominately between pH 4. 5- 6. 9. Only pectolytic *Bacilli* were recovered from the brines of softened cucumbers. Sterile cucumbers in 6% salt brines became soft when inoculated with representative pure cultures of these *Bacilli* if the pH was in a range suitable for their growth and elaboration of their pectolytic enzyme systems (inter science, 2006).

2.12.2. Cucumber softening Bacilli

Bacillus polyrnyxa, Bacillus macerans group like *Bacillus sub*tilis var. *ntterinaus* , *Bacillus. pumilus, Bacillus zaterosporus* could cause softening of cucumbers. Representative cultures of these bacteria cause rapid softening and gas formation in the polypectate gel medium.

They also ferment purified pectic acid and D-galacturonic acid as energy sources both with copious gas production. *In vitro* studies already reported indicate that these bacteria can survive and grow in environments having pH values as low as 3.8.If the pH of the brined cucumbers it is desirable for softening probably pH 5.5 or above (inter science, 2006).

2.12.3. Cucumber softening molds

This softening causes *Fusarium* (*Fusarium solani*), *Penicillium, Phoma, Cladosporium, Alternaria, Aspergillus, Mucar* (jay, 1996).

Commercially brined and fermented pickles have never been a reported source of *Clostridium botulism* poisoning (Hesseltine, 1983).

2.13. Identification of *Alternaria spp*

(Source: Snowdon A.N., 1990)

Fig. 2.6. *Alternaira* spp sporingiospores

2.13.1. Rate of growth -*Altenaria* grows rapidly and matures within 5 days

2.13.2. Colony morphology

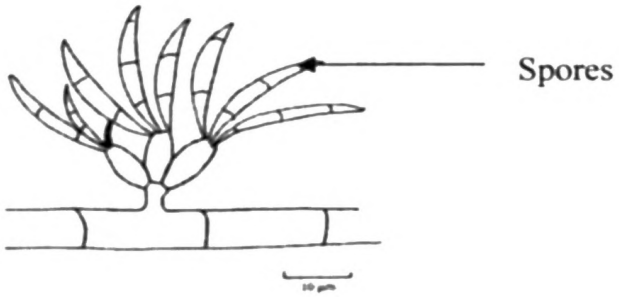
.The colony is flat, downy to cottony and may eventually be covered by grayish, short, aerial Hyphae. The reverse side is typically browny to black due to pigment production (Dubey, 2004).

2.13.3. Microscopic morphology

Alternaria has septate, dark hyphae. They bear simple or branched large conidia (8-16*23-50Mm) which have both transverse and longitudinal separations. These conidia may observe singly or in acropetal chains and may produce germ tubes. They are ovoid to oclavate, darkly pigmented, muriform, smooth or roughened .The end of the conidium nearest the conidiospores is round while it tapers towards the apex. This gives the typically bear or club-like appearance of the conidia (Dubey, 2004).

The dark brown spores are borne in simple or branched chains from the tips of simple dark conidiophores and are divided into several cells by transverse and vertical walls (Dubey, 2004; jay, 1996; Garrity, 2001).

2.14. Identification of *Fusarium spp*



(**Source:** Snowdon A.N., 1990)

Fig. 2.7.*Fusarium spp* spores

2.14.1. Rate of growth

It has Rapid growth rate, mature within 4 days (Dubey R.C., 2004).

2.14.2. Colony morphology

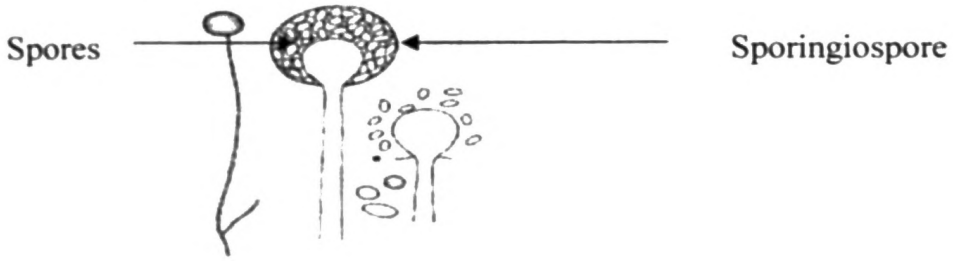
At first white and cottony, but often quickly develops a pink or violet center with a lighter periphery .Some species remain white or become orangey. *Fusarium solani* is unique in becoming blue green or bluwish brown where clusters of conidiogenous cells develop. Reverse is usually light, but may be deeply colored (Dubey R.C., 2004).

2.14.3. Microscopic morphology

Separate hyphae. There are two types of conidiation(1) unbranched or branched conidiospores with phialides that produce large (2-6*14-80)sickle or cacanoe-shapeed macro conidia(with 3-5 septa)and (2)long or short simple conidiospores bearing small (2-4*4-8Mm),oval,1-or 2- celled conidia singly or in clusters (Dubey R.C., 2004).

Most characteristic are the colorless spores (conidia), which are canoe-shaped in side view, have a distinct "foot cell" at the lower end, and are divided by several cross-walls (Dubey R.C., 2004)((jay, 1996) (.Garrity G. M, 2001).

2.15. Identification of *Mucor spp*



(**Source:** Snowdon A.N., 1990)

Fig 2.8. *Mucor spp* sporingiospores

2.15.1. Rate of growth

It has Rapid growth rate, mature within 4 days. Most species do not grow well at 37^0C (Dubey R.C., 2004).

2.15.2. Colony morphology

Quickly covered agar surface with fluff resembling cotton candy, and later turns gray or grayish brown. Reverse is white (Dubey R.C., 2004).

2.15.3. Microscopic morphology

Hyphae are wide (6-15Mm) and practically nonseptate. Sporangiospores are long and often branched and bear terminal round ,spores-filled sporangia(50-300Mm in diameter).The sporangial wall dissolves, scattering the round or slightly oblong spores (4-8Mm in diameter),revealing the columnella and sometimes leaving a collarette at the base of the sporangium. There is no apophysis, no rhizoids are formed(Dubey R.C., 2004).

Colonies grows fast, whitish to grayish, usually thick owing to the abundant upright sporangiophores. Spores (sporangiospores) produced inside spherical sporangia at the tips of the sporangiophores, brownish. Always with a large columella that remains after the sporangial wall is broken (a). Large dark zygospore may be produced. Common almost everywhere fungi occur (Dubey R.C., 2004; Jay, 1996; Garrity G. M., 2001).

2.16. Identification of *Actinomycetes*

Family *Actinomycetaceae* consist of heterogeneous group of filamentous organisms which resemble to fungi. However they are closely related to the bacteria in terms of size absence of nucleus membrane. The filaments are seldom more than 1 um in diameter.gram(+),arobic,catalase + (Mitruka.B.M., 1977).

2.17. Koch's postulates

Identify previously unknown disease Koch's rule is used. If the pathogen found seem to be the cause of the disease but no previously reports exist to support this then the following rules are taken to verify the hypothesis that the isolated pathogen is the cause of the disease (Agrios, 1997).

2.17.1. The steps of Koch's postulates.

1. The causative agent must be found in every case of the diseases.
2. The pathogen must be isolated and grown in pure culture on nutrient media, and it characteristics described (non obligatory parasites, or it must be grown on a susceptible host plant (obligatory parasite), and its appearance and effects recorded).
3. the pathogen from pure culture must be inoculated on healthy plant of the same species or verify on which the disease on the inoculated plants
4. The pathogen must be isolated in pure culture again and its characteristics must be exactly like these observed in step 2.

All above steps, usually known as Koch's rules and have been followed and proved true, the isolated pathogen is identified as organism responsible for the disease (Agrios, 1997)

2.18. Gherkin processing process

2.18.1. Gherkin grading

Grade	Avg. Fruit Count	Diameter	
		Fresh	Brined
200/300	250	10.5-12.5	10-12
160/250	205	11-14	10.5-13.5
80/200	140	12.5-18	12-18
80/160	120	16.5-18.5	13.5-17.5
80/120	100	18-20	16-18
60/80	70	18.5-23	17.5-19.5
40/80	60	20-22	18-22
40/60	50	17-25	19-21.5
30/80	55	20-25	16-24
30/60	45	22-25	19.5-24
30/40	35	22-30	21-24
20/40	30	30-38	21-29
10/20	**15**	**25-44**	**29-36**
5/30	17	27-34	24-43
27/34	14	34-40	27-33
10/15	12	38-44	32-38
5/10	08		36-42

Table 2.2. Gherkin grading

2.18.2. Fresh fruits and fermented fruits Quality characteristics

Quality characteristics of fresh fruits	Quality characteristics of fermented fruits
CRS (crooked fruits)	Fermented -100% fermented
Bell shaped fruits	Alkalinity- receiver perception
Wormed attached fruits	Color- olive green
Brown patches fruits	Diameter- receiver perception
Bloaters	Rigidity percentage - 2.5 or 3+
Short length fruits	Bloaters- reduced 2%
Surface smooth fruits	Other materials –none
Diameter of fruits	pH- receiver perception
Mechanical damaged fruits	Length - receiver perception
Rust patches	CRS Percentage- lowers than 3%
White fruits	Unrelated fruits - receiver perception
	Smell- lactic acid smell, acetic acid smell

Table 2.3. Fresh fruits and fermented fruits Quality characteristics

2.18.3. Materials for preparation cover brine (initial brine solution)

10% salt solution

2L. of Concentrated acetic acid

200Kg $CaCl_2$

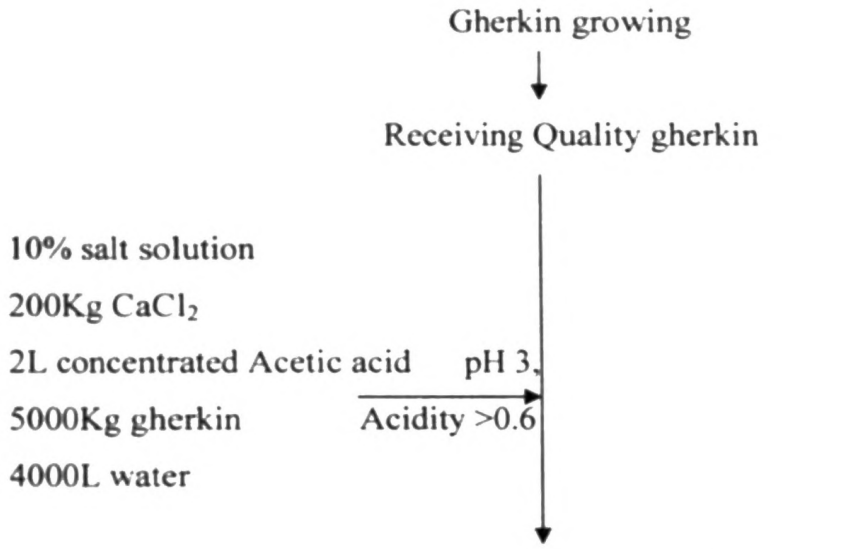
2L. concentrated acid

5000Kg gherkin

4000L. water

Initial Brine solution (cover brined) qualities (10% salts, pH 3.)

2.18.4. Production process



No of Days	Salinity (%)
1	10
At or < 3	5
14	7.5
30	8 plus(8.5)
45	10-12

Table 2.4. Salinity change with the time

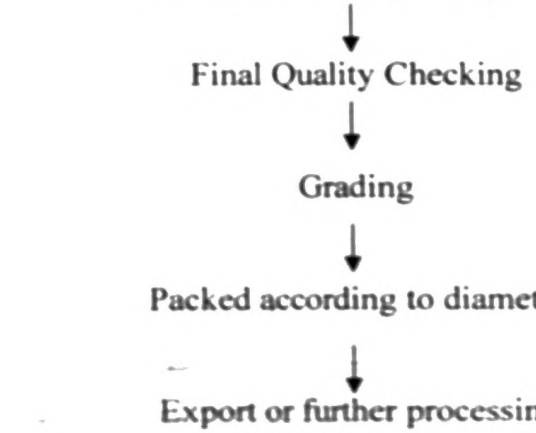


Fig.2.9. Production process flow chart

CHAPTER 3

Materials and Methodology

3.1. Materials

3.1.1. Sample collection-defected samples were collected from Sunfrost limited .Alawwa

3.1.2. Material common for all methods

3.1.2.1. Materials for glassware and media sterilization.

Materials: -

- Aluminum foil
- Cotton wool

Apparatus:-

Auto clave(100-135°C,Model:Compenstat)

Canisters

3.1.2.2. Material for the preparation of PDA

Materials:-

- Potato
- Bacteriological agar
- Glucose
- Tartaric acids
- Distilled water
- Cotton wool
- Muslin cloth

Apparatus:-

- Stain less steel knife
- Cutting board
- Measuring cylinders(500ml.,1000ml.)
- Beakers(500ml,1000ml)
- 10cm^3 graduated pipette
- Hot plate
- Magnetic stirrer
- Petri dishes

3.1.2.3. Culture Plates preparation

- Laminar flow cabinet (Model: EACI Environmental Air Controller, INC)

3.1.2.4. Inoculated plate incubation

- Incubator (0-50°C,Model:Lab line Instrument ,INC)

Experiment 1: Identification of defects of brined Gherkin Materials

Materials:-

- Defected fruits

Apparatus:-

- Hand lens

Experiment 2: Microscopic observation of defected area.

Materials: -

- Distilled water
- Defected fruits

Apparatus:-

- Light microscope, slides
- Stain less steel knife

Experiment 3: Humid Chamber Method.

Materials: -

- Defected fruits

Apparatus:-

- Hand lens
- Humid chamber

Experiment 4, 4.1, 4.2, 4.3–Determination of softening cause by obligatory parasite.

Materials: -

- sterilized brine solution
- fresh fruits

Apparatus:-

- sterilized slinger
- sterilized bottle
- lamina flow cabinet

Experiment 5-Identifying microorganisms by culturing defected pieces on PDA Plates

Materials:-

- 75% Alcohol
- sterilized distilled water
- watch glass
- prepared PDA Plates

Apparatus:-

- incubator

Experiment 6-Identification of Total *Coliform* test

Materials:-

- Maeconkey agar
- Distilled water

Apparatus:-

- starrier
- Petri dishes
- Beaker
- Autoclave

Experiment 7-Identification of bacteria

Materials:-

- Nutrient agar
- Distilled water

Experiment 8 –Determination of acidity, pH of cover brine in vats.

Materials:-

- Slandered NaOH
- Phenolphthalein

Apparatus:-

- pH Meter

Experiment 9 –Determination of vats dissolved oxygen (DO) concentration in vats

Apparatus:-

- Oximeter

3.2. Methodology

3.2.1. Methodology common for all experiments

3.2.1.1. Glassware, tools and media sterilization

a) Glass ware sterilization

- Petri dishes were placed in canisters and sterilized in the hot air oven at 180°C for 1 1/2 hrs.
- Flask and funnel were plugged with cotton wool and each was wrapped with an aluminum foil. They were sterilized in the autoclave at 121°C and 15 psi for 20 minutes (SLS 516, part 8, 1983).

b) Sterilization of tools

Inoculation loop-The loop was incinerated using a Bunsen burner until it become red hot. Then it was allowed to cool for about 10 seconds before use.

Spreader and the scalper were dipped in a small beaker containing 70% alcohol. Excess alcohol was allowed to drain and both were sterilized by passing through the Bunsen burner flame .then they were allowed to cool for about 10 seconds before use (Ileperuma I.A.T.K., 2006)

c) Sterilization and melt of the media

- Prepared liquid media were covered with cotton plug and autoclaved at 121°C, 15psi, for 15 minutes (SLS 510, part 10, 1983).
- Solidified media was remelted in the autoclave at100°C for 15minites.

d) Preparation of lamina flow cabinet

- Surface of the laminar was sterilized using 70% alcohol.

3.3. Methodology

3.3.1. Experiment 1 - Identification of defects of brined Gherkin

Procedure -Defected samples were collected and observed the defects using hand lens.

3.3.2. Experiment 2 -Microscopic observation of defected area

Procedure - Thin cross sections and longitudinal sections of the defected area of brined fruits and undefected area of brined fruits were observed through the low power of the light Microscope.

3.3.3. Experiment 3 -Humid Chamber Method

Procedure –Defected 5 fruits were cut into two through defected area and kept them in humid chamber for 5 days for observe fungal growth.

3.3.4. Experiment 4 -Determination of softening cause by obligatory parasite

Procedure –

Vlasette variety 25-44mm diameter size five fresh fruits were taken. Then fresh fruits were washed with 75% alcohol and then washed with sterilized distilled water. After defected fruits interior ooze was injected to sterilized fruits using sterilized slinger making mechanical damage. Fruits were dipped in sterilized brined solution. It was kept for 10 days and observed changers.

3.3.4.1. Experiment 4.1 –

Procedure

Above procedure was repeated by spreading ooze on the skin of the fresh fruits.

3.3.4.2. Experiment 4.2–

Procedure

Above procedure was repeated by spreading ooze on the skin of the semi processed 5 fruits

3.3.4.3. Experiment 4.3-

Procedure –Above procedure was repeated by injecting ooze in to the semi processed 5 fruits making mechanical damage.

3.3.5. Experiment 5–Identification of *Yeast* and molds (preparation of pure culture using streak plate technique)

Preparation of PDA Medium was prepared using potato. Then the Medium was autoclaved at 121°C for 15psi for 15 min. for sterilized the medium. (SLS 510, part 10, 1983)PDA Plates was Prepared PDA plates inoculated with defected fruits. Inoculated PDA plates were incubated at 25°C for 14 days in an inverted position after observations were taken.

3.3.5.1. Experiment 5.1.-Re inoculation of identified colonies.

Procedure

Identified colonies in experiment 5 were reinoculated to the fresh cucumber as the procedure in experiment 4 and kept with original condition, after 10 days observations were taken.

3.3.6. Experiment 5.2.-Identifying microorganisms by culturing defected pieces on PDA Plates.

Gherkin pieces were taken from defected fruits of defected area. Gherkin pieces were washed with 75% alcohol. Again pieces were washed with 75% alcohol. Next pieces were washed with sterilized distilled water. Then pieces were allowed to drain by keeping on them sterilized filter paper. Pieces were placed on PDA agar plates. PDA agar plates were labeled. PDA plates were incubated at 25°C for 14 days.

3.3.7. Experiment 6-Identification of *Coliforms*

Procedure

14g of Macconkey agar was weighed and dissolved it with 500ml of distilled water in a sterilized flask. Medium was sterilized at 121^{0}C and 15 psi for 15 min. 15ml of medium was transferred to each of the sterilized Petri dishes at laminar flow cabinet. It was allowed to cool. 2ml of brine solution and defected fruit ooze was spread on the plates and covered with parafilm. Plates were incubated at 37^{0}C for 48hr.After the 48 hour incubation the plates were examined for growth characteristics of coliform colonies.

3.3.8. Experiment 7-Identification of bacteria

3.3.8.1. Preparation of culture media

Procedure

14g of NA was weighed & dissolved with 500ml of distilled water. Mixture was well dissolved using a mechanical shaker. Media was sterilized in autoclave at 121^{0}C, 15psi, for15min.NA Plates was prepared pouring sterilized medium. Medium was Incubated at 35^{0}C for 2 days

3.3.8.2. Biochemical tests for bacteria identification

Bacteria Gram's staining

Procedure

Colony smear was prepared and it was heat Fixed. Then the smear was stained with crystal violet for about 30 seconds. After it was rinsed with water to remove the excess stain. Then the Smear was flooded with grams' iodine and allowed it to absorb for about 30 seconds. Again the smear was rinsed with water. Then smear was decolorized using 95% alcohol and the excess was removed by rinsing with water. Smear was counter stained with safranine for 20 -30 seconds Again smear was rinsed with water and blotted dry. Slide was examined under oil immersion lens (Harry W. *et al.*,1997)

Catalase test

Procedure

A colony of the bacteria was picked up from a plate and transferred on to microscope glass slide containing a drop of added distilled water Few drops of 3% H_2O_2 was Placed over the culture. It was observed the bubble formation within 20 seconds (Dubey R C , 2004).

3.3.9. Experiment 8 -Determination of acidity, pH of cover brine in vats

Procedure

Titrable acidity was measured titrating with slandered sodium hydroxide.

3.3.10. Experiment 9 -Determination of vats dissolved oxygen (DO)

Procedure

DO was measured. in vat upper layer and bottom layer using dissolved oxygen meter.

CHAPTER 4

Results and Discussion

4.1. Results

4.1.1. Experiment 1- Identification of defects of brined Gherkin

1) Bubble formation on the skin was mostly observed in 25-44 mm diameter size fruits of *Vllasette* variety.

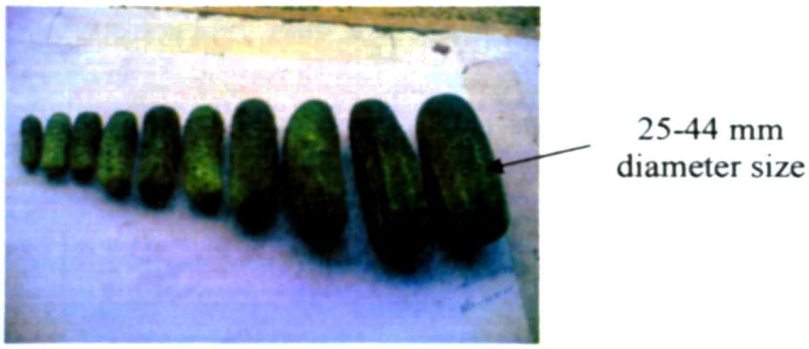


Fig. 4.1. Grades of cucumber

2) Defect was initially appeared as bubble on the skin. Bubble size varies from fruit to fruit. Bubble appears on the middle area of the fruit, but not at the two ends of the skin.

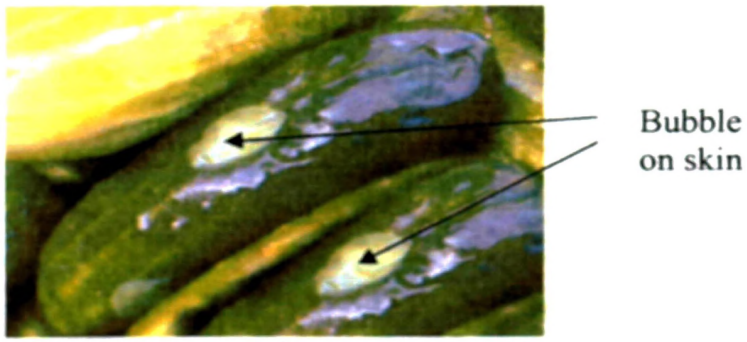


Fig. 4.2. Bubble on the fruits

3) After bubbles can be seen as holes

Fig. 4.3. Hole of fruits

4) Interior of the hole was filled with liquid.
5) Defect was initially shown in outer layer of the fruit.
6) Then the hole was spreaded to the interior of the fruits.
7) Finally, the whole interior flesh was destroyed remaining only the fruit skin.

Fig. 4.4. Final stage of the defect

Other observations.

1) Defected fruits were observed in the upper layer of the vats.
2) However only some fruits get defected.
3) To observe the defect it takes about 5-7 days.

4.1.2. Experiment 2 - Observation of defects through light microscope.

Softening is a reaction of destruction of middle lamellar containing pectic substances by pectolytic enzymes which causes loses of firmness and crispiness of the tissues of brined fruits. This can be identified by low power of microscope.

Undefected area

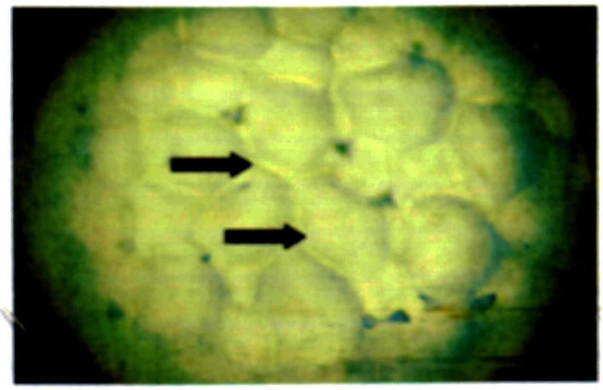


Fig.4.5. Brined fruit, Cross section, X100

Defected area

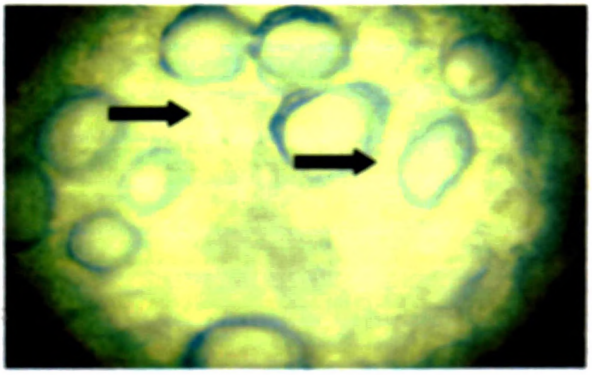


Fig.4.6.Brined fruit, Cross section, X100

→ Undestroyed middle lamellar in between cells

→ Destroyed middle lamellar in between cells

Undefected area **Defected area**

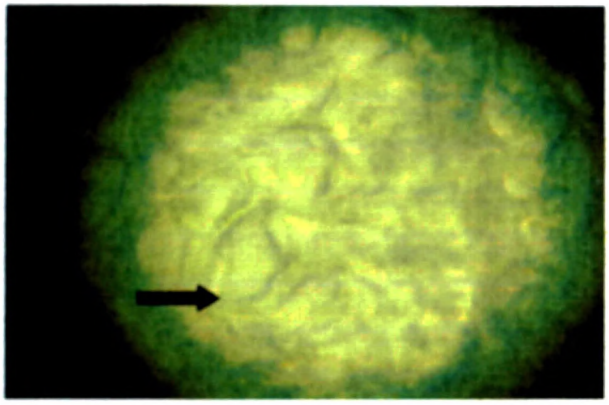


Fig.4.7. Brined fruit, longitudinal section,X100

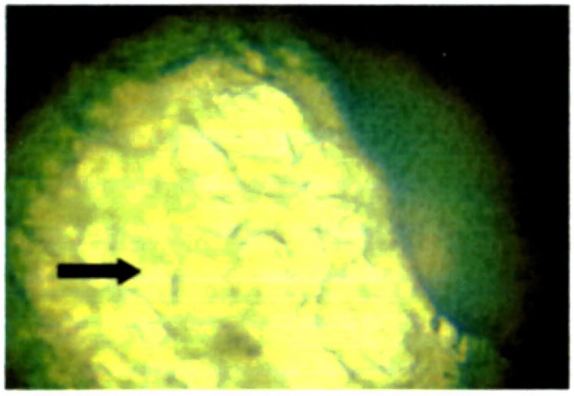


Fig.4.8. Brined fruit, longitudinal section, X100

→ Undestroyed middle lamellar in between cells

→ Destroyed middle lamellar in between cells

Observations-

1) Undefected fruits - Middle lamellar was clearly identified in between undestroyed parenchyma cells.
2) Cells were destroyed of the defected fruits.

Conclusion- Softening was occurred in brined gherkin

4.1.3. Experiment 3 -Humid Chamber Method

In the humid chamber it creates 100% atmospheric humidity. At this condition active spore of fungus is grown presence in defected area. And defect is enlarged. Thus this is test can be used to get a rough idea about fungus attack whether exist.

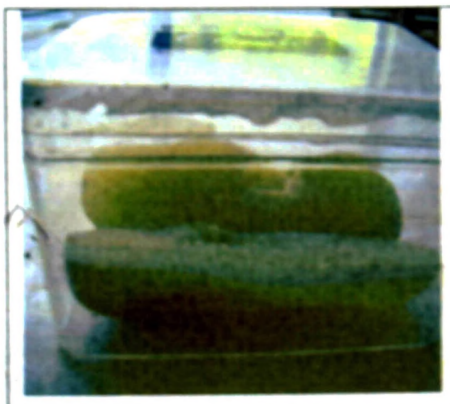


Fig.4.9. Defected Gherkin in Humid chamber

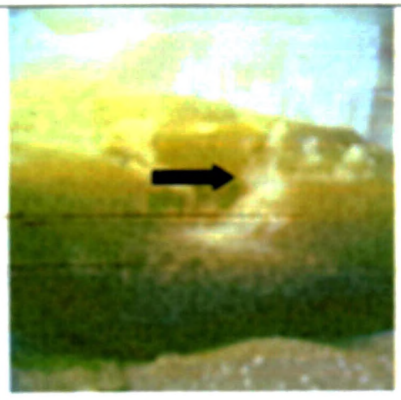


Fig.4.10. After 2 days in Humid chamber

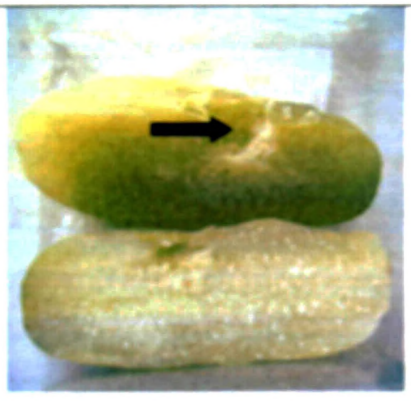


Fig.4.11. After 5 days in Humid chamber

Observations- In the five fruits that kept inside the humid chamber, fungal mycelia growth were not observed. *Yeast* growth was observed.

Conclusion- No mold growth, but *yeast* was present.

4.1.4. Experiment 4 –Determination of softening cause by obligatory parasite.

Obligatory parasites are microorganisms which grown in host only, not in artificial culture media. Microorganisms follow a life cycle, if miss any step growth of them might not observed. That is why defected fruit ooze spread on skin of the fruits as well as interior to prevent such miss of life cycle.

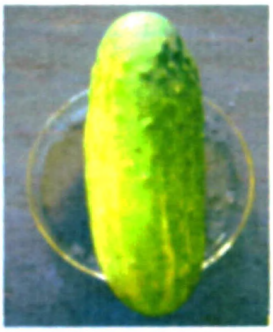


Fig.4.12.Row fruits used to Identify the defects caused by

Fig.4.13.Defected fruit ooze injected, fermented fruits for 10 days

Fig.4.14.interoir of (fig.4.13.)

Fig.4.15.semi fermented fruits used to identify the defects caused by obligatory parasites

Fig.4.16. Interior of (fig.4.13.)

Observations-Bubble defect was not observed in all fruits that used to check obligatory parasites (fruits were like quality brined fruits.)

Conclusion- This defect was not caused by any obligatory parasite.

4.1.5. Experiment 5–Identification of *Yeast* and molds (preparation of pure culture using streak plate technique)

A streak plate is prepared by dipping a sterile loop, into a medium containing a suspension of cell of the desired organism. The loop is used to make a series of parallel non overlapping streaks across the surface of the medium. As successive streak are made a number of cell is diluted to such a point that the final streak will usually yield separate and distinct pure colonies (Prescott and Dunn, 1987).

In identification of *Yeast*, it has characteristic formation of budding and ascospores in the cells. *Yeast* can see very clearly in medium power of the light microscope.

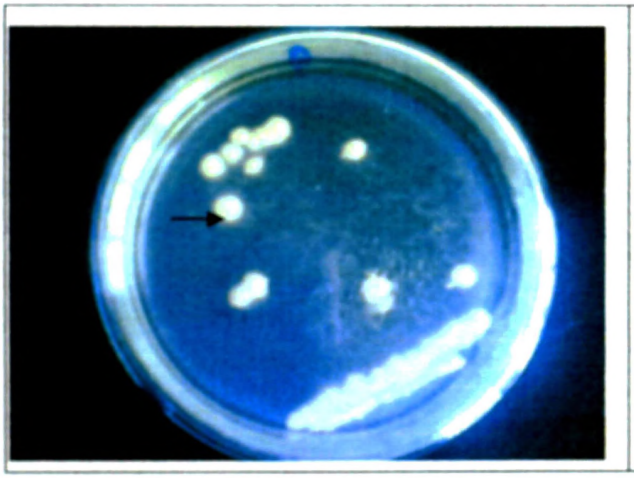


Fig.4.17. *Yeasts* colonies
→ *Yeast* colony

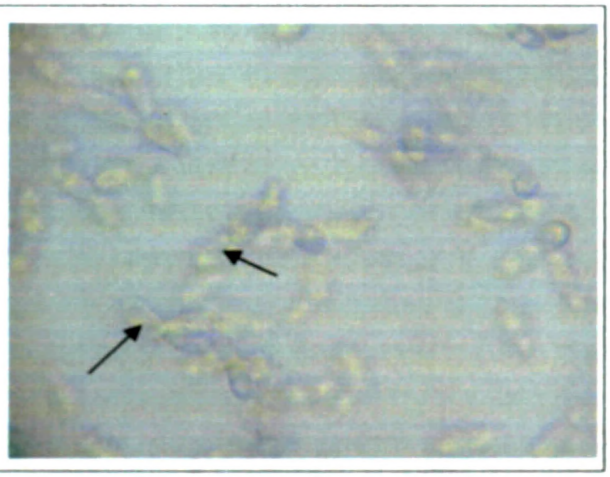


Fig.4.18. *Yeasts* cells, X400
→ Ascospores

Observations – (Colony characteristics)

- Colonies were formed, punched form, Unicellular budding cells
- Colony spreads mostly over bottom of the surface or some where middle part of the surface.
- Colonies are pasty in appearance, No any other fungal hyphae growth.

Conclusion-according to microscopic observation and colony morphology *Yeast* was identified. And also *Actinomycetes* was detected.

4.1.6. Experiment 5.1: - Re inoculation of identified colonies.

Re inoculation of pure culture to the fresh quality fruits it was not detected any defect similar to bubble formation. This procedure is called Koch's rule which is very important to identify the pathogenic disease. Here identified *Yeast* and *Actinomycetes* colonies re inoculate to the fresh fruits to detect this defects are caused by these organisms.

Observations of re inoculated fruits-

- The defect was not observed in re inoculated fruits

Conclusion-

- Defects are not caused by *Yeast* and *Actinomycetes*.

4.1.7. Experiment 5.2.-Identifying microorganisms by culturing defected pieces on PDA Plates.

Defected gherkin pieces can remain spore of softening organisms. Gherkin pieces were washed with alcohol to remove other unwanted microbial cells and sterilized distilled water was used to wash out excess alcohol. But it remains fungal spores if present which are grown.

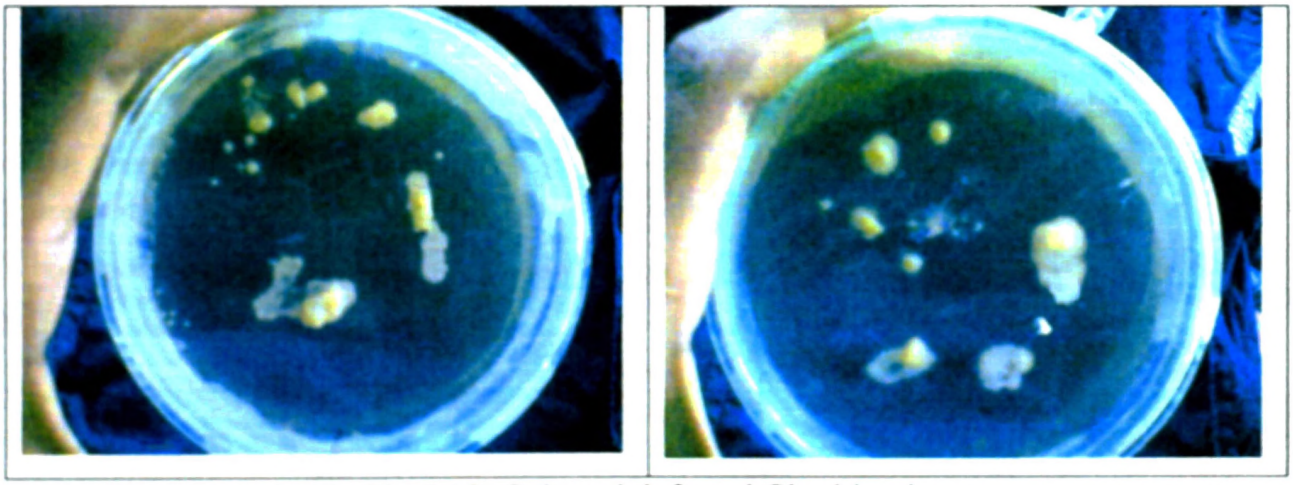


Fig.4.19. Cultured defected Gherkin pieces

Observations-Any kind of fungal mycelium was not observed.

Conclusion-Mold growth was not observed.

4.1.8. Experiment 6-Identification of *coliforms*

Coliform are microbes that can ferment simple sugar. If *Colifroms* present it observe as red color colonies. If these microorganisms entered to the fruits they can ferment sugar present in the fruits. Thus these microorganisms may be the causal organism. This experiment was used to culture *Coliforms* using pour plate technique to identify *Coliforms*.

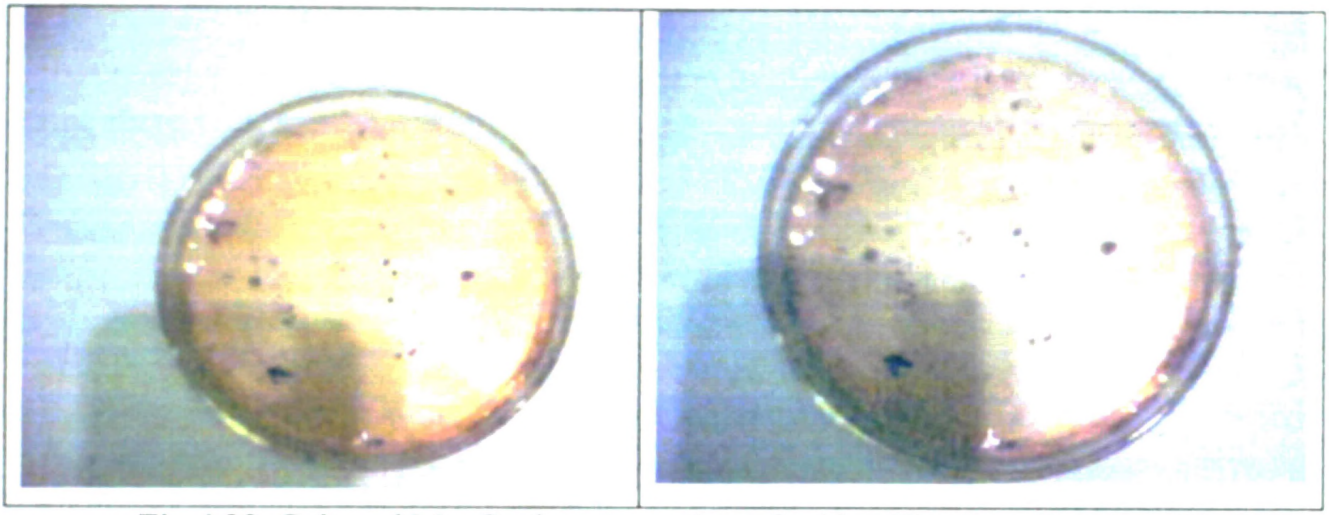


Fig.4.20. Cultured MacConkey Agar plates for identification of total *Coliforms*

Observations-Red color colonies were not observed

Conclusion-*Coliforms* are absent.

4.1.9. Experiment 7- Identification of Bacteria

Observations-

Two types of colonies were observed in NA media.

Morphological test

1. Colony form
2. Punched form
3. Colony spreads mostly over bottom of the surface or some where middle part of the surface.

Results of grams' Staining

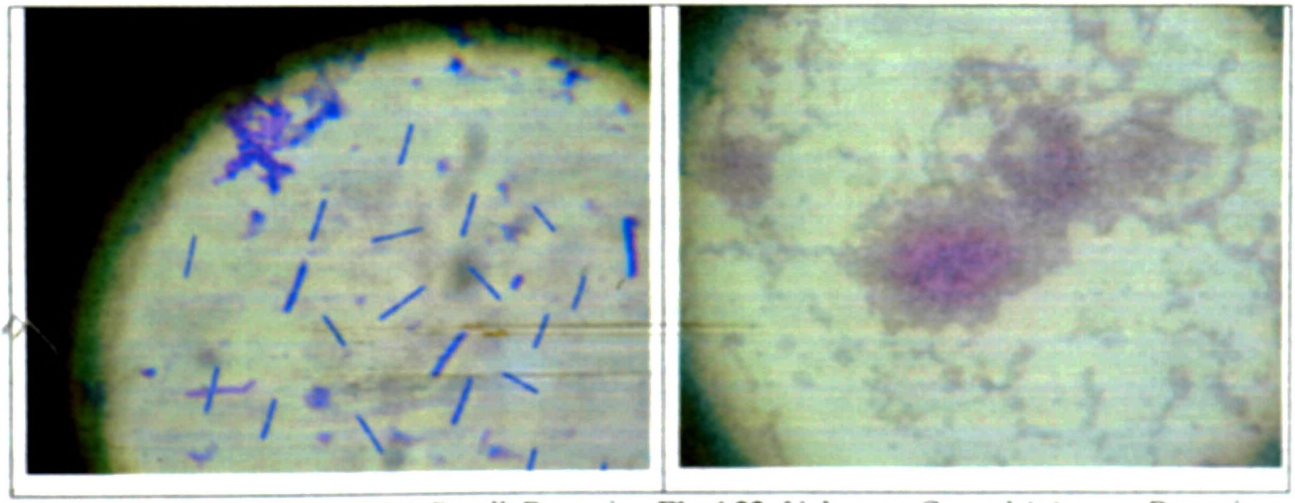


Fig.4.21. Unknown Grams' (+) *Bacilli* Bacteria. **Fig.4.22.** Unknown Grams' (+) *cocci* Bacteria.

Microscopic observations of above Unknown Grams' (+) *Bacilli* Bacteria	**Microscopic observations of above Unknown Grams' (+) *cocci* Bacteria**
Rod shape Cells (*diplobacilli*)	*Cocci* (through oil immersion lens)
Chemical tests	
Catalase test- catalase (-)	Catalase (-)
Grams' staining –Grams (+)	Grams (+)

4.1.10. Experiment 8-Determination of acidity, pH of cover brine in vats.

Observations-

In the process of Koch's postulation (re inoculation of pure cultures) it is needed to identify environmental condition to provide proper conditions. Because for growth of microorganisms pH value, salinity, temperature, water activity are very important.

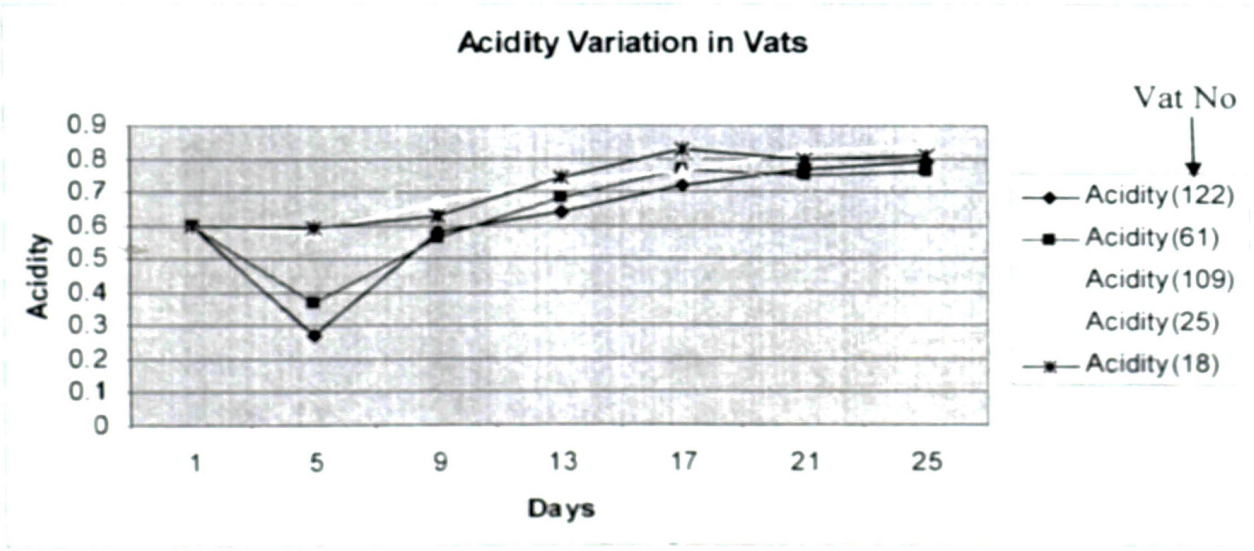


Fig.4.23.acidity variation in vats for first 30 days

Acidity of the vats varies during first 30 days in between 0.5-0.81%. Initially concentrated acetic acid was added to prepare 0.6% acid solution. That is for control the growth of unfavorable microorganisms.

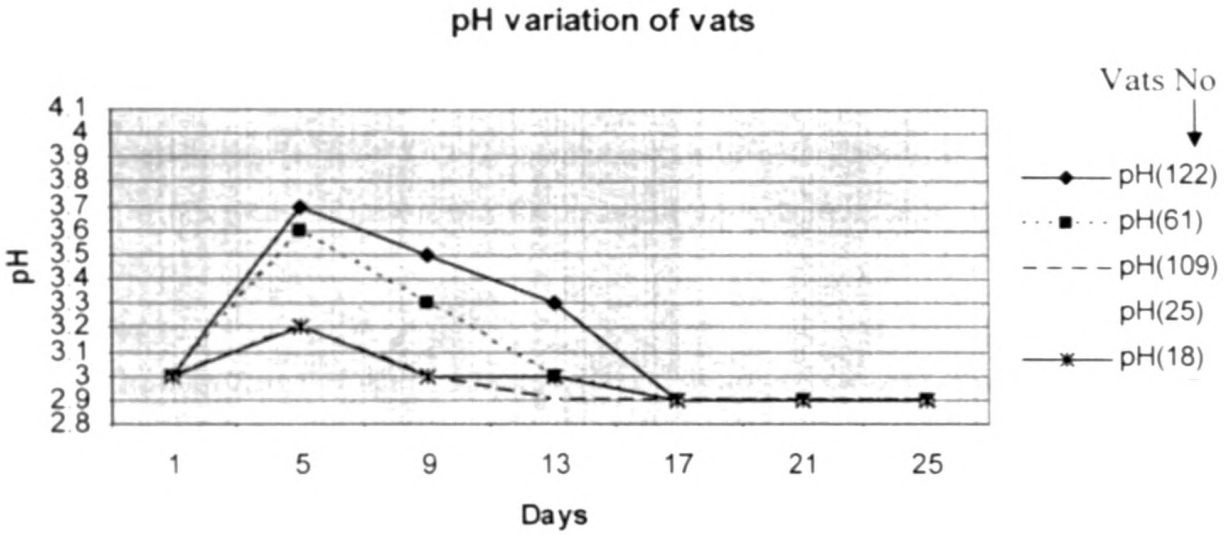


Fig.4.24. pH variation in vats for first 30 days

pH of the brine solution varies between 2.8-3.5 during first 30 days. These measurements are very important to decide whether pathogenic microorganisms are present. Because microorganisms has minimum and maximum pH value that they can alive. According to this result it can be proven that *Clostridium botulinum* cannot be grown at this condition, as the minimum pH values that needed for the growth of *Clostridium botulinum* is 4.6.

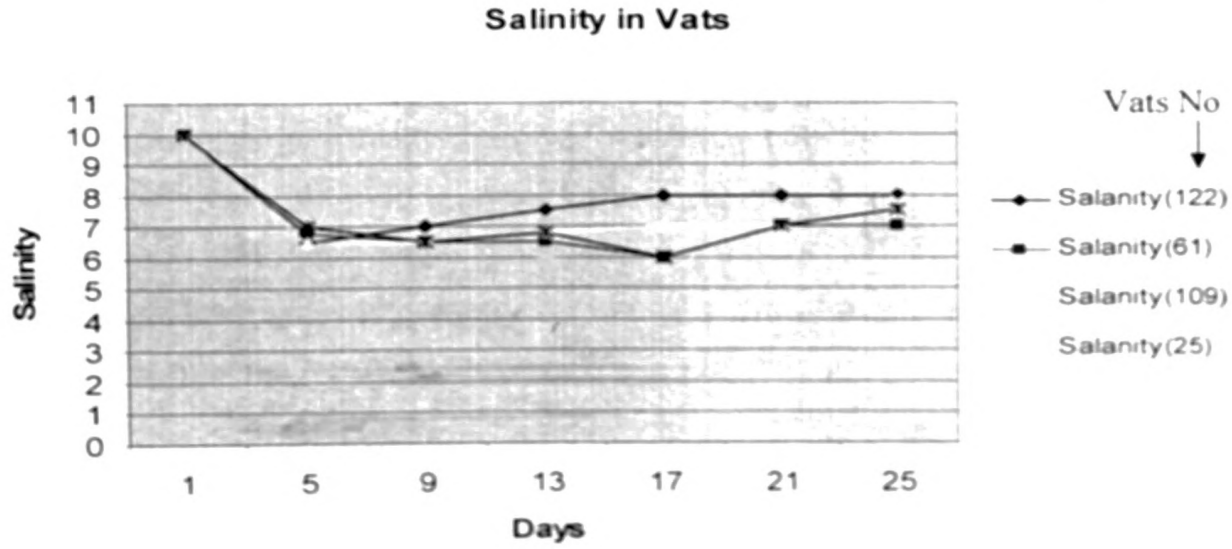


Fig.4.25. Salinity variation in vats for first 30 days

Water activity of a salt solution is normally 0.9. *Yeast* and mold, as well as some bacteria can grow at this condition.

Conclusion- pH of the brine solution varies between 2.8-3.5, acidity 0.5-0.81% and salinity 5 -8.5% within first 30 days of fermentation.

4.1.11. Experiment 9 -Determination of DO in cover brine in vats.

There is an optimum level of DO concentration in brine solution which is favorable for the growth of microorganisms. If the DO concentration is more than 1mg/L. mold growth can be observed.

Observations-

In top and bottom layer of the fermentation vats, dissolved oxygen concentration was not distinctively varies. Dissolved oxygen varies 6-8mg/L in vats.

Conclusion-According to this data, mold growth can be observed

4.2. Discussion-

Softening of gherkin in fermentation is a problem which causes profit losses. This type of defect previously was not identified and no earlier records about such a defect in detail. In brining process fresh quality fruits are graded according to the diameter of the fruits. This defect mostly identified in 25-44mm size brined fruits which is initially appeared as bubble on the skin of the *Ullasette* variety. But there is no evidence of this defect in *Ajex* variety. With the time it makes a hole in the flesh of the fruit and ultimately it spreads to the interior of the flesh and interior fills with liquid. Initially defect appears in outer flesh. With time the flesh gets totally destroyed remaining only the skin of the brined fruits. Defected fruits were observed in upper layer of the vats. However every fruits in every vat don't show this defect and it takes about 5-7 days to initiate the defects. Defected fruits can be identified totally dipped fruits in cover brine not submerged fruits. All these observations were not identified previously.

Also, these defects were showing only during the Yala season. The main differences of these two seasons are annual rain fall pattern and the environmental temperature. If these defects are caused by any kind of pathogenic organism, these two conditions (specially

the environmental temperature) can influence the growth of microorganisms. That may be the reason for above.

Further more, in here all these vats were opened to the ambient environment, where in other countries fermentation is done in covered conditions to prevent the contact of brine solution with the outside environment. Thus it can be mixed with birds droppings. It is a one source which can add pathogens to the medium. It has found that the softening resulted due to naturally existing enzymes. One of such softening has recorded to be initiated from the stem end. But this matter is not such a case, as large fruits contain high amounts of natural enzymes this can also be happened due to natural enzymes. Also it is needed to find out why this symptom is localized. Longitudinal cross sections of the defected area of brined fruits shows destructed middle lamellar and cells. But undefected area of brined fruits it appears undestroyed distinct middle lamellar in between cells. Thus, it is clear that this defect is softening.

The use of general purpose medium for detection of pectolytic microorganisms has a drawback in the study of microorganisms as this is a long process. There fore, it is easy to use a specific medium (poly pectate gel medium) for this purpose due to rapid screening.

As a presumptive test in humid chamber method it doesn't show any growth of fungal mycelium. If this softening cause by any mold it should be grown in defected area within about 5 days. Only *Yeast* and *Actinomycetes* growth were observed. How ever, as both *Yeast* and *Actinomycetes* do not secrete pectolytic enzymes, the softening of the gherkin can not be resulted due to them.

Defected fruit ooze was injected to the sterilized fruits and allowed to ferment as similar to the original process to find out whether the defect caused by obligatory parasites, because obligatory parasitic organisms grow only in host. This defect initiation can be happened due to attack of microbes to semi fermented fruits, but this is proven that after injecting the infected fruits ooze to semi fermented fruits the defect was not observed

In pure culture preparation of fungus on PDA media it doesn't identified any fungal mycelium growth except *Yeast*. It has recorded that *Coliforms* can live at this pH and they can ferment simple sugar if they anyhow entered the interior of the fruits. But this test proved that no *Coliforms* in brine solution.

In pure culture preparation of bacteria, the study was able to identify diplobacilli gram (+), catalase (+) bacteria which can be lactobacillus (See. Fig.4.17.). But, further biochemical tests are needed to identify cocci, gram (-), catalase (+) bacteria. (See. Fig.4.18.)

There are lots of factors to be considered for solving this problem. According to literature this softening identified to be caused by genera *Alternria* ,*Fusaiumand Mucor*. As it couldn't be observed any fungal growth in culturing on PDA it is proved that this defect is not caused by above mentioned genera.

In reinoculation process of *yeast* and *Actinomycetes*, it is necessary to prepare the brine solution conditions similar to original brine solution in order to use Koch's postulation accurately. According to the analysis of the brine solution, pH of the brine solution varies between 2.8-3.5, acidity 0.5-0.81% and salinity 5 -8.5% within first 30 days of fermentation. Dissolved oxygen concentration in brined solution is not distinctively varies between the top and bottom layer of the vats (See. App. 1).

The aim of the air purging is to prevent the bloater formation in the fruits. But this can cause negative effects as it can facilitate the growth of fungi. Because it creates aerobic condition in brine solution. If the dissolved oxygen concentration of brine solution is more than 1mg L. it can allow the growth of molds. During the first 14 days dissolved oxygen content varies between the ranges of 6-8mg L (See. App. 11).

Therefore, further studies should be conducted considering all these factors mentioned above in order to solve this matter.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

- *Yeast & Actinomycetes* were identified in defected area of the fruits.
- There is no positive correlation between pectinolytic *Fusarium, Alternria,* and *Mucor* with softening of gherkin.
- Defect is not caused by obligatory parasites
- Dissolved oxygen concentration of vats varies between 6-8mg/L during first 14 days.
- pH of the brine solution varies between 2.8-3.5, acidity 0.5-0.81% and salinity 5 - 8.5% within first 30 days of fermentation.

5.2. Suggestions for Further Studies

- According to the observations defects were identified mostly in 25-44mm diameter size fruits of *Ullasette* variety. Further studies are necessary to find the other varieties showing these defects.
- A detail investigation is needed to identify the reason for defect to appear only in the fruits in upper layers of the vat.
- Further studies are needed to identify the reasons for localized softening.
- More sophisticated biochemical tests are required to identify the exact type of organisms.

REFERENCES

- Adams, M.R., Moss, M.O., (1995). Food Microbiology, New Age International limited Publishers, 398p.
- Agriculture department, (2009). (Available from:http://www.agridept.gov.lk/more.php?morelink=Introduction%20%20&pagelink=Cucumber&heading=Vegetables Accessed on 25/02/2009)
- Agrios, G.N., (1997). Plant pathology, forth Edition, academic press, 635p.
- Barnwart, G.J., (1987). Basic food Microbiology, second edition, CBS publications & Distributors, 773p.
- Bell, T.A., Etchells, J.L., (1952). Food Technology, *sugar and acid tolerant of spoilage yeasts from sweet cucumber pickles. Vol. 22.* 468-472pp
- Bell, T.A., Etchells, J.R., (1950). Classification yeast from the fermentation of commercially brined cucumber, Farlowia, 87-112p.
- Breidt, F., (2006). Microbiology of Fruits and Vegetables, *Safety of minimally processed, acidified, and fermented vegetable products*,Vol.16, Boca Raton, CRC Press, Inc. 313-335p.
- Bell, T.A., Etchalls, J.L.,(1960). Journal of food science: *influence of salt on pectinolytic softening of cucumber*,*Vol.23* ,85-89pp
- Bell, C., Meaves, p., Williums, A P., (2005).Food microbiology and Laboratory Practices, Blak Well Publication ,324p.
- Cat Lab,(2007), Pectin - Introduction,Available From http://images.google.lk/imgres?imgurl http: edavies.files.wordpress.com/2007/04/pectin Accessed on 2009 01 13)
- Costilow,R.N.,Fabian,F.W.,(.1957)(Availablefrom.http://aem.asm.org/cgi/reprint/1/6/314.pdf Access on 2009 01 25)
- Robinson R.W.,Decker D.S.,(1997).Cucubits, cab international,226p
- Demain A L., Phaff H J. (1957). Agriculture and Food Chemistry *softening of cucumber during curing Journal*, Vol.5, 60-64pp
- DeVuyst, L., Vandamme, E.J., (1994). Antimicrobial potential of lactic acid bacteria. Bacteriocins of Lactic Acid Bacteria,941-1142p.
- Duber, R.C.,Maheshwari, D.K.,(2004). Practical Microbiology, First edition, S.cahnd & Company limited,350pp

- Dubey, R.C., Maheshwari, D.K.,(1999).A text Book of Micriobiology, First edition, S.Chand & Company LTD,681pp
- Etchells, J.L., Borg, A.F., Bell, T.A., (1968) Journal of food science: *factors influencing bloater formation in brined cucumber during controlled fermentation. Vol.16*, 569-575pp.
- Flaming, H.P., Thompson, R.L., Monroe R.J., (1978). Journal of food science *Susceptibility of pickle cucumber to boater damage by carbonation, Vol 43*: 892-896pp
- Fleming, HP., Daeschel, M.A., McFeeters, R.F., Pierson, M.D., (1989). Journal of Food Science: *Butyric acid spoilage of fermented cucumbers. Vol.54*, 636-639pp.
- Flaming H.P.,Thompson R.L.,Monroe R.J.(1978),susceptibility of pickle cucumber to bloater damage by carbonation., Journal of foods,43pp.842-896.
- Garbutt, J., (1997).Essentials of Food Microbiology, published in Great Britain in 1997 by Arnold, 251p.
- George, M. Garrity., (2001).Bergey's Manual of Systermatic Bactiriology ,Second edition, Springer Verlage Newyork Heidelberg,720pp.
- Gerard J. T., berdell, R., Chrstine L.,(2009).Microbiology An introduction, Eighth edition, dorling Kinder Sley(india) I.vd Ltd, 898pp.
- Harry, W.S., Vanderemark, J.P., Lee.J.J., (1997). A Laboratory Manual of Microbiology, W.H.Freeman and Company, fourth Edition, 351p.
- Homer, C., William, C.K., (1957). Vegetable crops, fifth edition, MC Graw Hill Book Company, INC, London, 635pp.
- Hutkins, R.W., (2006). Microbiology and Technology of Fermented Foods. Ames, Blackwell Publishing. 473 p.
- Ileperiuma, I.A I.K., (2006), screening of a potential biological control agent for the control of post harvest stem end rot of Banana and Papaya, 43p.
- Inter science, (2006), Institute of food technologist, Available on (http: www3.interscience.wiley.com journal 119780244, Access on 05 02 2009).
- Jay, J M., Loessner, M J., Golden, D.A., (2005). Modern Food Microbiology Springer New York, NY 790 p

- James, M. J., (1992), Modern Food Microbiology, chapmen and Hall Newyork London, 700p.
- Jay.J.M. (1996). Moderm food microbiology, champ and Hall Newyork London, 700p.
- Khetarpaul, N., (2005), Food microbiology, Daya Publisher House, 540p.
- Mavin, S.M., (1976). Compendium of methods for the microbiological Examination of foods, first edition, American Public Health Association, 702p.
- Norman N. Potter, Joseph H. Hotchkiss(1996),Food science, fifth edition, CBS Publishers & Distributors,608pp
- Pelczar, M. J., Chan, E.C.S., Krieg, N.R., (1993). Microbiology, Fifth Edition, Tata Mcgraw Hill Publishing Company limited, 917p.
- Mitruka, B.M., (1977).Methods of detection of Bacteria, CRS Press, INC Boca Raton,London, 256p.
- Panday, B.P.,(1994),Text Book of Botany, S. Chand & company limited, first edition, 324p,
- Pederson, C.S.,(1979).Microbiology in food fermentation, second edition, AVI publishing company, west port, conn,420pp
- Pitt, J.I., Hocking, A.D., (1999). Fungi and food Spoilage, second edition, An Aspen Publication, 593p.
- Prescott & Dunn's (1987), Industrial microbiology, fourth edition, Edited by Gerald reed, CBS publication & Distributors 883p.
- Prescott, L.M., Harley, J.P., Klein, D.A., (1987). Microbiology, New York, NY: McGraw-Hill, 992 p.
- Purseglove J.W.,(1968).Tropical crops-Dicotyledins, Longman group limited ,718p
- Ralph N. Costilow, Karen Gates, Melvyn L. Lacy, (1980). Applied and Environmental Microbiology, *Cause of Softening During Air-Purging of Fermentations. Vol.40,* 417-422p.
- Russell, I., Stewart, G.G., Yeast biotechnology, Landon Allen and Unwin Bostan, Sydney, Wellington, 539p.
- Salminen, S., (1998). Lactic acid bacteria, Second edition, Marcel Dekker, INC, 614p.

- Snowdan, A.L., (1990). A Colors Atlas of post Harvest; Disease and disorders of fruits and vegetables, volume 1, Wolfe Publishing LTD, 253p.
- Sri Lanka standards, Microbiological test methods, first revision, SLS 510, part 10, 1983).
- -- (1996), microorganisms in foods; microbial ecology of food commodities, an aspen publication.576pp
- Sri Lanka standards, microbiological test methods, first revision, SLS 516, part 2, 1991.
- Yadev, M., (2003).Microbiology, Discovery Publishing House, New Delhi, 316p.

Appendix 1

Results of Experiment 8:

		Vat No 122			Vat No 61	
Days	pH(122)	Acidity(122)	Salinity(122)	pH(61)	Acidity(61)	Salinity(6)
1	3	0.6	10	3	0.6	10
5	3.7	0.27	6.5	3.6	0.37	6.8
9	3.5	0.58	7	3.3	0.56	6.5
13	3.3	0.64	7.5	3	0.68	6.5
17	2.9	0.72	8	2.9	0.77	6
21	2.9	0.77	8	2.9	0.75	7
25	2.9	0.79	8	2.9	0.76	7

		Vat No 109			Vat No 25	
Days	pH(109)	Acidity(109)	Salinity(109)	pH(25)	Acidity(25)	Salinity(25)
1	3	0.6	10	3	0.6	10
5	3.2	0.58	6.5	3.2	0.63	6.5
9	3	0.67	6.5	3	0.74	6.4
13	2.9	0.73	6.8	3	0.77	6
17	2.9	0.76	7	2.9	0.81	5.5
21	2.9	0.82	7	2.9	0.72	7.5
25	2.9	0.84	7.5	2.9	0.73	7.8

		Vat No 18	
Days	pH(18)	Acidity(18)	Salinity(18)
1	3	0.6	10
5	3.2	0.59	7
9	3	0.63	6.5
13	3	0.74	6.8
17	2.9	0.83	6
21	2.9	0.8	7
25	2.9	0.81	7.5

Appendix 11

Results of Experiment 9:

No of days for fermentation	Salinity (%)	Salinity(mg/L)	
		Bottom layer	Top layer
Vat No 172			
1	6.5	6.4	6.9,6.7,7.1
7	7	6	5.9,6.1,6.2
10	7.5	6	6.2,6.5,6.0
Vat No 432			
4	5.5	7.5	7.9,7.9,7.9
10	7.5	6.3	6.7,6.8,6.6
14	8.0	6.0	6.5,6.3,6.2
Vat No 405			
3	5.5	6.7	7.3,7.2,7.5
10	8.0	6.5	7.1,7.2,6.9
Vat No 315			
5	7.5	6.5	7.1,7.0,6.8
11	8.0	6.0	6.1,6.9,6.5
Vat No 163			
2	5.5	7.3	7.5,7.9,7.8
8	7.5	6.6	6.8,7.3,7.4

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