# APPLICATION OF HAZARD ANALYSIS (HACCP) IN STARCH PRODUCTION BY THE WET MILLING OF MAIZE

BY

**R C SAMUELS**

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| **INTERNAL EXAMINER:** | Mr G J le Roux M.Sc. (Agric.) Microbiology |
| **EXTERNAL EXAMINER:** | Dr C J Jacobs Ph.D (Microbiology) |

# STATEMENT

**Masters Diploma in Food Technology**
**School of Physical Sciences**
**Cape Technikon**

I hereby declare that the contents of this research project is my own work, and opinions expressed and recommendations made are my own and are not necessarily that of the Cape Technikon.

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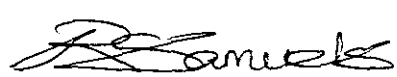

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# DEFINITIONS

The following definitions are from the guidelines of NACMSF (1992).

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| **HACCP** | Hazard analysis and critical control points. |
| **HACCP PLAN** | this is a written document which is based upon principles of HACCP and consist of prescribed procedures to be followed to assure the control of a process or procedure. |
| **HAZARD** | a biological, chemical and physical property that may cause a food to be unsafe for consumption. |
| **CONTROL POINT** | any point, step or procedure at which biological, physical or chemical factors can be controlled. |
| **CRITICAL CONTROL POINT (CCP)** | a point, step or procedure at which control can be applied and a food safety hazard can be prevented, eliminated or reduced to acceptable levels. |
| **CRITICAL DEFECT** | any source of contamination that will result in a hazard |

| | |
|---|---|
| **MONITOR** | observations or measurements, biological, physical or chemical, to assess when a CCP is under control. Recorded for use under verification. |
| **RISK** | an estimate of the likely occurrence of a hazard. |
| **SEVERITY** | the seriousness of a hazard |
| **TARGET LEVELS** | criteria which are used by an operator to reduce the risk of deviation. |
| **spp.** | species |
| **ppm.** | parts per million |
| **cfu.** | colony forming units |

# SUMMARY

This study is based on the Hazard Analysis in the Wet Milling of maize for the production of starch at the Bellville plant of African Products.

Wet milling of maize is a highly specific and completely integrated system developed to separate the major components of the kernel as completely as possible. Many microbiological problems existed in the process at this plant which could not be solved over the years.

Microbial analyses were done throughout the plant and high microbial counts were obtained at various sampling points. In applying HACCP, the following major hazards were identified:

The presence of Faecal Streptococci, *Staphylococcus aureus, Bacillus cereus*, Faecal coliforms, *Fusarium, Diplodia, Aspergillus, Penicillium* and various *Yeast* strains.

The following Critical Control Points (CCP's) were identified in the wet milling process:

Maize trucks, in-process water, steeping, storage tanks, Reineveld, wet mixing boxes, Laidlaw, drying and bagging off point.

The following were done as part of the HACCP plan:

i) modifications of the plant were suggested,
ii) different sanitation programmes were evaluated,
iii) monitoring of CCP's, and
iv) training of personnel.

In general, a regular sanitation programme need to be exercised in the wet-milling plant to prevent a build up of microbial populations at various sampling points. High $SO_2$ levels can

be maintained throughout the plant to achieve this. The final starch will then be used for Industrial starch.

Criteria to monitor the CCP's were suggested. Hazard Analysis is an effective method to improve the quality of the final product.

# OPSOMMING

Die studie is gebaseer op die identifisering van mikrobiese gevare (HACCP) tydens die nat maal proses van mielies in stysel produksie by die Bellville aanleg van African Products.

Die verwerking van stysel is 'n baie spesifieke en 'n geïntegreerde sisteem, ontwikkel om die grootste komponente van mielies te skei. Baie mikrobiologiese probleme wat bestaan het in dié proses by die aanleg kon nie deur die jare opgelos word nie.

Mikrobiese analises was dwarsdeur die proses gedoen en hoë mikrobiese tellings is gevind by verskillende punte. In die toepassing van HACCP is die volgende hoof mikrobiese gevare geïdentifiseer:

Fekale Streptokokki, *Staphylococeus aureus, Bacillus cereus,* Fekale kolivorme, *Fusarium, Diplodia, Aspergillus, Penicillium* en verskillende *gis* spesies.

Die volgende Kritiese Kontrole Punte (KKP's) is geïdentifiseer:

Mielietrokke, proses water, weking, stoortenke, Reineveld sentrifuge, mengbakke, droging en verpakking van stysel.

In die algemeen is 'n gereelde sanitasie program noodsaaklik in so 'n proses om die opbouing van mikroörganismes te verhoed. Wanneer daar hoë tellings teenwoordig is in die fabriek, kan hoë swaweldioksied vlakke aangewend word om die doel te bereik. Die finale stysel produk kan dan gebruik word vir industriële doeleindes.

Die volgende kriteria om KKP's te monitor is voorgestel as deel van die HACCP program:

i) verandering van die fabriek uitleg,
ii) verskillende sanitasie programme is ondersoek,
iii) monitering van KKP's, en
iv) bevestiging van finale produk kwaliteit.

Die gebruik van HACCP is 'n doeltreffende metode om die kwaliteit van die finale produk te verbeter.

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# INTRODUCTION

The Wet Milling of maize is a highly specific and completely integrated system developed to separate major components of the kernel as completely as possible. Many microbial problems exist in this process which could not be solved over the years.

Periodic investigations have been carried out, but not documented sufficiently to give a solution to the problem.

The aims of the study were:

i) to identify some of the microbiological hazards in the process and product by using the HACCP method, and

ii) to recommend a proper microbial quality assurance plan.

The investigation of the process at the Bellville Mill plant of African Products will provide answers to the problem of production on high quality starch for export or the local market. This study is important because starch is a major ingredient in many foods and pharmaceutical preparations.

# CHAPTER 2

# LITERATURE REVIEW

The process of separation of the major components of the kernels by wet milling is illustrated in Fig's. 1 to 3.

This highly complex system can be divided into three stages i.e. cleaning of grain, steeping, milling and fraction separation. The final products are bran, germ, gluten and starch.

During the wet milling of maize, various sources of microbial contamination exist, either in the raw maize kernels or during the process. The conditions during the process are conducive to rapid proliferation of microorganisms.

Spicher (1972) found large differences in microbial counts at various stages of the process and between different factories. Generally, numbers of mesophilic bacteria increased and reached a maximum on the wet starch ($10 \times 10^4$ to $10 \times 10^7$ cfu/g) and on dry starch were $1 \times 10^3$ to $10 \times 10^4$ cfu/g.

Numbers of moulds increased initially, ranging from 150 to 1 500/g on wet starch and from 150 to 1 000/g on dry starch. Numbers of sporeformers (mesophilic and thermophilic) ranged form 100 to 10 000 cfu/g on wet starch to 100 to 100 000 cfu/g on dry starch.

Spicher (1972) attributed the difference to contamination during the process and also the particular processing (without giving any details). One of the sources he identified however, was the steep water. There seems to be good correlation between the results of Spicher (1972) and Blennerhassett (1975) although the latter did only a limited study.

Both found unacceptably high counts in the dried starch at times and could not offer specific solutions to the problem.

## 2.1 RAW MAIZE

Maize is delivered in bulk rail cars and stored in silos. The maize is moved by a conveyor via an air cleaner which remove the grit, sand and other waste. Spicher (1972) found that counts on the raw maize were as follows: mesophilic bacteria ranged form 16,800 to 207,500 cfu/g, moulds 7 550 to 11 400 cfu/g and the sporeforming thermophilic bacteria 63 to 79 cfu/g.

Blennerhassett and Samuels (1988) found that the fungal population on maize kernels of third grade white maze were as follows: 58 % had *Diplodia* of which 36 % kernels germinated, 10 % had *Fusarium*, 1 % *Aspergillus* and 31 % unidentified moulds.

*Diplodia* causes cob and stalk rot in maize and the identified genera are all possible producers of mycotoxins. Maize is one of the grains that could be very high in mycotoxins.

## 2.2 STEEPING

Arnold (1978) and Watson (1984) described the steeping process as serving the following purposes:

i) softening of the grain to be ready for milling,

ii) leaching out of soluble protein from the grain,

iii) reducing or inhibition of the activity of the microorganisms that are brought into the mill, and

iv) completion of the cleaning of the grain.

Steeping is done with water with $SO_2$ levels maintained very high (1 000 - 2 000 ppm) in a battery of tanks.

The steep liquor is maintained to fill the cone of the steep tanks. This is imperative that the liquid always covers the maize, as it is added to float of detritus in the form of dust and straw. The steep liquor is circulated around the different steeps and its temperature is maintained at 50 °C to 52 °C.

Watson (1984) stated that the major part of the bacterial population is killed off by high $SO_2$ levels. The optimum temperature for steeping, according to him is between 45 °C to 55 °C. This is also the optimum temperature for certain *Lactobacillus spp* among others *L.bulgaricus*. These bacteria produce lactic acid which lowers the pH of the steeps, thus restricting the growth of other microorganisms. The lactic acid produced is absorbed into

maize kernels. This together with the high temperatures, kill the grain and increases membrane permeability, which leads to leaching out of solubles from the kernels.

Blennerhassett and Samuels (1988) studied the survival of *Diplodia* during the steeping process. They found that maize contaminated with *Diplodia* could be used in wet milling for the following reasons:

Firstly, the 36 hours of steeping time is insufficient for the multiplication and sporulation of *Diplodia*, especially because of the high $SO_2$ content and secondly because the temperature of 48 °C to 51 °C will prevent formation of spores. The temperature is however, not high enough to inactivate the fungus culture and steeped. When maize was treated with Diplodia culture and steeped all the Diplodia were killed off during the process.

Bennet *et al.* (1976) found that most aflatoxin from contaminated maize is retained in the steepwater. The starch was clear. The same was found with zearalenone which was retained mostly in gluten and bran (Bennet and Anderson, 1978). On completion of the steeping, the steeped maize is pumped into the hopper, from where it is drawn for milling.

## 2.3 MILLING

The milling process in the Bellville Mill of African Products will be described (Fig's 2 and 3).

The first grind mill is designed to open the kernel and release the germ, which is removed here. The mixture goes through the second grind mill for a further reduction in particle size.

From the second grind tank, the product is pumped to the third grind mills where it is finely ground.

### 2.3.1 GERM TREATMENT

The germ is pumped into a series of screens. The screens allows water bearing starch to pass through. The germ stays on the screen and passes from one screen to the next until the majority of the starch has been removed. From the final screen, the germ passes to the water expeller, which squeezes the germ without crushing it. The water from the expeller returns to the germ tank and the germ is conveyed to the germ drier.

### 2.3.2 BRAN TREATMENT

The product is pumped to the bran screens. After screening, the bran is dewatered, then fed to a paddle mixer where concentrated steep liquor from the evaporators is mixed back in. The bran is finally dried in a hot air, rotating drier.

## 2.4 STARCH REFINING

Primary separation now takes place where the major gluten part is separated from the starch (Fig's. 2 and 3). The refining takes place in three stages:

i) separation of the starch - gluten mixture,

ii) dewatering of starch, and

iii) drying and bagging of starch.

### 2.4.1 SEPARATION OF THE STARCH - GLUTEN MIXTURE

The starch milk containing gluten, flows into the Dorr Clones centrifuge for separation. The Dorr Clones are also a point where separation takes place. Fresh water enters the system at the Dorr Clones.

As the water flows countercurrent to the starch milk, it is enriched with gluten and the starch is impoverished.

From the outlet of the Dorr Clones, the starch slurry goes to the starch storage tanks from where it is pumped to dewatering centrifuges. The water is pumped to the Dorr Clone wash water tank and then back into the system at the centrifuges.

### 2.4.2 STARCH DEWATERING

Two different types of centrifuges are used i.e. Laidlaw and Reineveld. The objective of the dewatering centrifuges is to produce a starch cake, containing approximately 35 % water (Arnold, 1978).

#### 2.4.2.1 LAIDLAW

The Laidlaw has three sequences viz. *filling, spinning and cutting of starch.* The starch slurry (61 % water) is fed evenly into the drum rotating at about 720 revolutions per minute.

The starch slurry is pushed with centrifugal force against the filter cloth fitted on the inside of the drum wall. The water, with a small concentration of starch fines, passes outwards through the filter cloth and the perforated holes in the drum. All the overflow drains to the overflow tank.

*NOTE:* All Laidlaw units were subsequently replaced with Reineveld centrifuges.

### 2.4.2.2 THE REINEVELD

The Reineveld works on the same principles as the Laidlaw. Arnold (1972) suggested when cleaning the Reineveld, the centrifuge needs to be opened up. The excess starch must be scraped off the plough, the filter hosed and then removed to wash the centrifuge drum. Care should be taken to ensure that the filter cloth is correctly replaced as damage could be caused during start-up and operation.

Curry (1991) even suggested that the cloth should be changed and the side-panel of the Reineveld be thoroughly washed with a high pressure watering hose. He mentioned that this will control the fungal growth at the Bellville plant.

The cloth in the Reineveld is changed every two weeks at which the machine is thoroughly sanitized. When the cloth is changed the centrifuge supply tank is dosed with sodium hypochlorite and the mixture is flushed through the centrifuge and wet collector system.

Spicher (1972) found that mesophilic bacteria increased from 1 000 cfu/g to 3,2 x $10^5$ cfu/g after centrifuging. Mesophilic sporeformers were very low, ranging from less than 10 to 30 cfu/g. The thermophilic sporeformers were found to be more than mesophilic sporeformers,

ranging from 10 to 1 000 cfu/g. Increased mould counts were found at this point, ranging from 30 to 3 160 cfu/g in all three different factories tested.

### 2.4.3 PROCESS WATER

Watson (1984) described the use of water in wet milling as follows: Fresh water and steam are used to wash starch free from gluten before centrifugation and drying.

The following are reservoirs of process water:

#### 2.4.3.1 DORR CLONE WASH WATER TANK

Water from this tank is obtained from various sources. These are:

i) fresh water,

ii) fresh $SO_2$ water,

iii) Swenson process condensate (condensate which comes from the Swenson in the glucose section), and

iv) steam and filtrate tank.

#### 2.4.3.2 REINEVELD OVERFLOW TANK

The overflow from the Reineveld is transferred to a Reineveld overflow tank and then flows back into the Dorr Clone wash water tank (Arnold, 1972).

### 2.4.3.3 REINEVELD FILTRATE TANK

The filtrate from the Reineveld is transferred to a Reineveld filtrate tank and is then circulated back into the process (Arnold, 1972). Process water tanks are potential sources of contamination found with the steeps (Spicher, 1972). He studied the microbial counts on steep liquor and found that mesophilic bacteria ranged from 7 600 to 101 000 cfu/g, moulds 55 to 235 cfu/g, sporeforming mesophiles 152 to 1 200 cfu/g and sporeforming thermophilic bacteria 32 to 88 cfu/g. If the steep liquor is contaminated, it will transfer bacteria to the maize kernels.

## 2.5 DRYING OF STARCH

See Fig's 3 and 4.

### 2.5.1 FLASH DRIER

The objective of these driers is to dry the wet cake (approx. 35 % water) to a moisture of 12,5 % and for low moisture starch 6 %.

The driers are pneumatic conveyor driers which are particularly suited for drying heat sensitive materials such as starch. This is due to two main reasons (Arnold, 1972):

i) the short retention time which is a few seconds, and

ii) the low temperature of the outlet air of drier.

Knipe (1987) found that an outlet temperature of 45 °C corresponds to a moisture of 12,5 % and 65 °C for 6 % moisture respectively.

In a study of the efficiency of the flash drier, Blennerhassett (1975) examined samples taken at half hourly intervals at different points in the plant. Results were as follows:

i) starch prior to entering flash drier:

19 x $10^3$ to 22 x $10^6$ cfu/g,

ii) starch entering hammermill:

460 to 48 x $10^3$ bacteria/g,

iii) starch after storage in hopper:

350 to 5 x $10^3$ cfu/g

These counts were high despite the elevated levels of $SO_2$ (126 ppm). The microorganisms capable of withstanding the high $SO_2$ levels and low pH conditions were mostly yeasts. Blennerhassett identified these sources of contamination. The results showed that 120 bacteria/$cm^2$/hr were deposited in the flash drier. The air containing these organisms were drawn mainly from the drains and dirt around the air inlet.

Spicher (1972) found that drying usually reduced the numbers of all microbial groups but actual increases in moulds and total mesophiles were recorded. Numbers after the drying process were as follows:

mesophilic bacteria: 32 x $10^3$ cfu/g, mesophilic sporeformers: 10 x 100 cfu/g, moulds: 100 to 1 000 cfu/g.

Spicher concluded that the increase in moulds and mesophilic sporeformers can be due to infections during drying. It is logical from the relatively low temperatures and short retention times mentioned above.

### 2.5.2 HAMMER MILLS

Dried starch goes from the driers to the Hammer Mills and after sieving, into silos.

Spicher (1972) in his study of the microbial counts of starch at this point, found that previous processes can influence the bacterial counts greatly, due to contamination or infections. Hydrocyclones usually reduced bacterial and mould counts, but an increase was evident in certain plants.

### 2.5.3 STARCH AT THE BAGGING OFF POINT

The bacterial count on the final product depends on the efficiency of the flash drier and the degree of contamination at the bagging-off site.

Blennerhassett (1975) analyzed starch as it enters the bags to ascertain the bacterial resistance to storage at a low moisture content. Mesophilic counts varied from 310 to 1 890 cfu/g after storage for approximately one week.

## 2.6 STANDARDS IN A NORMAL PLANT

Burkett (1989) noted that the bacterial levels will be affected by control of temperature, pH and $SO_2$ levels in the wet starch. No specifications for these extrinsic parameters were given. African Products, Bellville have their own microbiological targets for the final product (Table 2). Starch is graded in three grades: Food, Pharmaceutical and as Industrial.

The end products must be low in microbial content as they are destined as ingredients of food and pharmaceutical products. **Food grade starch** vary in total plate count between 100 and 1 000 cfu/g; moulds and yeasts, maximum 250 cfu/g.

*Staphylococcus aureus, Escherichia coli, Clostridium perfringens, coliforms,* should be absent per gram and *Salmonella* and *Shigella* absent from 10 gram. Chemical targets should also be maintained with $SO_2$ levels between 80 to 100 ppm and pH levels ranging from 4,0 to 5,0.

## 2.7 USE OF SANITIZERS

Burkett (1990) studied the effect of oxidants (e.g. $H_2O_2$) on the microbial level of finished starch. When used it resulted in very low counts. Maximum levels found for **Food Grade Starch** were as follows: bacteria 500 cfu/g, moulds 50 cfu/g, yeasts 50 cfu/g.

He found that a food operating plant using $SO_2$ as a control at 100 ppm should have starches containing less than 500 bacteria/g and less than 100 yeasts and moulds/g. Unacceptably high counts of 10 to 50 x $10^3$ cfu/g were obtained, if the temperature and $SO_2$ levels were not under control.

Maximum levels obtained for **Industrial Starch** were as follows: bacteria 1 000 cfu/g, moulds 200 cfu/g, yeasts 100 cfu/g.

Good results can be obtained without any oxidants, but $SO_2$ levels must be at least 100 ppm. to get bacteria $<$ 500/g and moulds and yeasts $<$ 100/g.

# CHAPTER 3

# HAZARD ANALYSIS CRITICAL CONTROL POINT SYSTEM

## 3.1 INTRODUCTION

HACCP is a systematic approach to the identification and control of microbiological hazards in foods. This systems was developed by The Pillsbury Company in the early 70's to ensure the safety of foods for the United States space program (Bauman, 1974, Bauman, 1990).

It was rapidly incorporated into regulations for canned foods. It was easy to implement in canneries because the CCP's were easily controlled physically i.e. adequate heat treatment and soundness of the container. The rest of the food industry was slow to adopt this system, probably due to the complexity of processing of other products.

However, in the late 80's and this decade renewed interest were shown in the system. Individuals, companies and regulatory authorities were involved in numerous studies and recommendations (Buchanan, 1990). This was due to the tremendous increase in the incidence of foodborne diseases like *Salmonella* and recently *Campylobacter, Listeria, Yersinia* and others. There are an estimated 6.5 to 30 million cases of foodborne diseases in the United States every year (Snyder and Doland, 1990; Beran, Shoeman and Anderson, 1991).

## 3.2 BASIC STEPS

The HACCP system comprises of the following basic steps:

i) identification of hazards, their severity and their risks,

ii) determination of CCP's,

iii) establishment of CCP criteria,

iv) monitoring of CCP's

v) protocols for CCP deviation,

vi) verifications, and

vii) implementation.

### 3.2.1 IDENTIFICATION OF HAZARDS, THEIR SEVERITY AND THEIR RISKS

All the possible hazards in a food chain are identified, ideally covering all the steps from growing and harvesting to manufacturing, distribution, retailing and consumption of the product.

These would include foodborne disease, mycotoxins, toxic chemicals and foreign matter. Risk analysis consists of determining if a food has the following three major hazard characteristics:

i) if the product contain a "sensitive" ingredient or ingredients',

ii) the manufacturing process does not contain a controlled processing that effectively destroys harmful bacteria, and

iii) there is substantial potential of microbiological abuse in distribution or in subsequent handling that could render the product harmful when ingested (NACMCF, 1992).

### 3.2.2 DETERMINATION OF CCP'S

A CCP is any point in the process where lack of control may result in a hazard. Evaluations of all steps in the food production, preparation and processing are done including formulation and raw materials. Cleaning and sanitation was recently included in the HACCP program. This also includes drawing up a detailed flow chart of operations indicating hazards and CCP's.

### 3.2.3 ESTABLISHMENT OF CCP CRITERIA

Examples of criteria are: pH, temperature/time range for pasteurisation, maximum antibiotic residues, $SO_2$ levels and percentage moisture.

### 3.2.4 MONITORING OF CCP'S

Establish a regular plan to monitor the CCP's. An example of such a procedure is testing at hourly intervals. Responsibility for this type of testing needs to be assigned to a specific person. Records must be kept, preferably for each batch, for reference and verification. More commonly chemical, physical or microbiological tests (preferably rapid ones) are used for monitoring.

### 3.2.5 PROTOCOLS FOR CCP

Protocols are drawn up to determine which corrective action needs to be taken if a deviation occurs at a CCP. Corrective actions need to be clearly defined beforehand, with the responsibility for action assigned to an individual.

### 3.2.6 VERIFICATION

The first step in verification is to ascertain that CCP limits at CCP's are satisfactory. In verification one needs to make sure that the HACCP plan is functioning effectively. The last part of verification deals with the government's regulatory responsibility and actions to ensure that the establishment's HACCP system is functioning satisfactorily. This step will include traditional microbial counts and chemical analysis of the final product.

### 3.2.7 IMPLEMENTATION

To implement a HACCP program, the following actions need to be taken:

i) HACCP evaluations must be conducted of all the food production / processing / preparation steps,

ii) criteria for control must be established for each CCP; monitoring procedures need to be selected and implemented; and courses of action decided upon whenever the criteria are not met,

iii) a HACCP plan must be developed for all foods concerned,

iv) personnel need to be trained so that they will be aware of hazards and know the criteria for control, how to monitor the CCP's and the action to take when the criteria are not met,

v) the HACCP plan must be put into effect in the facility where food is produced, harvested, transported, stored, processed or prepared, and

vi) the HACCP plan and its implementation must be verified.

## 3.3 APPLICATIONS

HACCP should be applied to all the phases of food production, processing and sales. Numerous publications are available, covering a wide variety of food products. The ICMSF (1988) brought out a handbook concerning all aspects of HACCP and its application.

The application of HACCP in vegetable, fruit and grain production is important in order to produce a high quality product with less loss due to spoilage. The principal hazard is fungal spoilage in the field and foodborne pathogens and mycotoxins during storage of the product (ICMSF, 1988).

Other examples are seafoods (Garrett and Hudak-Roos, 1990) refrigerated and frozen foods (Goff, 1988; Bryan, 1990), animal foods (ICMSF 1988, Adams, 1990) and processed foods

(ICMSF, 1988). The food service industry is responsible for numerous outbreaks of foodborne disease and there HACCP was applied successfully (Sen, 1991).

### 3.3.1 THE ROLE OF THE GOVERNMENT

The first government to implement HACCP as part of their function is that of Canada. This will provide their fishing industry with an effective mechanism to ensure the protection of consumers world wide (Bryan, 1985; Bryan, 1990; Spencer, 1992). In South Africa it was suggested as a function of Environmental Health Officers (Von Holy, 1990 and Hughson, 1993).

## 3.4 STATEMENT OF PROBLEM

The existing problems in the Wet Milling Process at the Wet Milling Plant, African Products, Bellville, were investigated previously (Blennerhassett, 1975). The variation in microbial counts in the process and the finished products, persisted in the local plant over several years.

It is clear from the results reviewed that a minimum of work has been done on microbial contamination during wet milling. It is imperative that an in-depth study needs to be done to:

i) pin-point sources of contamination,

ii) determine growth patterns of various groups of microbes during processing,

iii) ascertain effect of various process parameters on populations, and

iv) maintain final microbial counts consistently within or below standards.

# CHAPTER 4

# METHODS

## 4.1 SAMPLING POINTS

Samples were collected at different sampling points (Table 1).

## 4.2 PREPARATION OF SAMPLES FOR THE ENUMERATION OF VARIOUS MICROORGANISMS

In-process samples were taken at various points, before and after sanitation. Samples were collected in 100 ml sterile sample jars and kept at 6 °C in the refrigerator until tested.

### 4.2.1 ANALYSIS OF THE IN-PROCESS SAMPLES

One ml or one gram of sample was weighed out into 99 ml of a quarter strength Ringer's (Wilson and Miles, 1975) solution. The sample was shaken well before further dilutions for plate counts were made. The standard plate count procedure was followed.

### 4.2.2 ANALYSIS OF DRY STARCH

Twenty grams of sample was weighed out in 80 ml of 1/4 strength Ringer's solution. The sample was shaken well before further dilutions for plate counts were made.

## 4.3 ENUMERATION OF VARIOUS MICROBIAL GROUPS

This was done according to the methods of CPC (CPC, 1990).

### 4.3.1 ENUMERATION OF MESOPHILIC BACTERIA

Enumeration of mesophilic bacteria was done on Plate Count Agar (Biolab, Merck). All plates were incubated at 32 °C for 48 hours.

### 4.3.2 ENUMERATION OF COLIFORMS

Enumeration of coliforms was done on Violet Red Bile Agar (Biolab, Merck) and incubated at 37 °C for 24 hours. All the dark red and pink colonies were counted as coliforms.

### 4.3.3 ENUMERATION OF THERMOPHILIC SPOREFORMERS

Dextrose Tryptone Agar (Oxoid, Milsch) was used for the enumeration of thermophilic sporeformers. The samples were heat treated at 80 °C for 15 minutes and incubated at 55 °C for 48 hours. All yellow colonies were reported as thermophiles.

### 4.3.4 ENUMERATION OF MOULDS AND YEASTS

Enumeration of moulds and yeasts were done on Potato Dextrose Agar (Oxoid, Milsch). Plates were incubated at 32 °C for 72 hours and mould and yeast colonies were counted separately.

### 4.3.5 ENUMERATION OF FAECAL STREPTOCOCCI

Enumeration of faecal streptococci was done on Bile Esculin Agar (Oxoid, Milsch). Plates were incubated at 37 °C for 24 hours and small dark-brown colonies were counted.

## 4.4 IDENTIFICATION OF ENTEROBACTERIACAEAE

### 4.4.1 ENRICHMENT SAMPLE PREPARATION

Twenty grams of sample was weighed out into 80 ml of Lactose Broth (Oxoid, Milsch) and incubated at 37 °C for 48 hours.

### 4.4.2 IDENTIFICATION OF ISOLATES

Identification of isolates of the different Enterobacteriaceae were done using the API 20 E method (Analytabs Products, New York).

## 4.5 IDENTIFICATION OF FUNGI

The growth medium used was Malt Extract Agar (Oxoid, Milsch) and plates were incubated at 25 °C for 5 days. Fungi were identified using colony morphology and microscopic features (Beuchat, 1987).

## 4.6 RAPID METHOD

The Malthus machine (Medical Distributors, Johannesburg) was tested as an alternative to traditional counts. This was done to try and ensure better in-process control at various CCP's. The samples were analyzed by Swift Laboratories. Results were compared with traditional counts done on duplicate samples.

## 4.7 CHEMICAL ANALYSIS

Sulphur dioxide determinations were performed according to the AOAC-methods (AOAC, 1980).

## 4.8 SANITATION METHODS

Plate counts of microbial population at different sampling points were counted before and after sanitation with the following:

### 4.8.1 50 % PEROXIDE SOLUTION - CONTACT TIME 36 TO 48 HOURS

### 4.8.2 HIGH $SO_2$ LEVELS (250 ppm) THROUGHOUT FACTORY

## 4.9 STATISTICAL ANALYSIS OF DATA

Analysis of variance was done using the ANOVA12 programme by Zar (1984).

# CHAPTER 5

# RESULTS

## 5.1 VARIATION OF POPULATION AT SAMPLING POINTS

### 5.1.1 STARCH STORAGE TANKS

Samples were drawn at points S6 - 1 and S6 - 2 (Fig. 2).

Counts were as follows: Mesophilic bacteria counts ranged from less than 1,000 cfu/g to 2,268 x $10^6$ cfu/g. Mould counts ranged from less than 1,000 cfu/g to 126,000 cfu/g. Yeast counts ranged from less than 1,000 cfu/g to 756,000 cfu/g. Coliforms counts were less than 100 cfu/g (Table 2 and 3).

### 5.1.2 DEWATERING CENTRIFUGES - LAIDLAW

Samples were collected at point S7 - 1 (Fig. 2) and the microbial populations were as follows: mesophilic bacteria counts ranged from 1,000 to 1,638 x $10^6$ cfu/g; mould counts ranged from less than 1,000 cfu/g to 378,000 cfu/g; yeast counts ranged from less than 1 000 to 756 000 cfu/g (Table 4).

### 5.1.3 REINEVELD

The microbial population at sampling point S7 - 2 (Fig. 2) was as follows: mesophilic bacteria counts ranged from 18 000 cfu/g to 3,024 x $10^6$ cfu/g; mould counts ranged from less than 1,000 cfu/g to 189,000 cfu/g; yeast counts ranged from less than 1 000 cfu/g to 2,898 x $10^6$ cfu/g and coliform counts ranged from less than 100 cfu/g to 14 000 cfu/g (Table 5).

### 5.1.4 PROCESS WATER

#### 5.1.4.1 STEEP LIQUOR

Analyses of the steep liquor were as follows: mesophilic counts were 71 000 cfu/g; mould counts 5 000 cfu/g and yeast count 1 000 cfu/g.

#### 5.1.4.2 REINEVELD OVERFLOW TANK

Counts at this sampling point (S9) were generally high. The mesophilic bacteria ranged from 58 000 cfu/g to 2,041 x $10^6$ cfu/g, mould counts ranged from less than 1 000 cfu/g to 484 000 cfu/g. Yeast counts ranged from less than 100 cfu/g to 315 000 cfu/g and coliform counts from less than 100 cfu/g to 50 000 cfu/g (Table 6).

### 5.1.4.3 REINEVELD FILTRATE TANK

Mesophilic counts at sampling point S10 (Fig 2) ranged from 75 000 cfu/g to 2,835 x $10^6$ cfu/g. Mould counts ranged from less than 1 000 cfu/g to 500 000 cfu/g; yeast counts from less than 1 000 cfu/g to 1 260 x $10^6$ cfu/g and coliform counts from 100 cfu/g to 189 000 cfu/g (Table 7).

### 5.1.5 WET MIXING BOXES

Results of samples (before drying) collected at sampling points S8 - 1 and S8 - 2 (Fig. 2) were as follows: mesophilic bacteria ranged from 15 000 cfu/g to 1,89 x $10^6$ cfu/g; mould counts ranged from less than 1 000 cfu/g to 441 000 cfu/g and yeast counts ranged from less than 1 000 cfu/g to 945 000 cfu/g (Table 8 and Table 9).

### 5.1.6 DRYING OF STARCH

The counts after drying at sampling points S12 (Fig. 2) were found to be as follows: mesophilic bacteria ranged from 200 cfu/g to 500 000 cfu/g; the moulds were 1 000 cfu/g, while occasionally *Staphylococcus aureus* and faecal streptocci were present in the 10 g and 1 g samples.

## 5.2 ANALYSIS OF FINAL PRODUCTS

The final products (Table 10 and Table 11) were analyzed and graded i.e. food, pharmaceutical or industrial according to results. Counts were as follows: mesophilic

bacteria ranged from 300 cfu/g to more than 500 000 cfu/g; mould counts ranged from less than 20 cfu/g to 200 cfu/g; yeast counts ranged from 80 cfu/g to 1 000 cfu/g. Some of the bacteria isolated were identified as follows: coliforms, *Escherichia coli* and *Staphylococcus aureus,* faecal streptococci and thermophilic bacteria.

## 5.3 IDENTIFICATION OF DIFFERENT BACTERIA AND FUNGI AT DIFFERENT SAMPLING POINTS

Different organisms were isolated at different sampling points. Results can be found in Table 12.

## 5.4 MEAN VALUES AT DIFFERENT SAMPLING POINTS FOR VARIOUS BACTERIA

In all cases mesophilic microorganisms were the dominant bacteria ( $\bar{x}$ : 217 000 to 100 000 cfu/g) with yeast being co-dominant ( $\bar{x}$ : 23 000 to 380 000 cfu/g) (Table 13).

Intermediate numbers were recorded for moulds ( $\bar{x}$ : 8 000 to 14 000 cfu/g) and the lowest for coliforms ( $\bar{x}$ : 100 to 36 000 cfu/g).

The highest microbial levels for mesophilic bacteria moulds and yeasts were found at sampling point S10 (Fig. 2). Yeasts yielded the highest levels at sampling point S7 - 2.

## 5.5 SANITATION METHODS

### 5.5.1 PEROXIDE SOLUTION (50 %) - CONTACT TIME 36 - 48 HOURS

#### 5.5.1.1 MESOPHILIC BACTERIA

At sampling point S4 an increase in the mesophilic count after sanitation was evident (Table 15). The value increased from 615 cfu/g to 1 x $10^6$ cfu/g. At points S6 - 1 and S6 - 2, a reduction in mesophilic counts were obtained. Values reported were 32 200 cfu/g before sanitation to 10 cfu/g after sanitation. Sampling points S7 - 1, S7 - 2, S8 - 1, S8 - 2, S9 and S10 had reduced counts after sanitation (Table 14 and Table 15; Fig. 4). Although there was a decrease in counts, mesophiles were still high.

#### 5.5.1.2 COLIFORM BACTERIA

Counts before sanitation ranged from 10 cfu/g to 190 500 cfu/g and after sanitation were generally low ranging from 10 cfu/g to 6 030 cfu/g (Table 16 and Table 17; Fig.6).

#### 5.5.1.3 MOULDS

There were large decreases in counts at sampling points S6 - 1, S6 - 2 and S9. Before sanitation counts were 24 500 cfu/g and 1 000 cfu/g and 34 600 cfu/g respectively, but after sanitation it had all been reduced to 10 cfu/g (Table 18 and Fig. 8).

#### 5.5.1.4 YEASTS

There were large decreases in counts after sanitation at the following sampling points: S6 - 2, S7 - 2, S8 - 1, S9. Before sanitation counts ranged from 10 cfu/g to 891 000 cfu/g. Counts after sanitation ranged from 10 cfu/g to 363 000 cfu/g. At the other sampling points no definite difference in counts were found (Table 19 and Fig. 9).

#### 5.5.1.5 FAECAL STREPTOCOCCI

Counts before sanitation ranged from 1 800 cfu/g to 199 500 cfu/g. Counts were reduced after sanitation and ranged from 500 cfu/g to 2 7 00 cfu/g. The sanitation method was effective (Table 20 and Fig. 10).

### 5.5.2 HIGH $SO_2$ LEVELS (250 ppm)

Two groups of bacteria were examined: Coliform and mesophilic bacteria:

#### 5.5.2.1 MESOPHILIC BACTERIA

There were decreases in counts after sanitation at sampling points S6 - 1, S6 - 2, S11 - 1, S11 - 2 and S12. Counts before sanitation ranged from 170 cfu/g to 758 500 cfu/g. Counts after sanitation ranged from 10 cfu/g to 380 100 cfu/g. Counts at the following sampling points, S7 - 1, S7 - 2, S8 - 1, S8 - 2 and S9, remained very high:

Counts ranged from 134 800 cfu/g to 758 500 cfu/g before sanitation and from 125 100 cfu/g to 380 100 cfu/g after sanitation (Table 21 and Fig. 5).

#### 5.5.2.2 COLIFORMS

A general decrease in the coliform counts were found. Counts ranged from 10 cfu/g to 24 500 cfu/g before sanitation and it decreased to a range of 10 cfu/g to 2 000 cfu/g after sanitation (Table 22 and Fig. 7).

### 5.5.3 EFFECT OF DIFFERENT SANITATION METHODS

The effect of peroxide on the mesophilic bacteria was significant ($p < 0.05$) in the B series (Table 15), and the effect of high $SO_2$ levels (250 ppm) was not significant ($p > 0.05$) (Table 21).

The effect of peroxide on the coliform counts was not significant ($p > 0.05$) (Table 17), and the effect of high $SO_2$ levels (250 ppm) was not significant ($p > 0.05$) (Table 22).

The effect of peroxide on the following groups was not significant: moulds ($p > 0.05$) (Table 18) yeast ($p > 0.05$) (Table 19) but was only significant on faecal streptococci ($p < 0.05$) (Table 20).

## 5.6 DETERMINATION OF OPTIMUM INTERVALS FOR SANITATION

The counts were monitored on different days:

i) before sanitation,

ii) two days after sanitation, and

iii) six days after sanitation.

### 5.6.1 MESOPHILIC BACTERIA

Counts were reduced at sampling points S4 and S7 - 2 and were less than 100 00 cfu/g. At sampling points S7 - 1, S8 - 1, S8 - 2, S9 and S10, counts were lower in comparison to counts before sanitation.

Counts before sanitation ranged from 33 000 cfu/g to more than 500 000 cfu/g and from 21 000 cfu/g to 500 000 cfu/g after sanitation.

After six days the mesophilic population in the factory was very high. Counts ranged from less than 1 000 cfu/g to more than 500 000 cfu/g (Table 23).

### 5.6.2 COLIFORM COUNTS

Two days after sanitation, counts were very low at sampling points S4, S6 - 1, S6 - 2 and S7 - 1. Counts before sanitation ranged from 600 cfu/g to 3 000 cfu/g. After six days the

coliform population in the factory was reported, less than 100 cfu/g due to high $SO_2$ levels (250 ppm).

Six days after sanitation, coliforms were expected to be high. Counts at all sampling points were less then 100 cfu/g except S9 and S10 which were 400 cfu/g and 1 600 cfu/g respectively (Table 24).

### 5.6.3 OPTIMUM SANITATION INTERVALS

Significant differences exist between number of both mesophilic bacteria ($p < 0.05$) but not coliform bacteria ($p > 0.05$) before sanitation and that recorded 2 days after (Table 23 and Table 24).

Measurements taken six days after were not significant for coliform bacteria ($p > 0.05$) and for mesophilic bacteria ($p > 0.05$).

Comparisons between the mean number of bacteria before and after sanitation reveal the results much clearer. The mean number of mesophilic bacteria before sanitation was much higher than that recorded two days after sanitation (401 667 cfu/g to 191 111 cfu/g).

However 6 days after sanitation, the number of mesophilic bacteria returned to pre-sanitation levels (401 667 to 352 000 cfu/g). In the case of coliform bacteria the numbers before sanitation were very high (13 133 cfu/g) in relation to that two days (876 cfu/g) and six days (300 cfu/g) after sanitation (Table 23 and Table 24).

## 5.7 COMPARISON OF COUNTING METHODS

### 5.7.1 MALTHUS METHOD

The sampling points, S4, S8 - 1, S8 - 2 and S9 showed low coliform counts (100 to 2 500 cfu/g) and the rest of the samples shown no coliform growth. The detection time for the coliform counts were between 5,6 and 9,4 hours, except for S6 - 1 and S6 - 2 which showed no growth for total microbial activity.

The detection time varied from 4,9 hours to 10,7 hours depending on the degree of contamination. Mould and yeast counts were only reported at sampling points, S8 - 1, S9 and S10. The rest of the samples showed no growth.

The detection time for mould and yeast counts, varied between 7,0 hours and 17 hours (Table 25).

### 5.7.2 STANDARD PLATE COUNT METHOD

The sample points S6 - 1, S6 - 2 showed no growth (Table 25). The rest showed total plate counts, varying from 2 000 to more than 500 000 cfu/g. Mould and yeast counts at S7 - 1, S7 - 2, S9 and S10 showed no growth.

### 5.7.3 COMPARISON OF PLATE COUNT AND RAPID METHOD

The mesophilic bacteria counts were the same at sampling points S6 - 1, S6 - 2 and S10 (Table 25).

At sampling points S7 - 1, S7 - 2, S8 - 1, S8 - 2 and S9 counts were not the same, but results from both methods indicate a high degree of contamination. Counts ranged from less than 1 000 cfu/g to 910 000 cfu/g.

Coliform counts were the same except at S8 - 1, S9 and S10. At S9 and S10 the Malthus test result showed no growth in comparison to the 1 600 cfu/g obtained from the standard plate count method.

The mould and yeast counts were the same, indicating no growth in these samples. Discrepancies were found at the following sampling points: S7 - 1, S7 - 2, S8 - 1, S9 and S10, where the Malthus test results showed no growth and the counts of the standard plate count method ranged from a 1 000 cfu/g to 27 000 cfu/g.

# CHAPTER 6

# GENERAL DISCUSSION

The significance of the results of each sampling point will be discussed briefly.

## 6.1 RAW MAIZE

In view of results mentioned in the review, the microflora of maize was not studied.

## 6.2 STEEPING

Counts in general were very low. The counts declined to 1 000 cfu/g and the mesophilic sporeformers were 3 000 cfu/g. Mould counts ranged from less than 10 moulds/g and less than 100 yeasts/g per steep. Similar patterns were found by Spicher (1972) and Blennerhassett (1975).

The steep liquor plays a major role in the process. Analysis of the steep liquor showed a high survival rate despite the high $SO_2$ level maintained.

The high counts can be due to the contamination of the steep liquor water during circulation for extended periods of time. Spicher (1972) also reported similar patterns on the analysis of steep liquor.

## 6.3 DEWATERING CENTRIFUGES

## LAIDLAW / REINEVELD

In general the mesophilic bacteria counts in the dewatered starch were very high (more than 500 000 cfu/g). We can therefore consider these sampling points as CCP's. (Table 6 and Table 7; Fig. 11).

The centrifuges need the most attention, due to the design of the machinery. The cloth inside the centrifuge is always wet and starch lumps build up on it. The wet starch with a water concentration of 75 % to 95 % can support the growth of microorganisms, especially at ambient temperatures.

## 6.4 PROCESS WATER

### 6.4.1 REINEVELD OVERFLOW TANK / REINEVELD FILTRATE TANK

Counts were in general very high before the modification of the plant. There was a significant decrease in the microbial counts after these tanks were removed from outside the factory to the inside (Fig. 2 and 3). Cleaning could take place more easily, because the asbestos tanks were replaced with stainless steel tanks.

### 6.4.2 WET MIXING BOXES

The microbial counts in the wet mixing boxes were always high. Similar patterns were found by Spicher (1972) and Blennerhassett (1972) for mesophilic bacterial counts. The mould counts are much higher than those found by Spicher (1972).

The design of the wet mixing boxes is an important factor in the growth of microorganisms. The auger was normally covered in lumps of starch. An increase in mould counts were found when these starch lumps loosened. More care should be taken during sanitation of the wet mixing boxes because the closed system is a suitable environment for the growth of microorganisms.

## 6.5 DRYING OF STARCH

In the literature study Spicher (1972) and Blennerhassett (1975) mentioned that the drying process can influence the bacterial count greatly. This is confirmed in this study by the mesophilic bacterial counts ranging from 200 to 500 000 cfu/g in dried starch. The mould count was 1 000 cfu/g. Occasionally *Staphylococcus aureus*, Coliform bacteria and faecal streptococci, were isolated and positively identified. The bacterial counts on the dry starch exceeds the specifications suggested by Burkett (1990).

## 6.6 IDENTIFICATION OF DIFFERENT BACTERIA AND FUNGI AT DIFFERENT SAMPLING POINTS.

Various bacteria and fungi were identified, especially at sampling points described as CCP's. The same microorganisms occurred at the dewatering centrifuges and the wet mixing boxes. Faecal streptococci, coliforms and yeasts in the steeps appears to be the general contaminants in the entire plant especially before modification (Fig's 2 and 3). *Fusarium* and *Diplodia* originated from the poor quality of crop accepted by the factory for processing. *Staphylococcus aureus* were isolated in the plant when the sanitary conditions of the dewatering centrifuges and wet mixing boxes were low.

## 6.7 MEAN VALUES OF VARIOUS MICROORGANISMS AT DIFFERENT SAMPLING POINTS

The mesophilic bacteria was the dominant group in the plant. Low coliform levels were found, due to the effect of the 50 % peroxide solution and high $SO_2$ levels (250 ppm). A very high coliform count was found at S10. The sampling point (S10), used to be a tank outside the factory and it has been replaced with a stainless steel tank inside the factory. The coliform counts can also be very high, due to the fact that it is a closed system and proper sanitation is not possible.

The yeast count at S10 is very low, because the mesophilic bacteria were the organisms that grew best at this sampling point. The yeast counts were influenced by temperature (37 °C) and the high $SO_2$ (250 ppm) levels at this sampling point. Sampling points S6 - 1, S6 - 2,

S7 - 1, S7 - 2, S8 - 1, S8 - 2, S9 and S10 needs priority cleaning, especially for the mesophilic bacteria.

## 6.8 DETERMINATION OF SANITATION INTERVALS

There was little difference in the mesophilic counts after sanitation. After six days there was a build up of microbial population, except at sampling points S4, S6 - 1 and S6 - 2 (Table 23).

There was a decrease in the coliform counts. Six days after sanitation, coliform growth was absent, except at sampling points S9 and S10. These are the in process water tanks. One have to keep in mind that the high $SO_2$ levels (250 ppm) at S4, S6 - 1 and S6 - 2 also have an effect on the coliform count.

The optimum sanitation interval is 6 days after sanitation. The mesophilic counts were all more than 500 000 cfu/g. The optimum sanitation interval is different for mesophilic and coliform bacteria due to the different growth rates of the bacteria. The growth rate of coliforms depends on the sanitary conditions in the plant, whereas mesophilic counts are the general population present in the factory. The mesophilic bacteria are also more resilient than the coliform bacteria after sanitation (Table 15 and Table 21). The coliform can also be controlled by the high $SO_2$ levels (96 - 245 ppm), whereas the mesophilic counts were not influenced.

## 6.9 COMPARISON OF SANITATION METHODS

### 6.9.1 50 % PEROXIDE SOLUTION

As seen from the results one can conclude that the 50 % peroxide had little effect on the mesophilic count (Fig. 5). A decrease in the counts occurred only at the Dorr Clone wash water tank (S4) and Dorr Clones (S5). The coliform count also show little decrease. The disinfectant was effective on the moulds which showed some improvement after sanitation.

The sanitation was not as effective as expected, probably due to inefficient cleaning beforehand. High counts will continue in the plant if sanitation is not done on a regular basis and more efficiently.

### 6.9.2 HIGH $SO_2$ LEVELS (250 ppm)

The coliform counts were in general low before and after sanitation, although the mesophilic counts were still high after sanitation. High counts were found at certain sampling points. The counts depends on the initial bacterial population and the $SO_2$ level. The $SO_2$ levels needs to be monitored at the different sampling points and in the final product.

### 6.9.3 EFFECT OF DIFFERENT SANITATION METHODS

Lower bacterial counts as a result of sanitation indicates that sanitation works. If the sanitation method with 50 % hydrogen peroxide is not possible, due to production, then high

$SO_2$ levels (250 ppm) can be used as an alternative. On the other hand, it appears that high $SO_2$ levels will result in a final product with a $SO_2$ level out of specification.

The effects of increased concentration and levels of both sanitation methods need to be examined in the future.

## 6.10 COMPARISON OF COUNTING METHODS

### 6.10.1 MALTHUS vs STANDARD PLATE COUNT METHOD

The Malthus method is quicker than the Standard plate count (SPC) method. Detection time varies from 4, 9 - 10, 7 hours in comparison with 48 hours for the incubation period. Although the capital cost of the Malthus is very high, it is faster and showed few discrepancies in the results compared to the SPC.

Moulds could not be detected with the Malthus test method, due to slow growth rate ,while plate count ranged from 1 000 cfu/g to 27 000 cfu/g.

The Malthus method can be applied to monitor the counts at the different CCP's. Data is available in a shorter period of time to ensure prevention of hazards.

# CHAPTER 7

# HACCP - IMPLEMENTATION OF THE PLAN

In the previous chapter it is clear that only a systematic approach (like HACCP) to the problem can solve it. It was therefore applied to the entire process following the guidelines set out in Chapter 3.

## 7.1 IDENTIFICATION OF HAZARDS

The hazards identified in the wet milling plant were as follows:

Bacteria: Faecal streptococci, *Staphylococcus aureus, Eschericha coli,* coliforms. These are foodborne pathogens or indicators of faecal contamination.

Fungi: *Aspergillus, Rhizopus, Diplodia, Fusarium* and *Penicillium.* All of these are potential mycotoxin producers and could do so in poor quality maize.

Thermophilic Bacteria: Sporeforming *Bacillus* spp and which are potential hazards in the canning industry, can originate from maize and dust and soil.

Sources from which food poisoning *Staphylococci* enter foods are usually humans and animals; boils and wounds and also the human skin may be sources of contamination.

Coliform bacteria could originate from bird droppings in the plant, but *Escherichia coli*, coliforms and faecal streptococci could also be from human faeces or carriers.

In general, high counts can cause quality hazards if it exceed the specifications for final products.

## 7.2 MAJOR CONTAMINATION POINTS

Major points of contamination and CCP's are indicated on the flowchart (Fig. 3).

### 7.2.1 MAIZE TRUCKS

Raw maize is delivered in bulk rail cars (Fig. 2) and is contaminated with dust, soil and airborne microorganisms. This is the major source of microorganisms, but some of these will be removed in the steeping process.

### 7.2.2 IN-PROCESS WATER

#### 7.2.2.1 DORR CLONE WASH WATER TANK

This is a major point of contamination. The mesophilic counts were even higher after sanitation. This is an indication of some contamination along the process line.

The sources of contamination can possibly be from Swenson process condensate or the

The Dorr Clone wash water tank is the only point in the system where fresh water is introduced into the in-process water. The entire system will be contaminated if the in-process water is circulated in the plant through the Dorr Clones.

#### 7.2.2.2 REINEVELD OVERFLOW TANK

Overflow water from the centrifuges were transferred to this tank outside the factory. This was found to be a major point of contamination early in the study.

#### 7.2.2.3 REINEVELD FILTRATE TANK

The filtrate from the centrifuges flows into this tank and is circulated back into the in-process water. Mesophilic bacteria, mould and yeast counts were high in general. This is due to the fact that the tanks were situated outside the factory and were exposed to different sources of contamination.

### 7.2.3 DEWATERING CENTRIFUGES

Due to the design of the centrifuges they are considered to be a major points of contamination. If not cleaned properly, this will be a suitable environment for the growth of mesophiles, moulds, yeasts and *Enterobacteriaceae*.

### 7.2.4 WET MIXING BOXES

The design of the wet mixing boxes allows areas where rapid microbial growth can take place, forming lumps of contaminated starch. They were open to the atmosphere, which allowed contamination from dust. The high counts indicate insufficient cleaning and/or rapid build up of microorganisms.

### 7.2.5 BAGGING-OFF POINT

Faecal streptococci, faecal coliforms and *Staphylococcus aureus* isolated on the final product could be due to workers at this point with dirty hands, or even the sanitary conditions in the plant.

## 7.3 CRITICAL CONTROL POINTS

Some of the major contamination points identified as CCP's were as follows:

Maize trucks

Reineveld Overflow tank

Reineveld Filtrate tank

Steeping

Storage tanks

Reineveld

Wet mixing boxes

Drying

Bagging-off point

## 7.4 CONTROL AND MONITORING OF CCP'S

### 7.4.1 MAIZE TRUCKS

The bacterial and fungal contamination cannot be controlled, but minimized. The quality of the raw maize needs to be monitored by the quality control department.

### 7.4.2 STEEPING

Bacterial populations can be controlled in the steeps by maintaining very low pH levels and high $SO_2$ levels.

Criteria for the steeps are as follows:

Check the pH, temperature and the $SO_2$ levels.

Maintain pH at less than 4,5, the temperature, between 50 °C and 52 °C; and the $SO_2$ will vary but aim for levels of 1 000 ppm to 2 000 ppm.

### 7.4.3 REINEVELD FILTRATE / OVERFLOW TANK

The disinfectants had minor effects on the microbial counts at this sampling point, because of the different contamination factors.

Control practised at this point was the removal of the fibre tanks outside the factory. They were replaced with stainless steel tanks. These tanks not only reduced the microbial risk, but it also simplified cleaning.

heck microbial count; clean and sanitize regularly. It also appears from the results that sanitation of the plant at Bellville Mill, should take place at least on a weekly basis. If possible, preferably at shorter intervals of four to five days, depending on the production level. Coliform bacteria will be under control if the $SO_2$ levels are maintained at a high level.

### 7.4.4 STARCH STORAGE TANKS

The bacterial counts in these tanks can be controlled. Effective sanitation is necessary at this point.

Monitor the retention time, temperature and microbial counts.

The following groups need to monitored daily:

Mesophilic bacteria, moulds and yeast, coliforms and faecal streptococci. The coliform counts should not exceed 300 cfu/g and mesophilic counts 20 000 cfu/g.

### 7.4.5 WET MIXING BOXES

The wet mixing boxes were changed to a closed system. Stainless steel covers were made for the boxes, but high microbial counts were maintained. The only method to prevent growth of microorganisms is by regular sanitation.

Periodical examination needs to be performed for mesophilic bacteria, yeasts and moulds. Mesophilic counts should not exceed 10 000 cfu/g and mould counts 5 000 cfu/g.

### 7.4.6 DRIER

The retention time and temperature need to be monitored to ensure that the drying process kills off most of the microorganisms. Temperature of the drier should be maintained at 165 °C and a retention time of at least 8 seconds.

### 7.4.7 BAGGING POINT

Personnel should be made aware of the importance of personal hygiene and sanitation. Monitor counts on final products at hourly intervals per batch. The final products need to conform to internationally accepted specifications.

*NOTE:* The bagging has now been automated, which will solve any previous contamination problems which originated here.

# CHAPTER 8

## 8.1 CONCLUSIONS

i) If the in-process water is contaminated, the entire plant will be infected with contaminants.

ii) In the past, although different disinfectant were used, no improvements occur in the counts. The 50 % peroxide solution and even high $SO_2$ levels are good disinfectants, but not very effective, if not applied regularly.

iii) It is evident that a regular sanitation programme should be exercised.

iv) The identification of CCP's and the following of a HACCP plan is important to solve quality problems in a production process.

v) The Malthus appears to be an answer to delays of despatch of final products. Rapid detection of in-process and post-manufacturing contamination is possible within hours instead of days.

## 8.2 RECOMMENDATIONS

i) Institute HACCP

ii) Monitor CCP's regularly. They are as follow: Starch storage tanks, wet mixing boxes, dewatering centrifuges, driers and bagging-off point.

iii) Exercise a regular sanitation programme:

- Maintain $SO_2$ levels at maximum 250 ppm and temperature 40 - 50 °C to get counts less than 100 000 cfu/g. These $SO_2$ levels need to be maintain at sampling points S4, S5 and S6 - 1 and S6 - 2. $SO_2$ levels need to be maintained the very first day after sanitation.
- If there is a build up of microbial population in the plant, high $SO_2$ levels can also be maintained throughout the plant. The final starch will then be used for industrial starch.
- The dewatering centrifuges need to be cleaned out on a weekly basis. Manual cleaning appears to be an appropriate method. The cloth should be replaced regularly and the wire basket needs to be cleaned manually. If sanitation is not taken care of at this point, continuing microbial problems will occur.
- If the plant was allowed to become heavily contaminated with microorganisms the entire system should be thoroughly sanitised. Follow up sanitation on a regular basis to prevent heavy growths from re-establishing themselves. This was verified during a visit at the plant and shown in lower counts.

## 8.3 GENERAL NOTE

African Products, Bellville Mill currently produces Pharmaceutical and Food grade starch, having solved most of the microbiological problems by following the recommendations. They have also implemented the ISO 9002 Total Quality Management System.

CHAPTER 9

# REFERENCES

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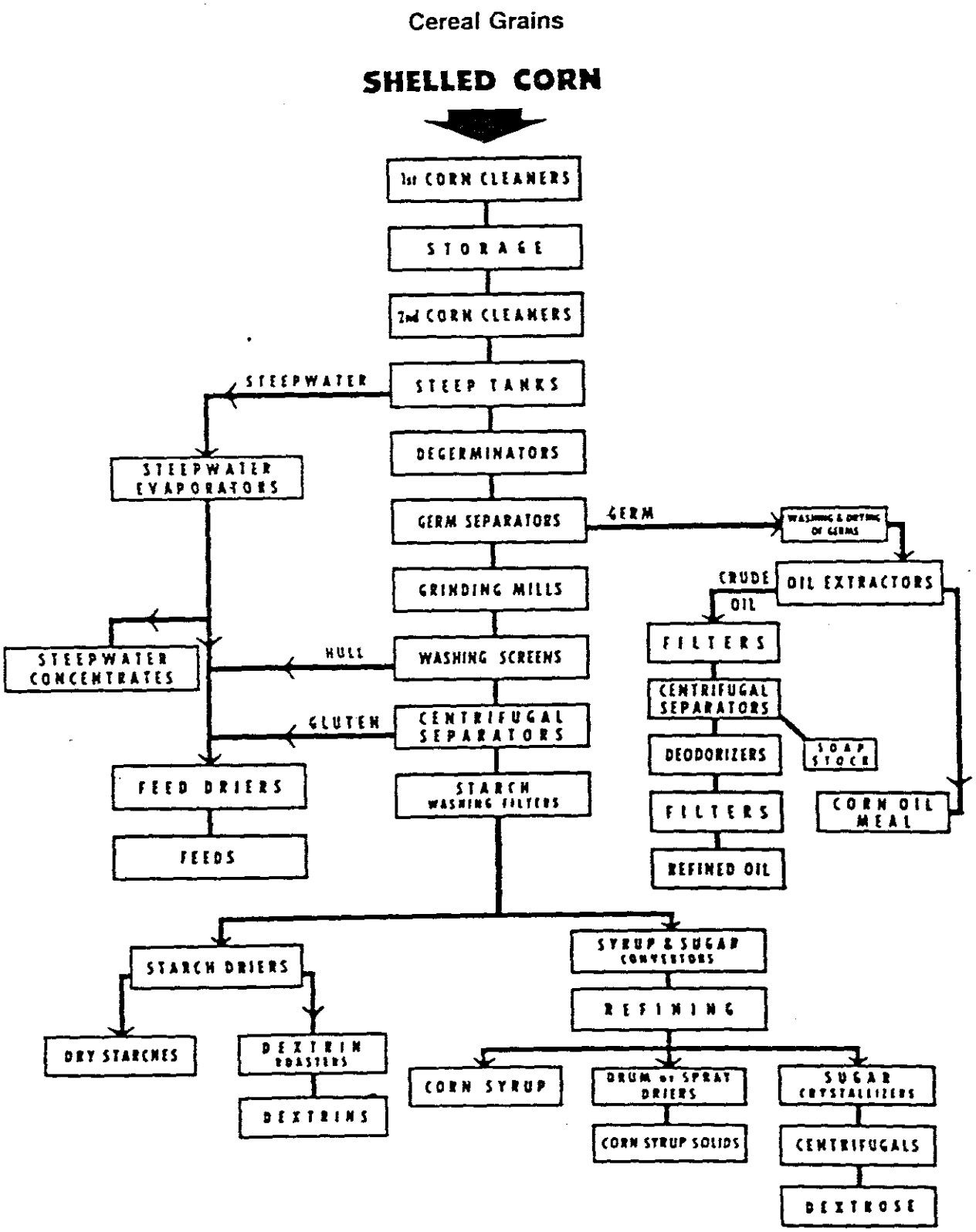
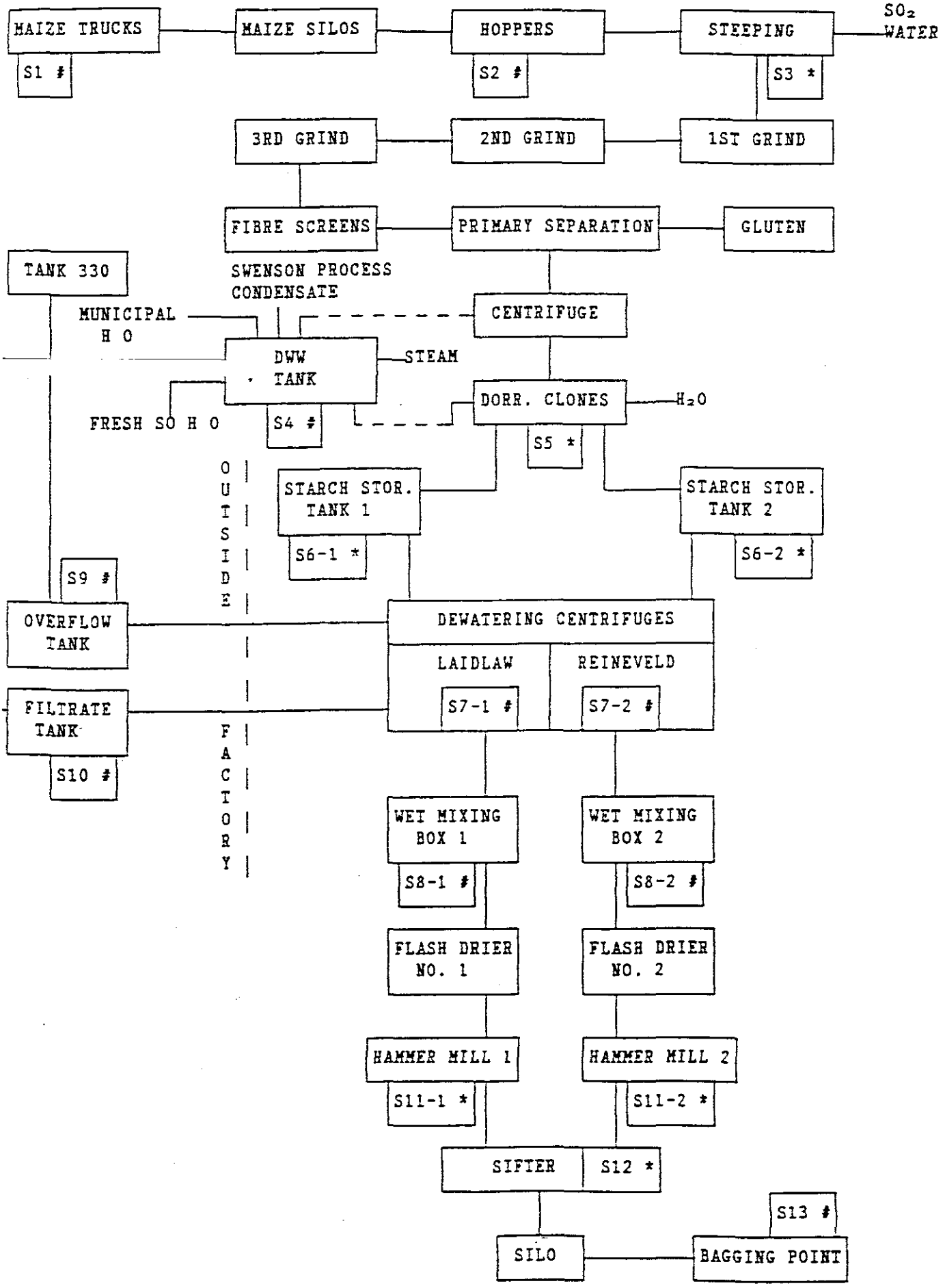



FIG. 1 Flow diagram of the wet-milling process. *Courtesy of Corn Refiners Association.*




2. Abridged flowchart before modification - minor (*) and major (#) contamination points (starch section); African Products, Bellville.

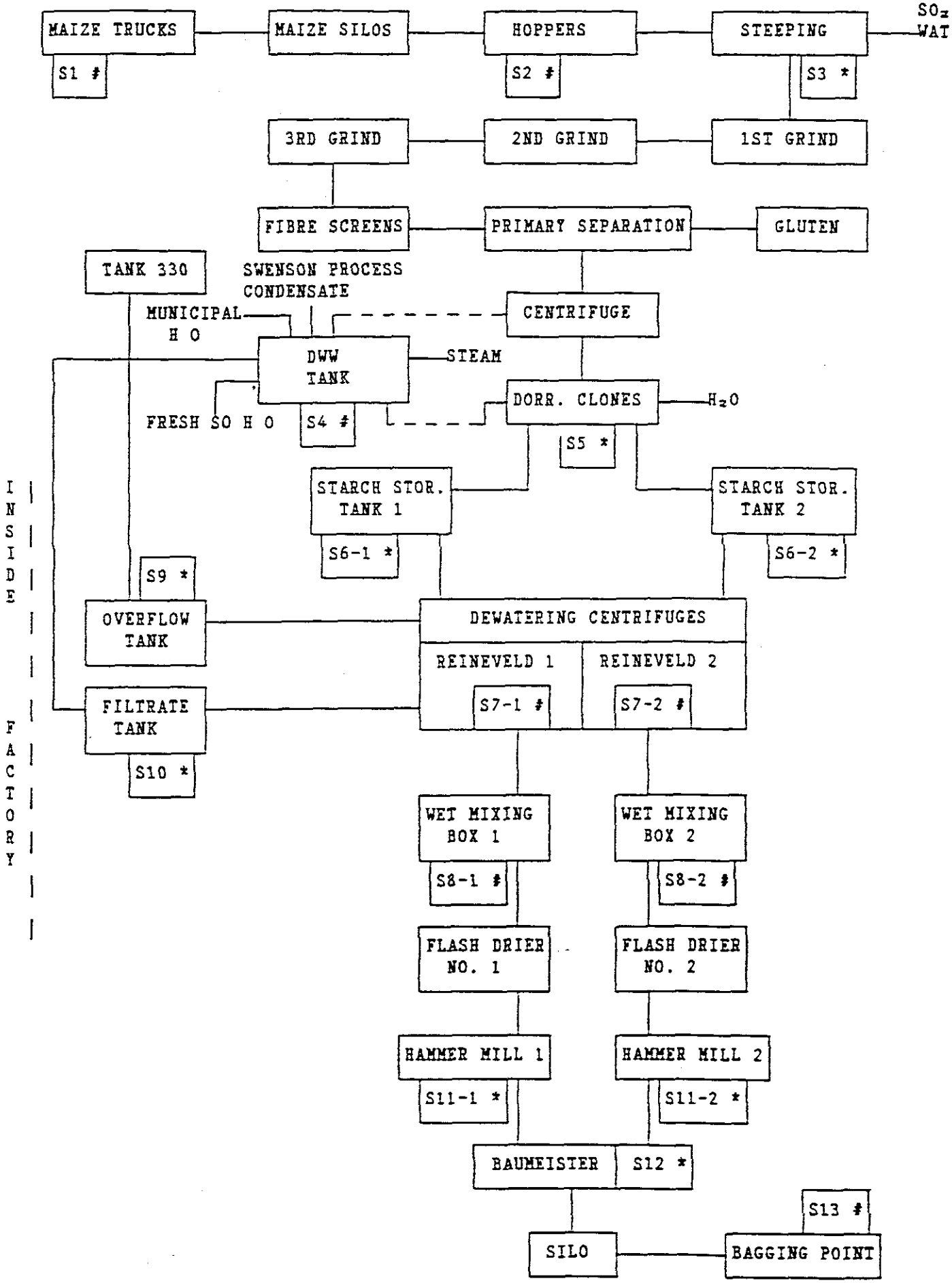



Fig 3. Abridged flowchart after modification: minor (*) and major (#) contamination points (starch section); African Products, Bellville.

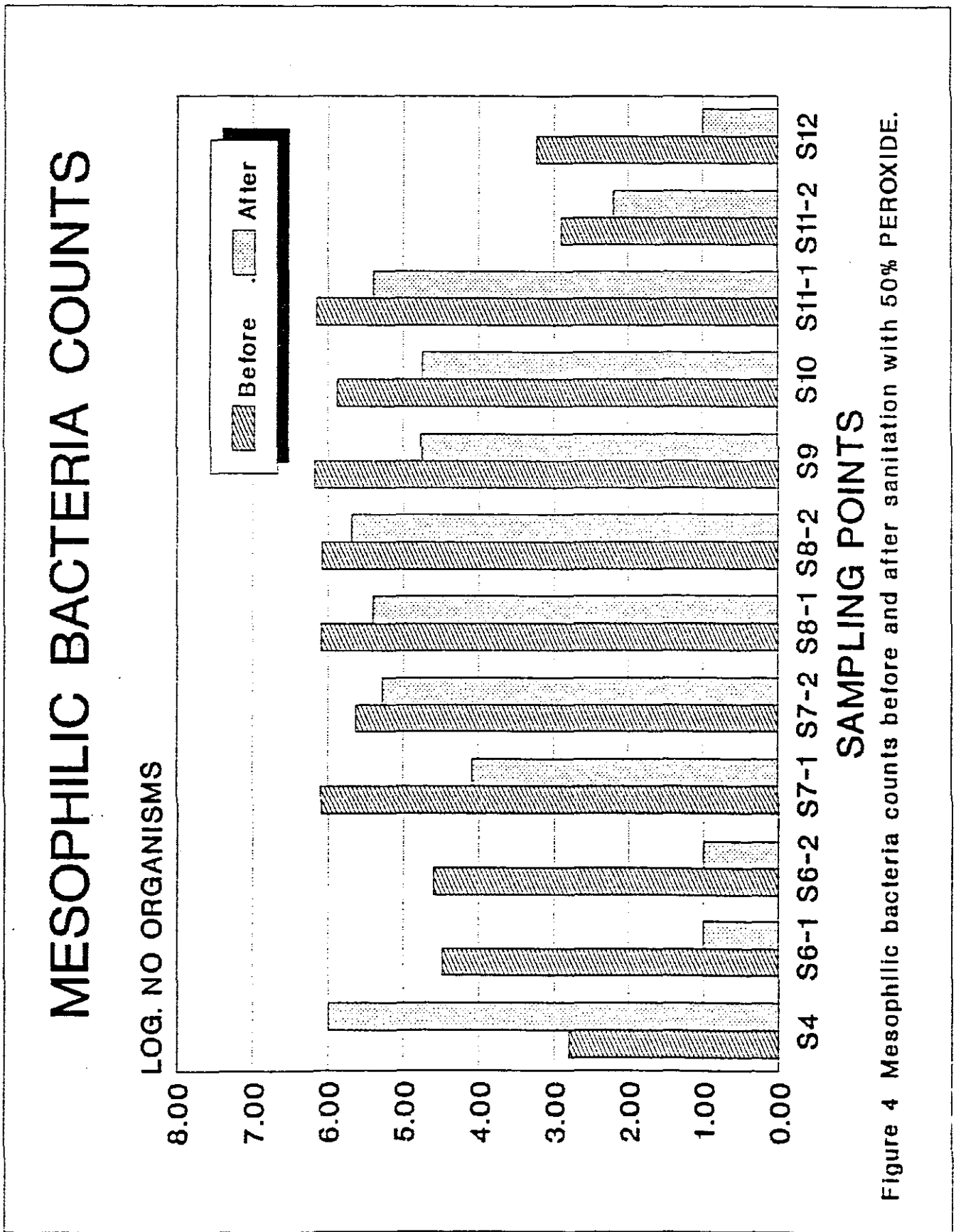



Figure 4 Mesophilic bacteria counts before and after sanitation with 50% PEROXIDE.

# MESOPHILIC BACTERIA COUNTS

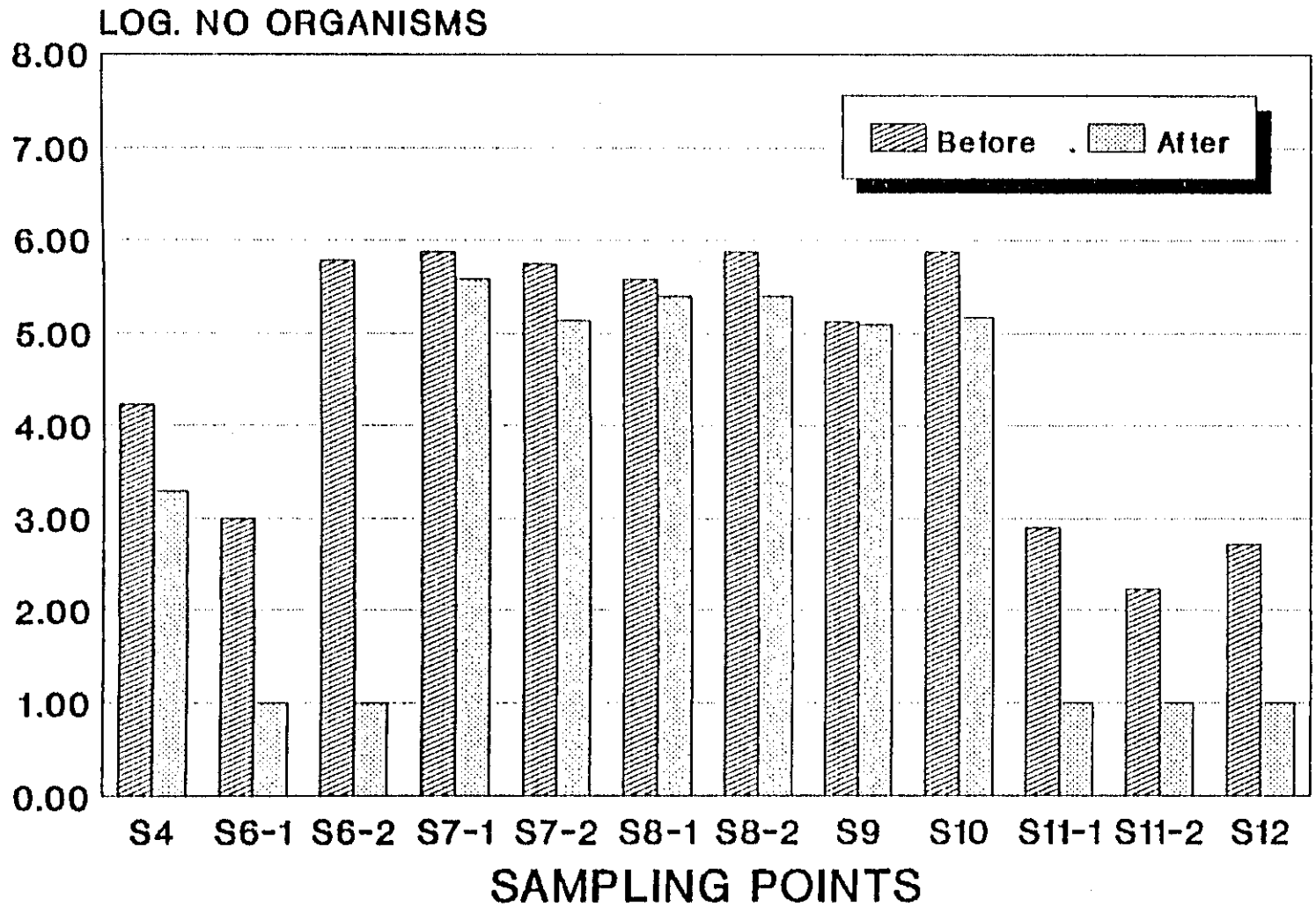



Figure 5 Mesophilic bacteria counts before and after sanitation with high SO2 levels (250 ppm).

# COLIFORM COUNTS

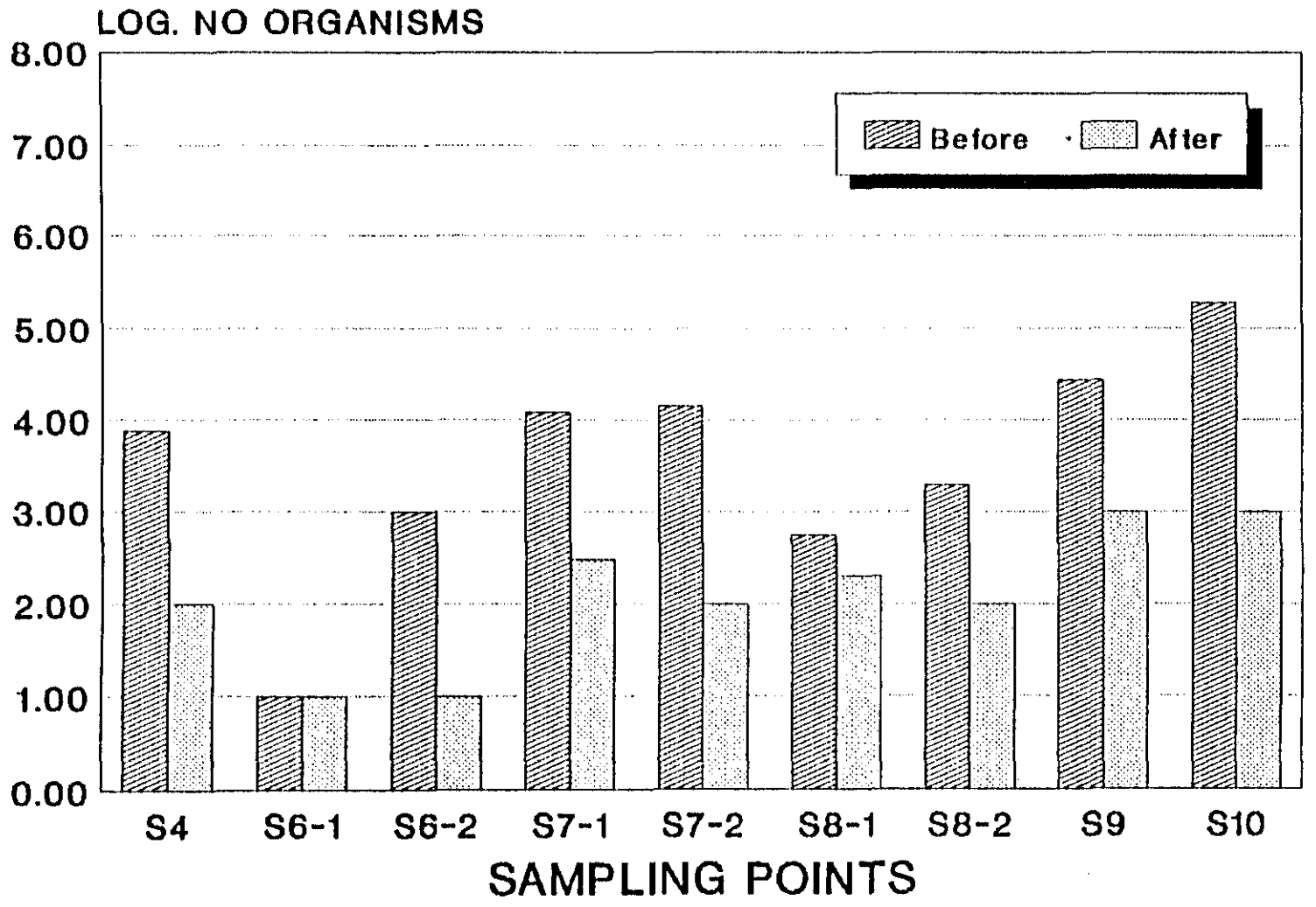



Figure 6 Coliform counts before and after sanitation with 50% PEROXIDE.

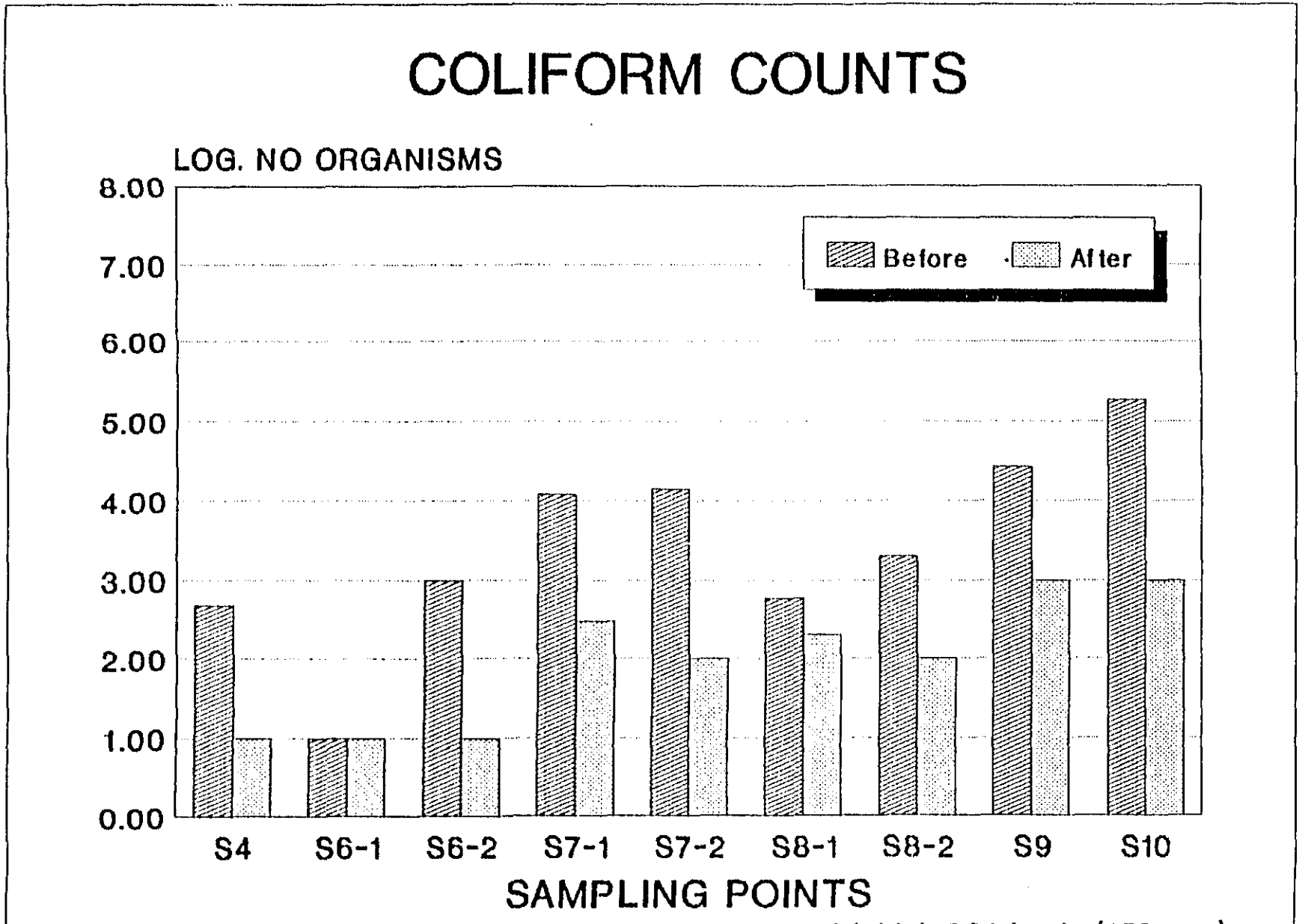



Figure 7 Coliform counts before and after sanitation with high SO2 levels (250 ppm).

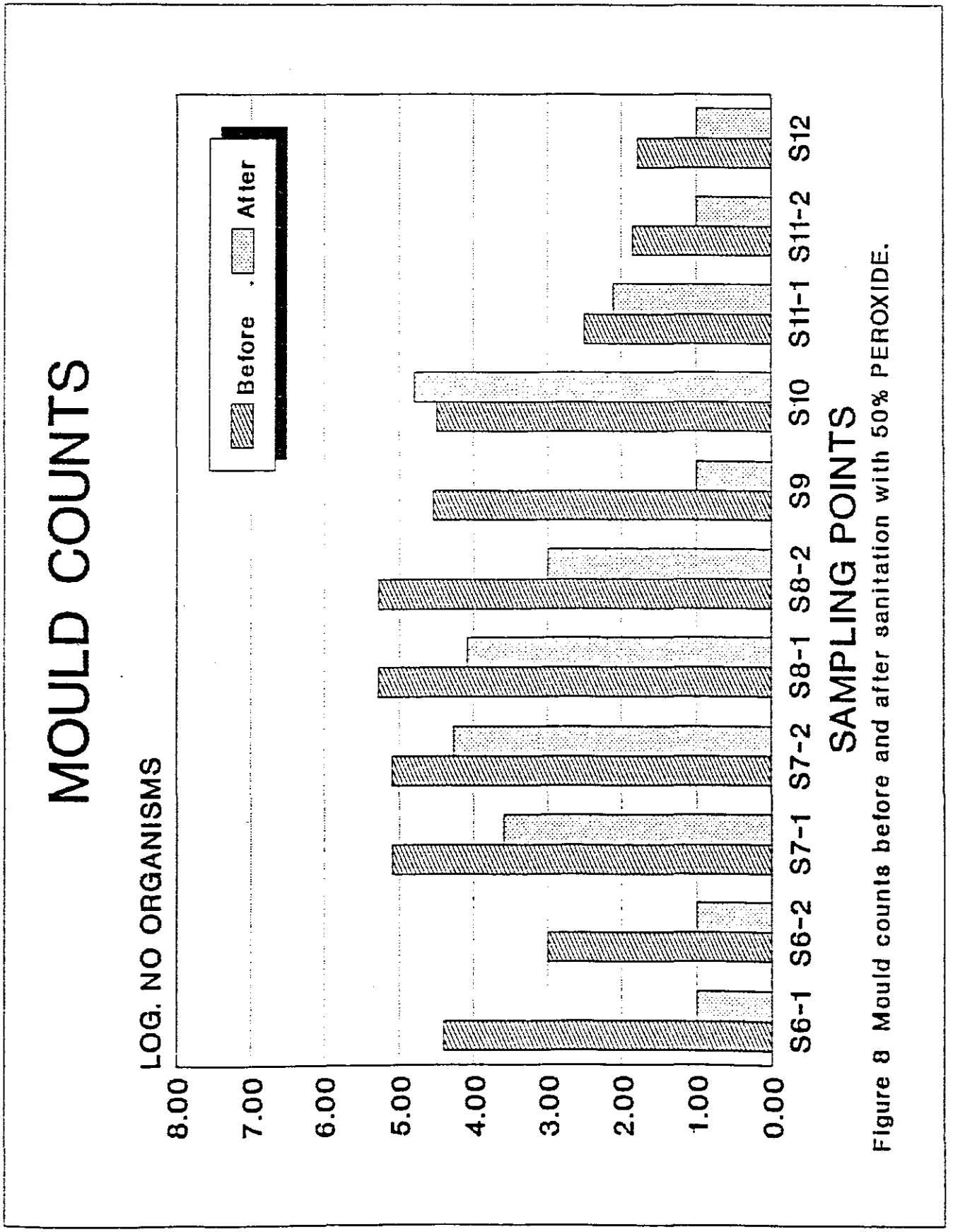



Figure 8 Mould counts before and after sanitation with 50% PEROXIDE.

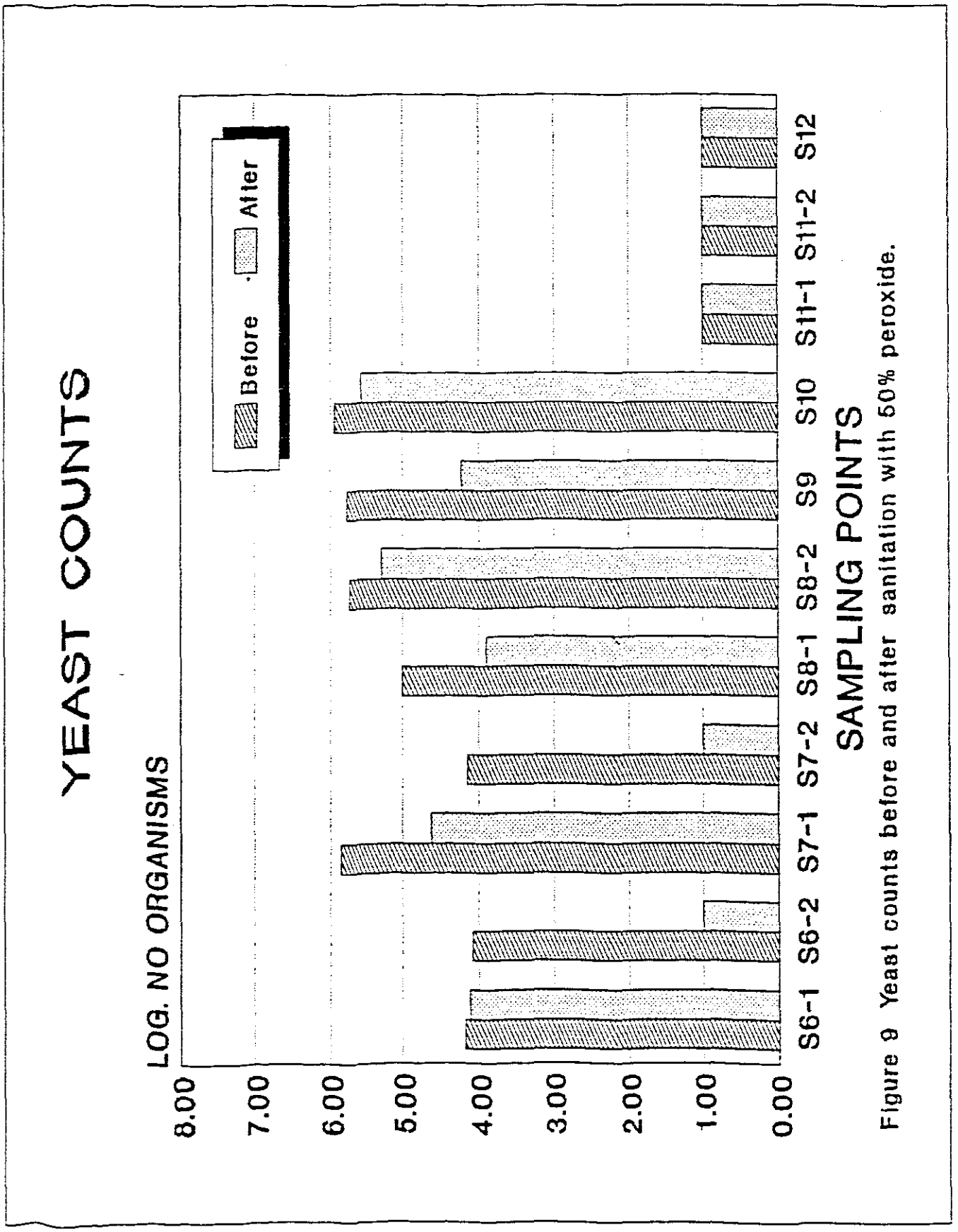



Figure 9 Yeast counts before and after sanitation with 50% peroxide.

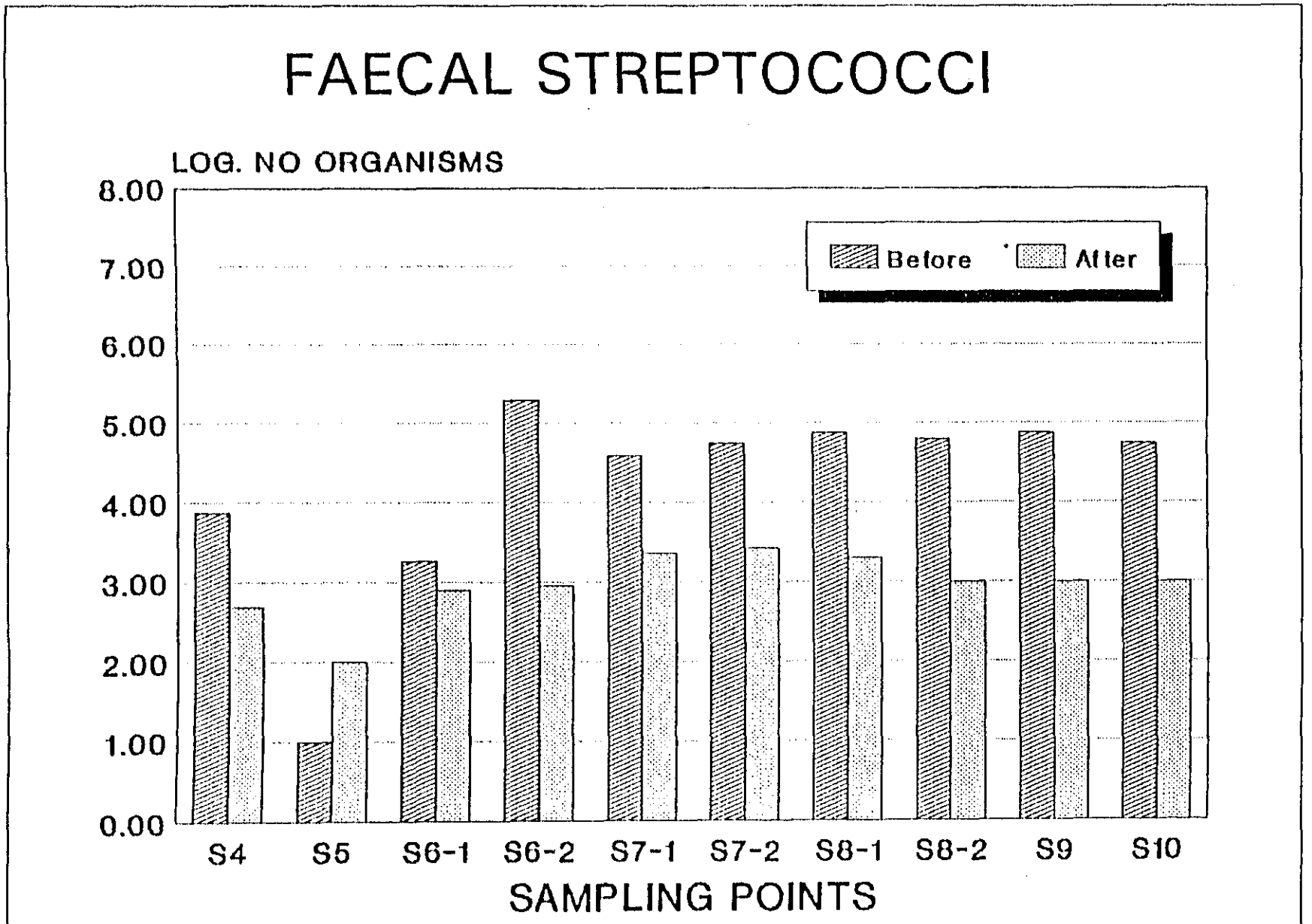



Figure 10: Faecal streptococci before and after sanitation with 50% PEROXIDE.

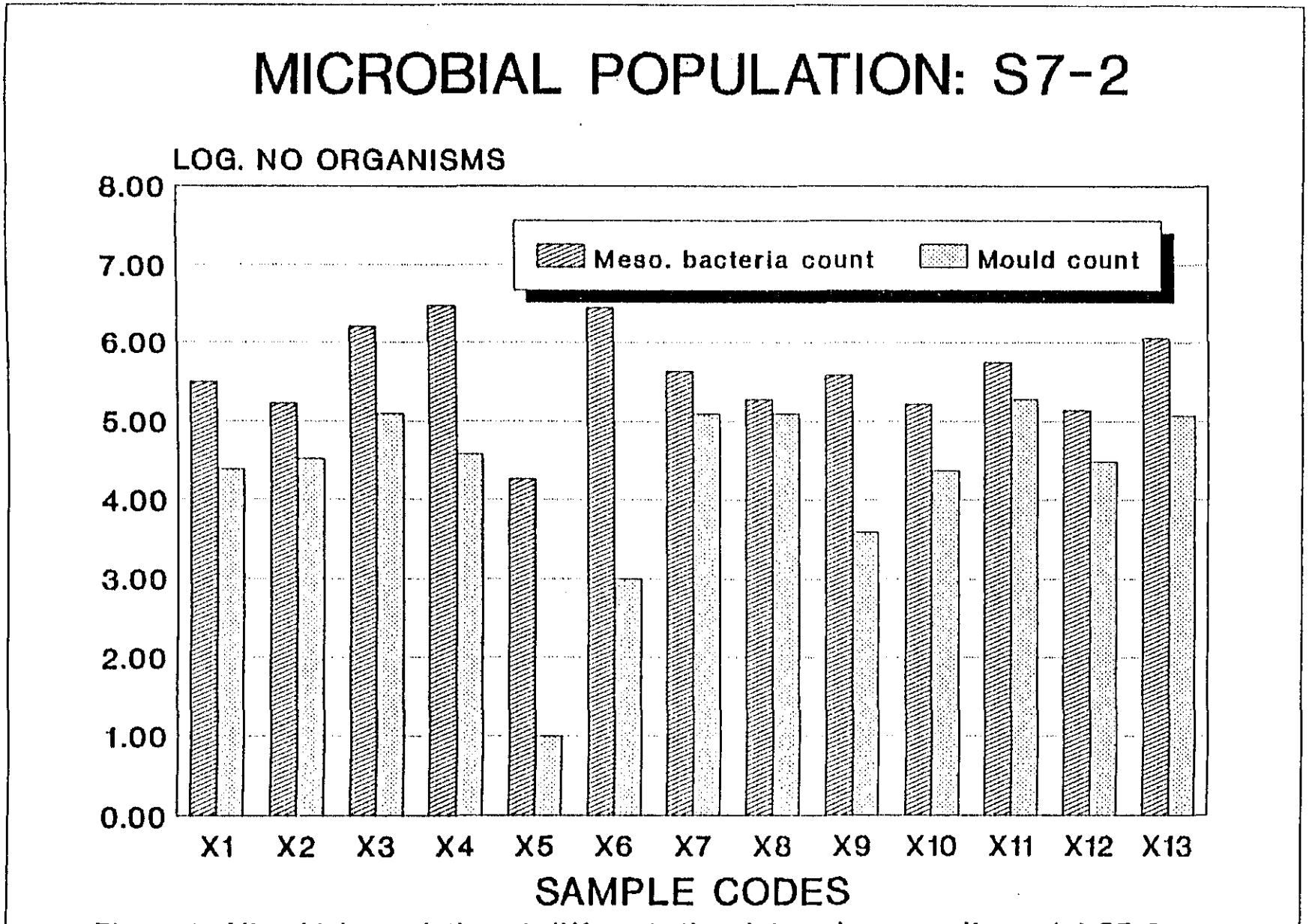



Figure 11. Microbial population at different time intervals - sampling point S7-2.

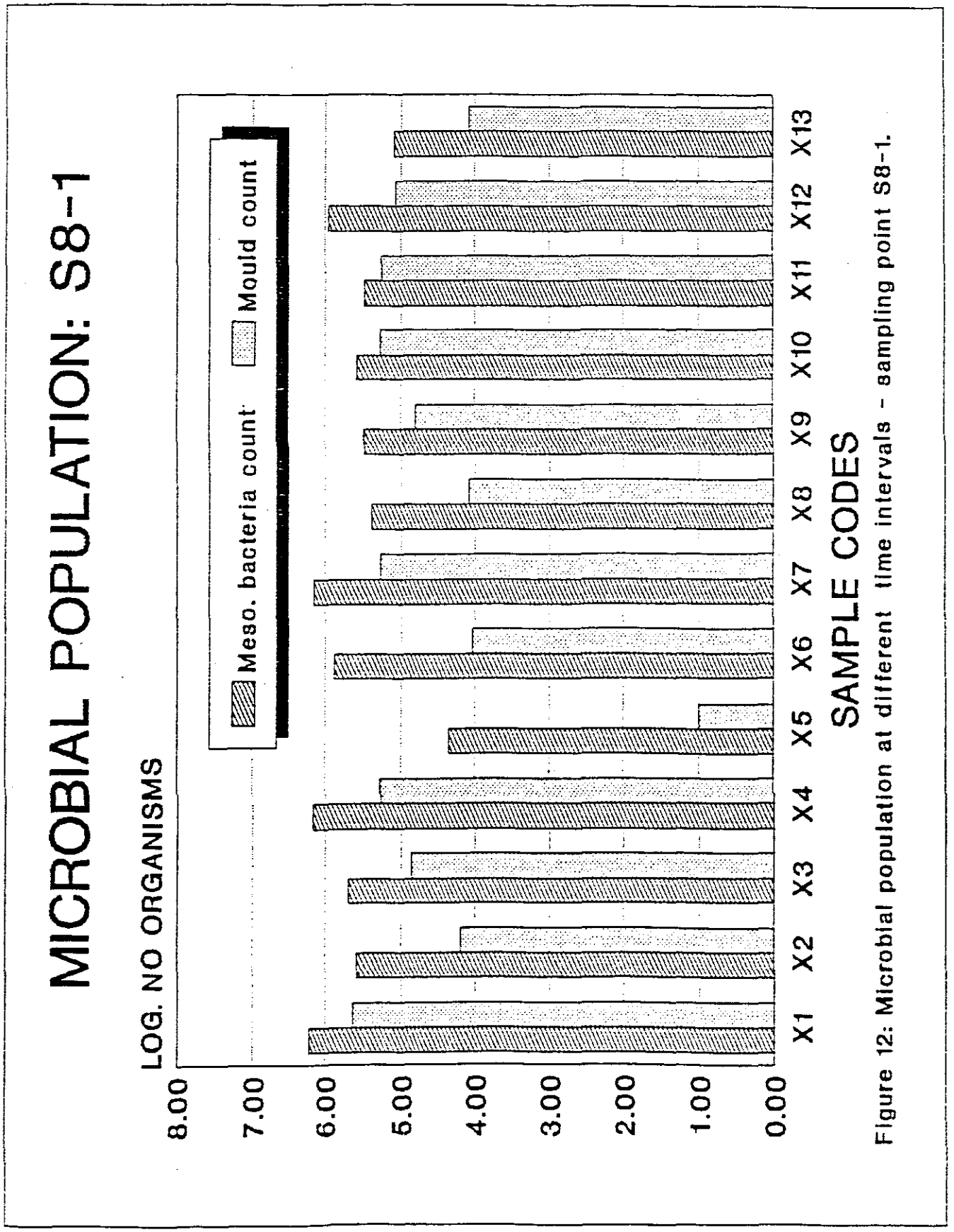



Figure 12: Microbial population at different time intervals - sampling point S8-1.

# TABLE 1

## VARIOUS SAMPLING POINTS IN STARCH SECTION, AFRICAN PRODUCTS, BELLVILLE

| SAMPLING POINTS | |
|---|---|
| S1 | MAIZE TRUCKS |
| S2 | HOPPERS |
| S3 | STEEPING |
| S4 | DORR CLONE WASH WATER TANK |
| S5 | DORR CLONES |
| S6-1 | STARCH STORAGE TANK 1 |
| S6-2 | STARCH STORAGE TANK 2 |
| S7-1 | LAIDLAW/REINEVELD 1 |
| S7-2 | REINEVELD/REINEVELD 2 |
| S8-1 | WET MIXING BOX 1 |
| S8-2 | WET MIXING BOX 2 |
| S9 | OVERFLOW TANK |
| S10 | FILTRATE TANK |
| S11-1 | HAMMER MILL 1 |
| S11-2 | HAMMER MILL 2 |
| S12 | GUMPSIFTER/BAUMEISTER |
| S13 | BAGGING POINT |

# TABLE 2

## MICROBIAL POPULATION AT SAMPLING POINT S6-1
## PERIOD 2 OCT. 1992 - 14 NOV 1992.

| SAMPLE CODE | DATE | MESOPHILIC TOT. COUNT | COUNT L. NO. | MOULDS | | YEAST | | COLIFORMS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. |
| X1 | 02/10 | 1 071 000 | 6.36 | 2 000 | 3.30 | 7 000 | 3.85 | < 100 | 1.00 |
| X2 | 04/10 | 1 000 | 3.00 | 2 000 | 3.30 | 1 000 | 1.00 | < 100 | 1.00 |
| X3 | 11/10 | 945 000 | 5.98 | 29 000 | 4.46 | 20 000 | 4.30 | < 100 | 1.00 |
| X4 | 16/10 | 315 000 | 5.49 | 126 000 | 5.10 | 189 000 | 5.28 | < 100 | 1.00 |
| X5 | 18/10 | 5 000 | 3.69 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 1 000 | 1.00 |
| X6 | 23/10 | 5 000 | 3.69 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 1 000 | 1.00 |
| X7 | 25/10 | 1 000 | 3.00 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X8 | 31/10 | 30 000 | 4.48 | 25 000 | 4.39 | 15 000 | 4.10 | < 100 | 1.00 |
| X9 | 03/11 | 12 000 | 4.08 | < 1 000 | 1.00 | 13 000 | 4.11 | < 100 | 1.00 |
| X10 | 05/11 | 34 000 | 4.53 | 8 000 | 3.90 | 34 000 | 4.53 | < 100 | 1.00 |
| X11 | 09/11 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X12 | 12/11 | 189 000 | 5.28 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X | | 217 417 | | 16 500 | | 23 667 | | 100 | |
| SD | | 382 578 | | 35 870 | | 53 091 | | 0 | |

# TABLE 3

## MICROBIAL POPULATION AT SAMPLING POINT S6-2
## PERIOD 2 OCT. 1992 - 14 NOV. 1992

| SAMPLE CODE | DATE | MESOPHILIC TOT. COUNT | COUNT L. NO. | MOULDS | | YEAST | | COLIFORMS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. |
| X1 | 02/10 | 2 258 000 | 6.03 | 6 000 | 3.78 | 756 000 | 5.88 | < 100 | 1.00 |
| X2 | 04/10 | 1 000 | 3.00 | 1 000 | 3.00 | 1 000 | 3.00 | < 100 | 1.00 |
| X3 | 11/10 | 31 000 | 5.98 | 2 000 | 3.00 | 2 000 | 3.00 | < 100 | 1.00 |
| X4 | 16/10 | 170 000 | 5.23 | 24 000 | 4.38 | 146 000 | 5.16 | < 100 | 1.00 |
| X5 | 18/10 | 10 000 | 4.00 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X6 | 23/10 | 5 000 | 3.69 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X7 | 25/10 | 2 000 | 3.30 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X8 | 31/10 | - | - | - | - | - | - | - | - |
| X9 | 03/11 | 14 000 | 4.15 | < 1 000 | 1.00 | 12 000 | 4.08 | < 100 | 1.00 |
| X10 | 05/11 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X11 | 09/11 | 630 000 | 5.79 | 59 000 | 4.77 | 20 000 | 4.08 | < 100 | 1.00 |
| X12 | 12/11 | 378 000 | 5.58 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X | | 319 091 | | 8 909 | | 85 636 | | 100 | |
| SD | | 677 775 | | 17 975 | | 226 437 | | 0 | |

# TABLE 4

## MICROBIAL POPULATION AT SAMPLING POINT S7-1
## PERIOD 2 OCT. 1992 - 14 NOV. 1992

| SAMPLE CODE | DATE | MESOPHILIC TOT. COUNT | COUNT L. NO. | MOULDS | | YEAST | | COLIFORMS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. |
| X1 | 02/10 | - | - | - | - | - | - | - | - |
| X2 | 04/10 | 126 000 | 5.10 | 378 000 | 5.58 | 112 000 | 5.05 | 12 000 | 4.08 |
| X3 | 11/10 | 1 890 000 | 6.28 | 1 000 | 3.00 | 212 000 | 5.33 | 12 600 | 4.10 |
| X4 | 16/10 | 1 000 | 1.00 | 126 000 | 5.10 | 756 000 | 5.88 | < 100 | 1.00 |
| X5 | 18/10 | 27 000 | 4.43 | < 1 000 | 1.00 | < 1 000 | 1.00 | 12 000 | 4.08 |
| X6 | 23/10 | 945 000 | 5.98 | < 1 000 | 1.00 | < 1 000 | 1.00 | 300 | 2.48 |
| X7 | 25/10 | 1 260 000 | 5.10 | 4 000 | 3.60 | 38 000 | 4.58 | 700 | 2.85 |
| X8 | 31/10 | 756 000 | 5.88 | 128 000 | 5.11 | 756 000 | 5.88 | < 100 | 1.00 |
| X9 | 03/11 | 35 000 | 4.54 | 26 000 | 4.44 | 441 000 | 5.64 | 100 | 2.00 |
| X10 | 05/11 | 756 000 | 5.88 | 16 000 | 4.20 | 31 000 | 4.49 | 700 | 2.85 |
| X11 | 09/11 | 378 000 | 5.58 | 16 000 | 4.20 | 189 000 | 5.28 | 8 100 | 3.91 |
| X12 | 12/11 | 1 638 000 | 6.21 | 126 000 | 5.10 | 31 000 | 4.49 | 200 | 2.30 |
| X | | 710 182 | | 74 818 | | 233 455 | | 4 264 | |
| SD | | 670 195 | | 114 343 | | 288 898 | | 5 600 | |

# TABLE 5

## MICROBIAL POPULATION AT SAMPLING POINT S7-2
## PERIOD 2 OCT. 1992 - 14 NOV. 1992

| SAMPLE CODE | DATE | MESOPHILIC TOT. COUNT | COUNT L. NO. | MOULDS | | YEAST | | COLIFORMS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. |
| X1 | 02/10 | 315 000 | 5.49 | 24 000 | 4.38 | 276 000 | 5.44 | 100 | 2.00 |
| X2 | 04/10 | 168 000 | 5.23 | 33 000 | 4.52 | 100 000 | 5.00 | 700 | 2.85 |
| X3 | 11/10 | 1 638 000 | 6.21 | 126 000 | 5.10 | 1 260 000 | 6.10 | 8 100 | 3.91 |
| X4 | 16/10 | 3 024 000 | 6.48 | 38 000 | 4.58 | 156 000 | 5.19 | 200 | 2.30 |
| X5 | 18/10 | 18 000 | 4.26 | < 1 000 | 1.00 | < 1 000 | 1.00 | < 100 | 1.00 |
| X6 | 23/10 | 2 838 000 | 6.45 | 1 000 | 3.00 | 2 898 000 | 6.46 | < 100 | 1.00 |
| X7 | 25/10 | 441 000 | 5.64 | 126 000 | 5.10 | 630 000 | 5.79 | 14 000 | 4.15 |
| X8 | 31/10 | 189 000 | 5.28 | 15 000 | 4.18 | 15 000 | 4.18 | < 100 | 1.00 |
| X9 | 03/11 | 378 000 | 5.58 | 4 000 | 3.60 | 378 000 | 5.58 | 800 | 2.90 |
| X10 | 05/11 | 164 000 | 5.22 | 23 000 | 4.36 | 44 000 | 4.64 | 800 | 2.90 |
| X11 | 09/11 | 567 000 | 5.75 | 189 000 | 5.28 | 378 000 | 5.58 | 12 000 | 4.08 |
| X12 | 12/11 | 140 000 | 5.15 | 30 000 | 4.48 | 15 000 | 4.18 | < 100 | 1.00 |
| X | | 823 333 | | 50 833 | | 512 583 | | 3 092 | |
| SD | | 1 076 941 | | 61 234 | | 832 776 | | 5 159 | |

# TABLE 6

## MICROBIAL POPULATION AT SAMPLING POINT S9
## PERIOD 2 OCT. 1992 - 14 NOV. 1992

| SAMPLE CODE | DATE | MESOPHILIC TOT. COUNT | COUNT L. NO. | MOULDS | | YEAST | | COLIFORMS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. |
| X1 | 02/10 | 2 041 200 | 7.30 | 126 000 | 3.78 | 315 000 | 5.10 | 27 000 | 4.43 |
| X2 | 04/10 | 630 000 | 5.79 | 126 000 | 3.78 | 315 000 | 5.10 | < 100 | 1.00 |
| X3 | 11/10 | 1 368 000 | 6.14 | 48 000 | 4.68 | 268 000 | 5.43 | 18 900 | 4.28 |
| X4 | 16/10 | 1 449 000 | 6.16 | 189 000 | 5.28 | 126 000 | 5.10 | 7 000 | 3.85 |
| X5 | 18/10 | 567 000 | 5.75 | 484 000 | 5.68 | 54 000 | 4.73 | 6 000 | 3.78 |
| X6 | 23/10 | 504 000 | 5.70 | 17 000 | 4.23 | 146 000 | 5.16 | 1 800 | 3.26 |
| X7 | 25/10 | 441 000 | 5.64 | 10 000 | 4.00 | 78 000 | 4.89 | 400 | 2.60 |
| X8 | 31/10 | 1 575 000 | 6.19 | 126 000 | 5.10 | 575 000 | 5.76 | 400 | 2.60 |
| X9 | 03/11 | 378 000 | 5.58 | < 1 000 | 1.00 | 63 000 | 4.80 | 500 | 2.69 |
| X10 | 05/11 | 136 000 | 5.13 | 7 000 | 3.85 | 17 000 | 4.23 | 1 700 | 3.23 |
| X11 | 09/11 | 126 000 | 5.10 | 6 000 | 3.78 | 13 000 | 4.11 | 200 | 2.30 |
| X12 | 12/11 | 1 890 000 | 6.28 | 7 000 | 3.85 | 13 000 | 4.11 | 1 800 | 3.26 |
| X | | 926 933 | | 95 583 | | 165 250 | | 5 483 | |
| SD | | 692 394 | | 138 432 | | 171 622 | | 863 | |

# TABEL 7

## MICROBIAL POPULATION AT SAMPLING POINT S10
## PERIOD 2 OCT. 1992 - 14 NOV. 1992

| SAMPLE CODE | DATE | MESOPHILIC TOT. COUNT | COUNT L. NO. | MOULDS | | YEAST | | COLIFORMS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. |
| X1 | 02/10 | 1 000 000 | 6.00 | 315 000 | 5.49 | 1 260 000 | 6.10 | 189 000 | 5.27 |
| X2 | 04/10 | 1 008 000 | 6.00 | 3 000 | 3.48 | 74 000 | 4.87 | 12 000 | 4.10 |
| X3 | 11/10 | 1 575 000 | 6.19 | 126 000 | 5.10 | 378 000 | 5.58 | 16 300 | 4.21 |
| X4 | 16/10 | 2 835 000 | 6.45 | 38 000 | 4.58 | 378 000 | 5.58 | 160 000 | 5.20 |
| X5 | 18/10 | 75 000 | 4.88 | < 1 000 | 1.00 | < 1 000 | 1.00 | 1 000 | 3.00 |
| X6 | 23/10 | 1 449 000 | 6.16 | 25 000 | 4.39 | 360 000 | 5.56 | 900 | 2.95 |
| X7 | 25/10 | 500 000 | 5.69 | 500 000 | 5.69 | 500 000 | 5.69 | 100 | 2.00 |
| X8 | 31/10 | 756 000 | 5.88 | 128 000 | 5.10 | 882 000 | 5.95 | 200 | 2.30 |
| X9 | 03/11 | 2 268 000 | 6.36 | 20 000 | 4.30 | 268 000 | 5.43 | 50 000 | 4.69 |
| X10 | 05/11 | 378 000 | 5.58 | 252 000 | 5.40 | 315 000 | 5.49 | 1 300 | 3.11 |
| X11 | 09/11 | 756 000 | 5.88 | 189 000 | 5.28 | 189 000 | 5.28 | 500 | 2.69 |
| X12 | 12/11 | 152 000 | 5.18 | 128 000 | 5.10 | 74 000 | 4.87 | 1 000 | 3.00 |
| X | | 1 062 667 | | 143 750 | | 389 917 | | 36 025 | |
| SD | | 841 584 | | 151 239 | | 359 823 | | 66 512 | |

# TABLE 8

## MICROBIAL POPULATION AT SAMPLING POINT S8-1
## PERIOD 2 OCT. 1992 - 14 NOV. 1992

| SAMPLE CODE | DATE | MESOPHILIC TOT. COUNT | COUNT L. NO. | MOULDS | | YEAST | | COLIFORMS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. |
| X1 | 02/10 | 1 710 000 | 6.23 | 441 000 | 5.64 | 465 000 | 5.67 | 21 000 | 4.32 |
| X2 | 04/10 | 378 000 | 5.58 | 15 000 | 4.18 | 270 000 | 5.43 | < 100 | 1.00 |
| X3 | 11/10 | 504 000 | 5.70 | 72 000 | 4.86 | 156 000 | 5.19 | 1 000 | 3.00 |
| X4 | 16/10 | 1 510 000 | 6.18 | 189 000 | 5.28 | 567 000 | 5.75 | 2 000 | 3.30 |
| X5 | 18/10 | 22 000 | 4.34 | < 1 000 | 1.00 | < 1 000 | 1.00 | 3 000 | 3.48 |
| X6 | 23/10 | 756 000 | 5.88 | 11 000 | 4.04 | 102 000 | 5.00 | 1 800 | 3.26 |
| X7 | 25/10 | 1 449 000 | 6.16 | 189 000 | 5.28 | 378 000 | 5.58 | 100 | 2.00 |
| X8 | 31/10 | 252 000 | 5.40 | 12 000 | 4.08 | 252 000 | 5.40 | 300 | 2.48 |
| X9 | 03/11 | 315 000 | 5.49 | 63 000 | 4.79 | 252 000 | 5.40 | 1 000 | 3.00 |
| X10 | 05/11 | 378 000 | 5.58 | 189 000 | 5.28 | 378 000 | 5.58 | 100 | 2.00 |
| X11 | 09/11 | 315 000 | 5.49 | 185 000 | 5.27 | 150 000 | 6.18 | < 100 | 1.00 |
| X12 | 12/11 | 126 000 | 5.10 | 12 000 | 4.08 | 110 000 | 5.04 | < 100 | 1.00 |
| X | | 642 917 | | 114 917 | | 256 750 | | 2 556 | |
| SD | | 582 575 | | 130 449 | | 165 607 | | 5 888 | |

# TABLE 9

## MICROBIAL POPULATION AT SAMPLING POINT S8-2
## PERIOD 2 OCT. 1992 - 14 NOV. 1992

| SAMPLE CODE | DATE | MESOPHILIC TOT. COUNT | COUNT L. NO. | MOULDS | | YEAST | | COLIFORMS | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. | TOT. COUNT | L. NO. |
| X1 | 02/10 | 1 710 000 | 6.23 | 441 000 | 5.64 | 465 000 | 5.67 | 21 000 | 4.32 |
| X2 | 04/10 | 1 890 000 | 6.28 | 136 000 | 5.13 | 304 000 | 5.48 | 12 000 | 4.05 |
| X3 | 11/10 | 1 764 000 | 6.25 | 126 000 | 5.10 | 945 000 | 5.98 | 700 | 2.85 |
| X4 | 16/10 | 15 000 | 4.18 | < 1 000 | 1.00 | < 1 000 | 1.00 | 600 | 2.78 |
| X5 | 18/10 | 1 260 000 | 6.10 | 189 000 | 5.28 | 567 000 | 5.75 | 200 | 2.30 |
| X6 | 23/10 | 504 000 | 5.70 | < 1 000 | 1.00 | 189 000 | 5.28 | 400 | 2.60 |
| X7 | 25/10 | 252 000 | 5.40 | 126 000 | 5.10 | 252 000 | 5.48 | 100 | 2.00 |
| X8 | 31/10 | 315 000 | 5.49 | 189 000 | 5.28 | 150 000 | 6.18 | 200 | 2.30 |
| X9 | 03/11 | 756 000 | 5.88 | 120 000 | 5.08 | 112 000 | 5.05 | < 100 | 1.00 |
| X10 | 05/11 | - | - | - | - | - | - | - | - |
| X11 | 09/11 | 378 000 | 5.58 | 120 000 | 5.08 | 252 000 | 5.48 | | |
| X12 | 12/11 | 315 000 | 5.49 | 12 000 | 4.08 | 72 000 | 4.86 | | |
| X | | 832 636 | | 132 818 | | 300 818 | | 3 209 | |
| SD | | 692 820 | | 122 910 | | 270 316 | | 6 876 | |

# TABLE 10

## ANALYSIS OF FINAL STARCH PRODUCTS

| SAMPLE NO. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| **COUNTS** | | | | |
| MESOPHILIC BACTERIA | 300 | > 500 000 | 1 000 | > 500 000 |
| YEAST COUNT | 80 | 1 000 | 300 | 300 |
| MOULD COUNT | 20 | 200 | 130 | 100 |
| *Escherichia coli* | +/g | +/g | +/g | - |
| Coliforms | +/g | +/g | +/g | +/g |
| *Staphylococcus aureus* | +/10g | +10/g | - | +10/g |
| *Faecal Streptococci* | +/g | +/g | +/g | +/g |
| Thermophilic bacteria | < 10 | < 10 | < 10 | < 10 |

# TABLE 11

## STARCH: FINAL PRODUCT RELEASE SPECIFICATION

| 1. MICROBIOLOGICAL TARGETS | FOOD GRADE | PHARMACEUTICAL GRADE |
|---|---|---|
| TOTAL PLATE COUT/g | 3 000 | 1 000 |
| TOTAL MOULD/g | 250 | 100 |
| TOTAL YEAST/g | 250 | 100 |
| FAECAL COLIFORMS/10g | absent | absent |
| | | |
| **PATHOGENS** | | |
| *Clostridium welchi*/g | absent | absent |
| *Pseudomonas aeruginosa*/g | absent | absent |
| *Staphylococcus aureus*/g | absent | absent |
| *Salmonella spp*/10g | absent | absent |
| *Shigella spp*/10g | absent | absent |
| | | |
| **2. CHEMICAL TARGETS** | | |
| pH | 40 - 50 | 4.2 - 5.0 |
| $SO_2$ LEVELS | MAX. 100ppm | MAX 80ppm |

**NOTE:** If microbiological counts/chemical results exceed the above targets, then the batch will be classified as Industrial Starch.

# TABLE 12

## IDENTIFICATION OF BACTERIA AND FUNGI AT VARIOUS SAMPLING POINTS

| SAMPLING POINTS | BACTERIA AND FUNGI IDENTIFIED |
|---|---|
| **DEWATERING CENTRIFUGES** | |
| LAID LAW | Faecal streptococci, *Staphylococcus aureus*, Faecal coliforms. *Fusarium spp, Aspergillus spp, Peniccilium spp* and *Geotrichum* |
| REINEVELD | Faecal streptococci, *Staphylococcus aureus*, Faecal coliforms. *Fusarium spp, Aspergillus spp, Penicillium spp* and *Geotrichum spp.* |
| **PROCESS WATER** | |
| REINEVLED OVERFLOW TANK | Faecal streptococci, *Staphylococcus aureus*, Faecal coliforms *Fusarium spp, Aspergillus spp, Penicillium spp.* |
| REINEVELD FILTRATE TANK | Faecal streptococci, *Staphylococcus aureus*, Faecal coliforms, *Fusarium spp, Aspergillus spp, Penicillium spp.* |
| WET MIXING BOXES | Faecal streptococci, *Staphylococcus aureus*, Faecal coliforms, *Fusarium spp, Aspergillus spp, Penicillium spp, Mucor spp, Rhizopus spp* |

# TABLE 13

MEAN VALUES OF DIFFERENT MICROORGANISMS AT VARIOUS SAMPLING POINTS

| SAMPLING POINTS | MESOPHILES | MOULDS | YEASTS | COLIFORMS |
|---|---|---|---|---|
| S6 - 1 | 217 417 | 16 500 | 23 667 | 100 |
| S6 - 2 | 319 091 | 8 909 | 85 636 | 100 |
| S7 - 1 | 710 182 | 74 818 | 233 455 | 4 264 |
| S7 - 2 | 823 333 | 50 833 | 512 583 | 3 092 |
| S8 - 1 | 642 917 | 114 917 | 256 750 | 2 550 |
| S8 - 2 | 832 636 | 132 818 | 300 818 | 3 209 |
| S9 | 926 933 | 95 583 | 165 250 | 5 483 |
| S10 | 1 062 667 | 143 750 | 389 917 | 36 025 |

# TABLE 14

MESOPHILIC BACTERIA COUNTS BEFORE AND AFTER SANITATION WITH 50 % PEROXIDE SOLUTION (SERIES A)

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | 12 800 | 4.11 | 10 | 1.00 |
| S6 - 1 | 301 900 | 5.48 | 7 070 | 3.85 |
| S6 - 2 | 158 400 | 5.20 | 114 800 | 5.06 |
| S7 - 1 | 204 100 | 5.31 | 173 700 | 5.24 |
| S7 - 2 | 724 400 | 5.86 | 676 000 | 5.83 |
| S8 - 1 | 891 200 | 5.95 | 676 000 | 5.83 |
| S8 - 2 | 724 400 | 5.86 | 676 000 | 5.83 |
| S9 | 1 870 900 | 5.94 | 676 000 | 5.83 |
| S10 | 891 200 | 5.95 | 676 000 | 5.83 |
| | | | | |
| | F = 1,15 | | P < 0,299 | |

# TABLE 15

MESOPHILIC BACTERIA COUNTS BEFORE AND AFTER SANITATION WITH 50 % PEROXIDE SOLUTION (SERIES B)

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | 600 | 2.79 | 1 000 000 | 6.00 |
| S6 - 1 | 30 000 | 4.48 | 10 | 1.00 |
| S6 - 2 | 40 000 | 4.60 | 10 | 1.00 |
| S7 - 1 | 1 260 000 | 6.10 | 12 000 | 4.00 |
| S7 - 2 | 436 500 | 5.64 | 190 500 | 5.20 |
| S8 - 1 | 1 260 000 | 6.10 | 251 000 | 5.40 |
| S8 - 2 | 1 202 200 | 6.08 | 501 100 | 5.70 |
| S9 | 1 548 800 | 6.19 | 57 500 | 4.76 |
| S10 | 758 500 | 5.88 | 56 2000 | 4.75 |
| S11 - 1 | 1 445 400 | 6.16 | 251 100 | 5.40 |
| S11 - 2 | 790 | 2.90 | 160 | 2.20 |
| S12 | 1 730 | 3.24 | 10 | 1.00 |
| | | | | |
| | F = 5,31 | | P < 0,031 | |

# TABLE 16

## COLIFORM COUNTS BEFORE AND AFTER SANITATION WITH 50 % PEROXIDE SOLUTION (SERIES A)

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | 7 600 | 3.89 | 100 | 2.00 |
| S6 - 1 | 10 | 1.00 | 10 | 1.00 |
| S6 - 2 | 1 000 | 3.00 | 10 | 1.00 |
| S7 - 1 | 12 000 | 4.08 | 300 | 2.48 |
| S7 - 2 | 14 100 | 4.15 | 100 | 2.00 |
| S8 - 1 | 500 | 2.75 | 200 | 2.30 |
| S8 - 2 | 2 000 | 3.30 | 100 | 2.00 |
| S9 | 27 000 | 4.43 | 1 000 | 3.00 |
| S10 | 190 500 | 5.28 | 1 000 | 3.00 |
| | | | | |
| | F = 1,87 | | P < 0,19 | |

# TABLE 17

## COLIFORM COUNTS BEFORE AND AFTER SANITATION WITH 50 % $P_{EROXIDE\ SOLUTION}$ (SERIES B)

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | 500 | 2.69 | 10 | 1.00 |
| S6 - 1 | 10 | 1.00 | 10 | 1.00 |
| S6 - 2 | 10 | 1.00 | 10 | 1.00 |
| S7 - 1 | 12 000 | 4.08 | 300 | 2.48 |
| S7 - 2 | 12 000 | 4.08 | 10 | 1.00 |
| S8 - 1 | 700 | 2.85 | 125 | 2.10 |
| S8 - 2 | 600 | 2.78 | 200 | 2.30 |
| S9 | 7 070 | 3.85 | 6 030 | 3.78 |
| S10 | 158 490 | 5.20 | 1 000 | 3.00 |
| | | | | |
| | F = 1.40 | | P < 0,24 | |

# TABLE 18

## MOULD COUNTS BEFORE AND AFTER SANITATION WITH 50 % PEROXIDE SOLUTION

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | - | - | - | - |
| S6 - 1 | 24 000 | 4.39 | 10 | 1.00 |
| S6 - 2 | 1 000 | 3.00 | 10 | 1.00 |
| S7 - 1 | 125 800 | 5.10 | 4 000 | 3.60 |
| S7 - 2 | 125 800 | 5.10 | 18 500 | 4.26 |
| S8 - 1 | 190 500 | 5.28 | 12 000 | 4.08 |
| S8 - 2 | 190 500 | 5.28 | 24 000 | 4.39 |
| S9 | 34 600 | 4.54 | 10 | 1.00 |
| S10 | 30 000 | 4.48 | 62 000 | 4.79 |
| S11 - 1 | 300 | 2.50 | 130 | 2.11 |
| S11 - 2 | 70 | 1.85 | 10 | 1.00 |
| S12 | 60 | 1.78 | 10 | 1.00 |
| | | | | |
| | F = 3,75 | | P < 0,06 | |

# TABLE 19

## YEAST COUNTS BEFORE AND AFTER SANITATION WITH 50 % PEROXIDE SOLUTION

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | - | - | - | - |
| S6 - 1 | 15 000 | 4.18 | 13 000 | 4.11 |
| S6 - 2 | 12 000 | 4.08 | 10 | 1.00 |
| S7 - 1 | 758 000 | 5.88 | 44 000 | 4.64 |
| S7 - 2 | 14 000 | 4.15 | 10 | 1.00 |
| S8 - 1 | 102 300 | 5.01 | 8 000 | 3.90 |
| S8 - 2 | 562 300 | 5.75 | 190 500 | 5.28 |
| S9 | 616 500 | 5.79 | 17 000 | 4.23 |
| S10 | 891 000 | 5.95 | 363 000 | 5.56 |
| S11 - 1 | 10 | 1.00 | 10 | 1.00 |
| S11 - 2 | 10 | 1.00 | 10 | 1.00 |
| S12 | 10 | 1.00 | 10 | 1.00 |
| | | | | |
| | F = 3,52 | | P < 0,07 | |

# TABLE 20

## FAECAL STREPTOCOCCI COUNTS BEFORE AND AFTER SANITATION WITH 50 % PEROXIDE SOLUTION

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | 7 500 | 3.88 | 500 | 2.69 |
| S6 - 1 | 1 800 | 3.26 | 800 | 2.90 |
| S6 - 2 | 199 500 | 5.30 | 900 | 2.95 |
| S7 - 1 | 38 000 | 4.58 | 2 300 | 3.36 |
| S7 - 2 | 56 000 | 4.75 | 2 700 | 3.43 |
| S8 - 1 | 76 000 | 4.88 | 2 000 | 3.30 |
| S8 - 2 | 63 000 | 4.80 | 1 000 | 3.00 |
| S9 | 75 800 | 4.88 | 1 000 | 3.00 |
| S10 | 56 000 | 4.75 | 1 000 | 3.00 |
| | | | | |
| | F = 10,57 | | P < 0,005 | |

# TABLE 21

MESOPHILIC BACTERIA COUNTS BEFORE AND AFTER SANITATION WITH $SO_2$ LEVELS (250ppm)

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | 16 900 | 4.23 | 1 990 | 3.30 |
| S6 - 1 | 1 000 | 3.00 | 10 | 1.00 |
| S6 - 2 | 616 500 | 5.79 | 10 | 1.00 |
| S7 - 1 | 758 500 | 5.88 | 380 100 | 5.58 |
| S7 - 2 | 562 300 | 5.75 | 141 200 | 5.15 |
| S8 - 1 | 380 100 | 5.58 | 251 100 | 5.40 |
| S8 - 2 | 758 500 | 5.88 | 125 100 | 5.40 |
| S9 | 134 800 | 5.13 | 125 800 | 5.10 |
| S10 | 758 500 | 5.88 | 151 300 | 5.18 |
| S11 - 1 | 790 | 2.90 | 10 | 1.00 |
| S11 - 2 | 170 | 2.23 | 10 | 1.00 |
| S12 | 520 | 2.72 | 10 | 1.00 |
| | | | | |
| | F = 5,08 | | P < 0,034 | |

# TABLE 22

## COLIFORM COUNTS BEFORE AND AFTER SANITATION WITH HIGH $SO_2$ LEVELS (250 ppm)

| SAMPLING POINTS | BEFORE SANITATION | | AFTER SANITATION | |
|---|---|---|---|---|
| | TOTAL COUNTS | LOG. NO. | TOTAL COUNTS | LOG. NO. |
| S4 | 12 000 | 4.08 | 2 000 | 3.30 |
| S6 - 1 | 10 | 1.00 | 10 | 1.00 |
| S6 - 2 | 10 | 1.00 | 10 | 1.00 |
| S7 - 1 | 12 000 | 4.08 | 10 | 1.00 |
| S7 - 2 | 10 | 1.00 | 10 | 1.00 |
| S8 - 1 | 800 | 2.90 | 200 | 2.30 |
| S8 - 2 | 10 | 1.00 | 10 | 1.00 |
| S9 | 3 900 | 3.59 | 800 | 2.90 |
| S10 | 24 500 | 4.39 | 1 800 | 3.26 |
| | | | | |
| | F = 3,50 | | P < 0,08 | |

# TABLE 23

MESOPHILIC COUNT
DETERMINATION OF OPTIMUM INTERVALS FOR SANITATION
PERIOD 26 JUNE TO 4 JULY 1992

| SAMPLING POINTS | BEFORE SANITATION | 2 DAYS / AFTER SANITATION | 6 DAYS / AFTER SANITATION |
|---|---|---|---|
| S4 | 33 000 | 21 000 | 11 000 |
| S6 - 1 | 500 000 | 500 000 | 156 000 |
| S6 - 2 | 82 000 | 500 000 | < 1 000 |
| S7 - 1 | > 500 000 | 143 000 | > 500 000 |
| S7 - 2 | > 500 000 | 84 000 | > 500 000 |
| S8 - 1 | > 500 000 | 101 000 | > 500 000 |
| S8 - 2 | > 500 000 | 125 000 | > 500 000 |
| S9 | > 500 000 | 125 000 | > 500 000 |
| S10 | > 500 000 | 134 000 | > 500 000 |
| X | 401 667 | 191 111 | 352 000 |
| SD | 195 508 | 178 732 | 226 197 |

# TABLE 24

## COLIFORM COUNTS
## DETERMINATION OF OPTIMUM INTERVALS FOR SANITATION
## PERIOD 26 JUNE TO 4 JULY 1992

| SAMPLING POINTS | BEFORE SANITATION | 2 DAYS / AFTER SANITATION | 6 DAYS / AFTER SANITATION |
|---|---|---|---|
| S4 | 200 | < 100 | < 100 |
| S6 - 1 | 4 000 | < 100 | < 100 |
| S6 - 2 | 4 000 | < 100 | < 100 |
| S7 - 1 | 100 | < 100 | < 100 |
| S7 - 2 | 500 | 800 | < 100 |
| S8 - 1 | 5 100 | 600 | < 100 |
| S8 - 2 | 4 300 | 900 | < 100 |
| S9 | > 50 000 | 3 000 | 400 |
| S10 | > 50 000 | 2 100 | 1 600 |

| | | | |
|---|---|---|---|
| X | 13 133 | 867 | 300 |
| SD | 20 989 | 1 031 | 497 |

# TABLE 25

## COMPARISON BETWEEN MALTHUS AND THE STANDARD PLATE COUNT METHOD AFRICAN PRODUCTS, BELLVILLE

| SAMPLE | TEST TYPE | MALTHUS | | STANDARD PLATE METHOD | |
|---|---|---|---|---|---|
| | | COUNT cfu/g | DETECTION TIME (hrs) | COUNT (cfu/g) | INCUBATION TEMP/PERIOD |
| S4 | TMA/TPC | 27 000 | 10,7 hrs | 12 000 | 32 C/48 hrs |
| | Coliforms | < 100 | negative | < 100 | 37 C/48 hrs |
| | Moulds/Yeasts | < 100 | negative | < 1 000 | 32 C/48 hrs |
| S6 - 1 | TMA/TPC | no growth | negative | < 1 000 | 32 C/48 hrs |
| | Coliforms | no growth | negative | < 100 | 37 C/24 hrs |
| | Moulds/Yeasts | no growth | negative | < 1 000 | 32 C/48 hrs |
| S6 - 2 | TMA/TPC | no growth | negative | < 1 000 | 32 C/48 hrs |
| | Coliforms | no growth | negative | < 100 | 37 C/24 hrs |
| | Moulds/Yeasts | no growth | 17 hrs | < 1 000 | 32 C/48 hrs |
| S7 - 1 | TMA/TPC | 120 000 | 8,3 hrs | > 500 000 | 32 C/48 hrs |
| | Coliforms | no growth | 8,0 hrs | < 100 | 37 C/24 hrs |
| | Moulds/Yeasts | no growth | negative | 1 000 | 32 C/48 hrs |
| S7 - 2 | TMA/TPC | 230 000 | 8,2 hrs | > 500 000 | 32 C/48 hrs |
| | Coliforms | no growth | negative | < 100 | 37 C/24 hrs |
| | Moulds/Yeasts | no growth | 8,7 hrs | 1 000 | 32 C/48 hrs |
| S8 - 1 | TMA/TPC | 2,0 x 10 | 4,9 hrs | > 500 000 | 32 C/48 hrs |
| | Coliforms | 2 500 | 5,6 hrs | < 100 | 37 C/24 hrs |
| | Moulds/Yeasts | < 100 | 7,0 hrs | 1 000 | 32 C/48 hrs |
| S8 - 2 | TMA/TPC | 910 000 | 7,1 hrs | > 500 000 | 32 C/48 hrs |
| | Coliforms | < 100 | 8,3 hrs | < 100 | 37 C/24 hrs |
| | Moulds/Yeasts | no growth | no growth | <1 000 | 32 C/48 hrs |
| S9 | TMA/TPC | 740 000 | 7.4 hrs | > 500 000 | 32 C/48 hrs |
| | Coliforms | < 100 | 9,4 hrs | 1 600 | 37 C/24 hrs |
| | Moulds/Yeasts | < 100 | 9,4 hrs | 2 000 | 32 C/48 hrs |
| S10 | TMA/TPC | 500 000 | 6,3 hrs | > 500 000 | 32 C/48 hrs |
| | Coliforms | no growth | negative | 1 600 | 37 C/24 hrs |
| | Moulds/Yeasts | < 100 | 7,8 hrs | 2 000 | 32 C/48 hrs |

**CODES:**

**TMA** - TOTAL MICROBIAL ACTIVITY

**TPC** - TOTAL PLATE COUNT

**cfu/g** - colony forming untis per gram