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ABBREVIATIONS

cfu/ml	:	colony forming unit per millilitre
CO ₂	:	Carbon dioxide
DFA	:	Discriminant Function Analysis
EHMF	:	2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone
FID	:	Flame ionisation detector
FPD	:	Freeze point depression
GC	:	Gas chromatography
HDF	:	4-hydroxy-2,5-dimethyl-3(2H)-furanone
MS	:	Mass spectrometry
PCA	:	Principle Component Analysis
QF	:	Quality factor
SPC	:	Standard plate count
SCC	:	Somatic cell count
UHT	:	Ultra high temperature
VOC	:	Volatile organic compounds

ABSTRACT

There are many ways for milk and dairy products to develop flavour defects. Sensory evaluation, has been the traditional approach to characterize off flavours. The need for odour sensing devices becomes greater when volatile and semi-volatile organic compounds are present in the product in parts per billion or even in the parts per trillion concentration range that cause off flavours. Today, sophisticated, sensitive instrumental tests such as electronic nose technology coupled with gas chromatography are capable of detecting, identifying and quantifying the specific chemical agents responsible for off flavours.

This study focused on the use of the electronic nose as a novel technology for the detection and monitoring of milk quality by testing the effects of heat treatment at 63°C and shelf life. Microbiological testing, sensory evaluation and gas chromatographic analysis were carried out together with aroma profiling using the electronic nose to determine milk quality.

In testing the effects of heat treatment the electronic nose was able to detect changes in the volatile chemistry of the milk by heating at 63°C for 9 min. Sensory evaluation and gas chromatography was able to detect nonanal and hexanal as compounds present in the sample at this time. In assessing the quality of milk by shelf life testing, using the electronic nose as an assessment tool, we were able to detect changes in the aroma profile as early as day 7. Sensory evaluation and gas chromatography was able to detect common odourants hexanal and diacetyl. However as the day intervals increased, gas chromatography was unable to detect predominant odour descriptors identified by sensory evaluation.

The results of this study show that the electronic nose is a technology that can be effectively used by the dairy industry for measuring the effect of pasteurization temperatures and time, and can indicate risks for milk spoilage. This technology is rapid, sensitive, economical and easy to use. It could also be used by small scale entrepreneurs as well as large dairy concerns.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

Quality, in its most general sense, is the ability to meet specific customer requirements. In production and food quality control protection is of utmost importance, that is, ensurance of the health and life of consumers by taking appropriate action at every stage of production and food distribution. For the consumer, in turn, sensory quality is equally important and often decisive in the purchase. Among the many sensory quality components such as colour, rheologic properties and packaging, the taste and flavour profile plays a particularly important aspect to the consumer, i.e. the odour and taste sensations received while eating. Thus the presence, contents and composition of volatile substances (viz. flavour and aroma) in food have a substantial influence on its quality. What is more, each product has a characteristic and unique composition of volatile components that make up the aroma. The aroma of most food products consists of complicated mixtures, sometimes consisting of several hundred compounds. An analysis of odour, its identification and quantitative evaluation, can constitute a valuable source of information on the quality of food, which includes both the sensory quality and the consumer's health and safety.

South Africa produces 2, 2-million tons of milk annually, of this 60% is used for fresh milk and fresh-milk products, and the remaining 40% for concentrated products, which includes milk powders and cheeses such as cheddar and gouda. Currently fresh milk is usually quality controlled using chemical and microbiological tests. These tests are retrospective and are based on the evaluation of the nutritional information and the extent of the changes in odour, taste and texture together with the number and type of bacteria present in the milk. There is a need for more accurate determination of shelf life and for the development of rapid methods for monitoring growth of the microorganisms responsible for spoilage, development of specific spoilage indicators and rapid on-line measurements of spoilage. The aim of such methods is to obtain increased accuracy in quality and assurance and more satisfied customers by ensuring that products do not spoil until after their use by date.

The aim of this study was to use electronic nose technology to detect aromas of microbiological spoilage organisms present in milk. This detection process can be used for monitoring of pasteurisation and shelf life of milk. The objectives were to: (i) ascertain the microbiological status and olfactory methods to measure pasteurisation and shelf life of milk; (ii) investigate the predominant volatile compounds present during pasteurisation and shelf life of milk; (iii) investigate electronic nose technology for determining pasteurisation and shelf life of milk; and (iv) to evaluate the accuracy, sensitivity and specificity of the electronic nose technology against other methods investigated.

1.2. TECHNIQUES USED TO MEASURE MILK QUALITY

The quality of milk plays an important role in the production of dairy products, affecting both the yield as well as the quality characteristics of the product. The use of traditional techniques for the determination of milk quality and authenticity of dairy products include microbiological, physico-chemical analysis, liquid and gas chromatographic techniques, rheological techniques and the use of sensory analysis.

1.2.1. Microbiological techniques

Microbiological techniques used to measure milk quality are based on the initial microflora of raw milk, the thermotolerant microflora, the psychrotrophic bacteria and the coliforms present.

1.2.1.1. The initial microflora of raw milk

The numbers and types of micro organisms in milk immediately after production (that is the initial microflora), directly reflects microbial contamination during production, collection and handling. The microflora in the milk when it leaves the farm is influenced significantly by storage temperature and elapsed time after collection. Generally milk is stored at $\leq 4^{\circ}\text{C}$, which helps in delaying bacterial multiplication for at least 24 hours. A useful indicator for monitoring the sanitary conditions present during the production, collection and handling of

raw milk is the 'total' bacterial count or standard plate count (SPC) (Mostert and Jooste, 2002).

1.2.1.2. Thermotolerant microflora

The initial microflora of freshly pasteurised milk usually reflects the Gram positive thermotolerant microflora present in raw milk. Although the raw milk supply is generally considered to be the principle source of thermotolerant species (including the *Bacillus* spp.) that are present in pasteurised milk, an improperly cleaned dairy plant processing system may also contribute large numbers of these organisms. In-plant contamination with these microbes can occur before, during or after the pasteurisation process (Te Giffel *et al.*, 1997). Thermotolerant strains of *Bacillus*, *Microbacterium*, *Micrococcus*, *Enterococcus*, *Streptococcus*, *Arthrobacter*, *Lactobacillus* and *Clostridium* have been isolated from processed milk products (Martin, 1981; Hull *et al.*, 1992; Kikuchi *et al.*, 1996; Raylea *et al.*, 1998). Heat processing characteristics affect the relative proportions of bacterial types that survive pasteurisation (Cromie *et al.*, 1989). Gram negative bacteria generally do not survive pasteurisation (Cousin, 1982), unless total bacterial numbers in the raw milk exceed the thermal destruction capability of the pasteurisation process (Patel and Blackenagel, 1972). Not all thermotolerant strains of bacteria are capable of reproducing in pasteurised milk under conditions of refrigerated storage. Small numbers of bacterial contaminants of this nature are unlikely to cause product spoilage within a typical fluid milk product shelf life. Milk defects that have been associated with *Bacillus* include bitter, yeasty, unclean and rancid off-flavours as well as coagulation of milk proteins. Although commonly present in milk, *B. cereus*, a potential food borne pathogen, may not grow as well at lower refrigeration temperatures as compared to other species.

1.2.1.3. Psychrotrophic bacteria

'Psychrotrophs' are ubiquitous in nature and common contaminants of milk. Although non-conventional psychrotrophic bacteria are heat labile, their heat resistant metabolites or enzymes (lipases and proteinases) can cause major flavour and textural defects in processed dairy products. Psychrotrophic bacteria are able to spoil milk by biochemically altering the

compounds present in milk. Psychrotrophs can cause decomposition of urea, hydrolyzation of starch, reduction of nitrate to nitrite and hydrolysis of proteins and lipids at temperatures as low as sub-zero. During the early stages of growth of psychrotrophs, biochemical changes occur at a low level resulting in a lack of freshness or a stale taste. At the later stages, biochemical transformations gain velocity, and aroma and flavour defects become apparent. Development of these off-flavours and odours are due to proteolysis and/or lipolysis, and both are a major concern to the dairy industry. Off flavours may be described as stale, bitter, fruity, putrid and rancid, and are related to casein degradation, such as coagulation and thickening. These detrimental effects result from the survival of heat stable proteases and lipases, proteolytic damage to the casein, and an increase in low molecular weight nitrogen compounds that act as nutrients for post pasteurization contaminants (Fairbairn and Law, 1986).

1.2.1.4. Coliform bacteria

Incidences of coliforms belonging to the genera *Enterobacter* and *E. coli* in raw milk have receive considerable attention. This is partially due to their association with contamination from faecal origin. It is now well recognised that the presence of coliforms in raw milk is not evident of direct faecal contamination and should not be relied upon to detect inadequate udder cleaning prior to milking. Coliforms can grow rapidly in moist milky residues (biofilms) on milking equipment and then become a major source of contamination of the milk being collected. Coliform counts found regularly in excess of 100/ml are considered by some sanitarians to be evidence of unsatisfactory production practices leading to environmental contamination of the milk. Although coliforms are relatively ubiquitous in the environment, their presence can be useful in assessing ware supplies for dairy production and milk collection activities (Mostert and Jooste, 2002). Currently, coliform tests and several DNA based methods are used to monitor the presence of pathogenic organisms (Mostert and Jooste, 2002).

1.2.1.5. Molecular biological techniques for microbial detection

Molecular methods commonly used to detect microbial pathogens include DNA/RNA probes, polymerase chain reaction (PCR) techniques and amplified fragment length polymorphism (AFPL). The principles and applications of these methods are described in Table 1.1.

Table 1.1: Molecular methods for detecting microbial pathogens

Method	Principle and Application	References
DNA/RNA probes	A probe is a nucleic acid sequence typical of the organism of interest, used to detect homologous DNA or RNA sequences in the target organism. RNA as target sequence has an advantage in having 10 ⁴ copies per cell versus DNA which has only one or two copies per cell. Nucleic acid fragments for testing are prepared using restriction endonucleases.	Wallbanks <i>et al.</i> , (1989) Waites (1997)
Polymerase chain reaction (PCR)	An amplification technique to increase low numbers of DNA molecules to detectable levels (10 ⁶ copies of the target sequence). PCR can increase one molecule of DNA to produce 10 ⁷ identical copies. Advantages: speed (4h), sensitivity (1 cell) and high specificity.	Waites (1997)
Amplified fragment length polymorphism (AFPL)	A method for the genomic typing of micro organisms based on DNA sequence polymorphism. The method is reproducible and highly discriminatory and is used for identification and classification of bacteria, yeasts and fungi. It is an advancement on related techniques such as PFGE, BRENDA, RAPD	Aarts (1999)

1.2.1. 6. Bacterial counts, pasteurization and shelf Life

The shelf life of pasteurised fluid milk is influenced by the number of micro organisms (Table 1.2) and raw milk quality. The microbial count and somatic cell count (SCC) determine the load of heat resistant enzymes in milk. Generally, high levels of psychrotrophic bacteria in raw milk are required to contribute sufficient quantities of heat stable proteases and lipases to cause breakdown of protein and fat after pasteurisation (Barbano *et al.*, 2006). Sanitation, refrigeration and the addition of CO₂ to milk are used to control both total and psychotrophic bacterial counts. When starting with raw milk that has a low bacterial count and in the absence of microbial growth in pasteurised milk, enzymes associated with high SCC will cause protein and fat degradation during refrigerated storage, and produce off-flavours (Barbano *et al.*, 2006). As the ability to kill, remove or control microbial growth in refrigerated pasteurised milk continues to improve, the original milk SCC will be the factor limiting the time of refrigerated storage before development of an off-flavour in milk.

Table 1.2: Criteria for the acceptability of pasteurised milk (Government Gazette, 1997)

Criteria	Limits
Newly pasteurised milk	
Total plate count (30°C for 72 hours)	<1 x 10 ⁴ cfu/ml
Thermophillic count	<1 x 10 ³ cfu/ml
Psychrotrophic count	<1 x 10 ³ cfu/ml
Coliform count	<10 /ml
Phosphatase test	Decolourisation > 30 min
Freeze point depression (FPD)	0.512 °C ≥ 0.53 °C

Bacterial count can be limited by a good veterinary follow-up of the animal and good hygiene practice in milking. Cold storage (4°C) is used to stabilize milk on the farm. It limits the development of the total bacterial count and some pathogens, but increases the growth of psychrotrophs such as *Pseudomonas*. Bacteria, with lipolytic and proteolytic enzymes

contribute to the progressive degradation of milk proteins and lipids not only during storage, but also after heat treatment because these enzymes are heat resistant.

1.2.2. Physico-chemical techniques

The chemical analysis of flavour in milk and milk products is complicated by the heterogeneous nature of milk. Significant levels of proteins, lipids and carbohydrates in milk make it difficult to separate flavour active chemicals based on general properties and volatility.

1.2.2.1. Milk constituents

Milk is one of the most nutritionally complete foods around. It is composed of water (87.6%), lactose (4.8%), fat (3.7%), 3.2% of casein (of which 2.4% is casein and 0.8% is whey protein), minerals and vitamins (0.8%) (Shearer *et al.*, 1992). It is essential in the human diet and represents one of the best natural sources of essential amino acids.

Though its structure appears to be continual and homogeneous, milk is composed of at least five physically and functionally discrete phases, namely whey, fat globules, casein micelles, membrane vesicle and milk cells, which is represented in Fig. 1.

Whey: is the medium in which all compartments are homogeneously dispersed and composed of various organic molecules, proteins and peptides.

Fat: is dispersed in milk as small droplets that are enveloped by a plasma membrane rich in phospholipids and commonly known as the milk fat globule membrane (MFGM).

Casein Micelles: are arranged as large colloidal particles which are spherical particles in the range of 200 nm, the surface of the micelle is not smooth but contains gaps between the tubular sub structures (Dalglish *et al.*, 2004).

Membrane vesicles: two types of vesicles are secreted by cells:

Exosomes: typically 40 -100 nm in diameter, originate from endocytic multivesicular bodies, and are released in an exocytic manner. Although the functions of exosomes remain largely unresolved, they are thought to play immuno-regulatory and antitumoral roles (Fevrier and Raposos, 2004).

Microvesicles: with a diameter in the range of 100 – 1000 nm, originate from the cell membrane and are shed directly into extracellular space, a process that seems to be important for membrane turnover, tumor ganglioside metabolism and vascular regulation (Hugel *et al.*, 2005). The process of exosome secretion and membrane shedding are poorly understood.

Milk cells: Various mammals contain a heterogeneous population of cells, commonly referred to as somatic cells (SC). In most species, the predominant cells are leukocytes, composed of lymphocytes, polymorpho-nuclear neutrophils (PMNs) and macrophages, which serve as important components in the mammary defense system against potential pathogens, mostly bacteria (Paape *et al.*, 2003).

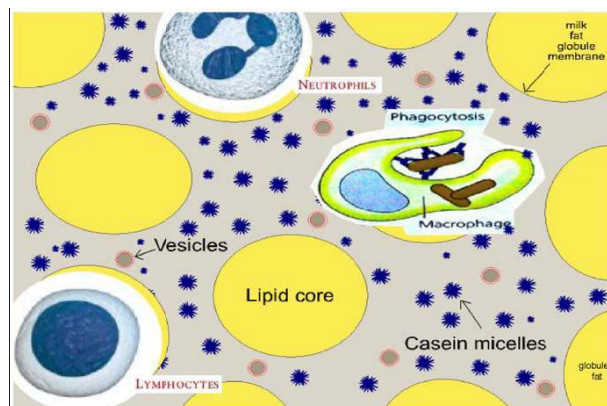


Figure 1.1: Schematic representation of physical phases of milk. The area between the milk particles represents the milk serum (whey), the phase in which all other phases are homogenously dispersed (Silanikove *et al.*, 2006)

The composition of milk varies considerably with the breed of cow, stage of lactation, feed, season of the year, and many other factors. However, some relationships between constituents

are very stable and can be used to indicate whether any tampering with the milk composition has occurred. For example, a milk of normal composition has a specific gravity that varies normally from 1.023 to 1.040 (at 20°C) and a freezing point that varies from -0.518 to -0.534°C. Any alteration, by addition of water for example, can be easily identified because these characteristics of milk will no longer be within the normal range. Milk is a highly perishable product that should be cooled to about 4°C as soon as possible after collection. Extremes of temperature, acidity (pH) or contamination by micro organisms can rapidly decrease its quality (Wattiaux, 2005).

1.2.2.2. Nutritional constituents

The nutritional value of milk is greater than the value of its individual nutrients because of its unique nutritional balance. The amount of water is that balance. This in turn is regulated by the nutrients in milk i.e., carbohydrates, proteins, fats and minerals.

Water

The amount of water in milk reflects that balance. In all animals, water is the nutrient required in the greatest amount and milk does supply a great amount of water—it contains approximately 87% water. The amount of water in milk is regulated by the amount of lactose synthesized by the secretory cells of the mammary gland (Wattiaux, 2005). Freezing point is normally constant owing to the fact that when lactose content decreases, chloride concentration increases.

Carbohydrates

The principal carbohydrate in milk is lactose. Although it is a sugar, lactose is not noticeably sweet to taste. The concentration of lactose in the milk is relatively constant and averages about 4.8%. As opposed to the concentration of fat in milk, lactose concentration is similar in all dairy breeds and cannot be altered easily by feeding practices. The molecules from which lactose is made are found in much lower concentrations in milk: glucose (14 mg/100 g) and galactose (12 mg/100 g). In a significant portion of the human population, the deficiency of

the enzyme lactase in the digestive tract results in the inability to digest lactose (Wattiaux, 2005).

Proteins

Most of the nitrogen in the milk is found in the form of protein. The building blocks of all proteins are the amino acids. The concentration of protein in milk varies from 3.0 to 4.0%. The percentage varies with the breed of the cow and in proportion to the amount of fat in the milk. There is a close relationship between the amount of fat and the amount of protein in milk-the higher the fat, the higher the protein. The protein falls into two major groups: caseins (80%) and whey proteins (20%) (Wattiaux, 2005). A high percentage of proteins and minerals favour the activity of the lactic acid bacteria. Milk with high protein and especially high casein content has high buffering properties. So, any modification aiming at increasing this content such as concentration by micro-filtration can contribute to increasing this property. Conversely, any method making it possible to maintain constant biochemical and especially protein composition such as the addition of milk powder or use of ultra-filtration can contribute to limiting the variations in acidification.

Fat

Normally, fat (or lipid) makes up from 3.5 to 6.0% of milk, varying between breeds of cattle and with feeding practices. A ration too rich in concentrates that do not elicit rumination in the cow may result in milk with a depressed percentage of fat (2.0 to 2.5%). Fat is present in milk in small globules suspended in water. Each globule is surrounded by a layer of phospholipids, which prevents the globules from clumping together by repelling other fat globules and attracting water. As long as this structure is intact, the milk fat remains as an emulsion. The majority of milk fat is in the form of triglycerides formed by the linking of glycerol and fatty acids. The proportions of fatty acids of different lengths determine the melting point of fat and thus the consistency of the butter derived from it. Milk fat contains predominantly short-chain fatty acids (chains of less than eight carbon atoms) (Wattiaux, 2005). Fat content varies greatly among the different breeds. Supplementing the diet with lipid can notably increase the fat percentage and may avoid this risk. Moreover, with an adequate choice of lipid supplementation, milk can present interesting nutritional characteristics such as high

concentrations of conjugated linoleic acid (CLA) or linolenic acid (C18:3) (Chillard *et al.*, 2003).

Minerals and vitamins

Milk is an excellent source of most minerals required for the growth of the young. The digestibility of calcium and phosphorus are unusually high, in part because they are found in association with the casein of the milk. As a result, milk is the best source of calcium for skeletal growth in the young and maintenance of bone integrity in adults. Another mineral of interest in the milk is iron. The low iron concentration in milk cannot meet the needs of the young, but this low level turns out to have a positive aspect because it limits bacterial growth in milk-iron is essential for the growth of many bacteria (Wattiaux, 2005).

Immunoglobulin G (IgG) content

Milk produced during the colostrum period may not be sold. This is usually fed to the calf. The legislation restricting IgG content was established following the negative effects of colostrum in milk: fouling of pasteurisation equipment leading to less effective pasteurisation (Maurice, 1979). Feagan (1979) also reported a decrease in the heat stability of milk and off-flavours in pasteurised milk which was linked to the increase in total soluble protein content. These effects depend on the age of the colostrums.

1.2.3. Gas chromatographic techniques

Gas chromatography with various detection methods has found greatest application in the determination of volatile aromatic substances. Most often the flame ionisation detector (FID) is used, which as a universal detector is allowed to obtain a full aromagram of a given product. In order to obtain the aroma profile connected with a selected class of compounds e.g. sulphur compounds such as alkyl sulphides and thiols Hill and Smith, 2000 and Mestres *et al.*, 1998) and nitrogen compounds such as alkyl pyrazines in fermented products (Sala *et al.*, 2000; Sala *et al.*, 2002) it is also possible to use selective detectors.

Determination of mentioned selected classes of compounds can be a good indicator of the quality, as the influence of these fermentation side-products on the bouquet is negative and very strong. An excellent solution is the application of gas chromatography along with a mass spectrometer, because it permits simultaneous identification of aromatic components of a food product. The possibility of sensoric analysis and semi-quantitative evaluation of components, which really influence the aroma among all the volatile compounds present in a sample, i.e. components sensorically active, is provided by the olfactometric detector, which allows direct olfactory evaluation of substances eluted from a column (Boudhrioua *et al.*, 2003; Farkaš *et al.*, 1997; Ferrari *et al.*, 2004; Ferreira *et al.*, 2001; Ferreira *et al.*, 2002; Frank *et al.*, 2004; Ruth *et al.*, 1995; Rychlik and Grosch, 1996). Quantitative olfactometric methods can be divided into two groups: methods which evaluate the intensity of odour of analytes as a function of time during a single chromatographic analysis (e.g. OSME) and methods based on thresholds of sensoric perceptibility, where the analysis of a series of dilutions of an odour extract is conducted, until reaching the moment when no more odour is detectable in the eluent from the column (e.g. CHARM, AEDA) (Mariaca and Bosset, 1997; Pollien *et al.*, 1997). Most often an olfactometric detector is employed together with other detectors (FID, MS) which permit the identification and exact analysis of components essential for the odour of a product.

The quality and quantity of flavour constituents analysed by gas chromatography can be used as an efficient tool for authenticity testing. Headspace gas chromatography has also proven to be a very accurate technique for the rapid examination of milk powder, to monitor flavour defects caused by autoxidation (Ulberth and Roubicek, 1995). It was found that fresh whole milk powder analysed within 24 hrs contained less than 10 $\mu\text{g kg}^{-1}$ hexanal, whilst those stored at room temperature (20-22°C) had up to 28 $\mu\text{g kg}^{-1}$ of hexanal.

For authentication of dairy products, headspace analysis would be interesting when distinct volatile compounds either produce by bacteria or only in exceptional cases, incorporated from feed could be related to certain regions. Distinct proportions of such compounds could also be indicative. However, bacterial populations continuously change over time, and this might affect the key volatile compounds and their proportions.

In contrast to gas chromatography, liquid chromatography is rarely used in odour analysis, chiefly as a preparative column chromatography as a stage in the preparation of a sample before the chromatographic analysis itself (Bhattacharjee *et al.*, 2003; Ferreira *et al.*, 2002; Guth, 1997, Ledauphin *et al.*, 2004; Mallouchos *et al.*, 2003a; Mallouchos *et al.*, 2003b; Rychlik and Grosch, 1996). Another application of liquid chromatography is the determination of thermally unstable compounds such as amines or aldehydes or compounds relatively sparingly volatile. However, this is usually connected with the necessity of converting determined substances into derivatives permitting detection with the use of detectors popular in liquid chromatography, such as a fluorescent detector (FD) or a photodiode array detector (PAD) which complicates the analysis (Wilkes *et al.*, 2000).

1.2.4. Rheological techniques

The rheological characterisation of dairy products is important as a means of determining body and texture for quality, and to identify as a function of composition, processing techniques and storage conditions.

Most of the research papers on milk showed the influence of three important variables of food processing on its rheological behaviour which include thermal treatment, milk composition and temperature storage. Gryzowska and Tuszynsky (1973) reported that temperature less than 50°C had no effect on the viscosity of skim milk, whilst temperatures above 60°C caused an increase in the viscosity, even when applied for a short time. These results were in partial agreement with those of Jeurink and De Kruif (1993) who found that the viscosity of skim milk increased after heat treatment at temperatures above 70°C. Although the importance of the techniques used is unquestionable, these methods are hardly possible to implement for practical use when samples need to be analysed on-line or at-time in the food industry. For practical reasons, the quality criteria of milk should be easily measurable. Simple and rapid methods are needed for quality control and for screening many samples in a research and development situation.

1.2.5. Sensory techniques

Sensorial analysis provides unique information about the degree of acceptance of a food and is widely utilised for the determination of overall quality (Di Natale, 1998). Aspects concerning taste and odour are evaluated by panels of specially trained people. Acceptance and preference are greatly dependent on how foods appeal to all senses. Therefore the dairy industry depends on analysts who have developed expertise in sensory evaluation for examination of representative samples in the receiving of raw materials, during product development, in processing, in storage and in distribution. Expert sensory analysts are capable of recognising both desirable and undesirable characteristics and can relate what their senses perceive to causative factors.

Sensorial analysis is an experimental method of food analysis which makes use of human senses as instruments. In this frame, the aspects concerning taste and odour are evaluated by panels of specially trained people. Sensorial analysis provides unique information about the degree of acceptance of a food and it is also widely utilised for the determination of overall quality. Problems affecting sensorial analysis include the standardisation of measurements, the correctness of training, of stability and reproducibility of the evaluation, as the most important ones. They affect the measurements which are also scarcely comparable between different panels. A key role in the technique is played by the translation of the olfactive stimuli into a numeric value utilised to represent the output of the sensorial analysis. It has to be noted that the communication of olfactive experiences, contrary to those related to sight or hearing, is quite vague and rather subjective.

Nevertheless, the importance of the panels is growing along with the necessity of improving the reliability of their results, since legislation in various countries is also giving legal value to sensorial analysis. In sensorial analysis, three senses are mainly involved: sight, olfaction and taste. While the sense of sight has been widely studied and many artificial systems are able to mimic some functions of the vision (e.g. the recognition of colours and forms). Taste and olfaction, which are the chemical interfaces of the living being, are not so well known and many aspects of their working principles are still unclear. For these intrinsic difficulties in

understanding the nature of the sense, only sporadic research on the possibility of fabricating artificial olfactory systems was performed for many years.

1.2.5.1. Odour measurements techniques

Human perception of odour is descriptive. Scales of human perception are subjective, yet carefully designed questions can produce surprisingly precise answers. In evaluating a particular odorous compound or mixture, typical questions to which a psychophysical scale can be assigned are the following: How potent is the odour? Are there different notes identifiable? How pleasant or unpleasant is the odour? These are the usual unconscious assessments that one makes in everyday life for food, drink and other odours of the immediate environment.

There are two main concepts used in the measurement of odour, detect ability and intensity. Olfactory psychophysics specifies selectivity and discrimination in terms of either the absolute or differential threshold. Methods commonly used in the food and beverage industry include:

Triangular test

In this test the panellist is presented with six flasks for each stimulus concentration, or stimulus class depending on what is being measured. The stimulus to be measured is presented in a carefully balanced arrangement or randomised arrangement of all the groups of stimuli. There are six possible arrangements of the flasks. Usually the task faced by the panellist is to pick out the sample that is different from the others after being told that two of the samples are the same. This method may be used with both quantitative and qualitative differences (Persaud and Travers, 1997).

Paired comparison test

In this test the panellist is offered two samples for comparison based on a specified property. The panellist indicates whether there is a difference or not. The panellist will be informed that the two samples may differ or not. The test is repeated many times and the presentation changed at will. One out of the two is correct.

Duo-Trio test

In this test three samples are offered, two identical and one different. One sample is the standard and this is presented first, this is followed by the two others, one of which is the same as the standard (first). The panellist must indicate which sample is the same as the standard. The standard sample can remain the same or be changed.

Descriptive analysis

At the heart of sensory analysis lies the complex field of language or descriptive analysis. Whilst at first it seems easy to describe a food sample, individuals familiar with a specific food may use many terms to describe the fine nuances. Some of these are idiosyncratic, but many are standard terms easily understood. Since long back researchers have tried to create a standardised list of terms by which to summarise and classify sensory perceptions.

Panellists process a constant amount of information when they describe products. When asked to describe a wide range of qualitatively different products during a single test, panellists overlook the nuances and focus on the more general terms that differentiate this broad array of qualitatively different stimuli. For products that vary only slightly from each other panellist use the more rarely used terms, focusing on minor differences and highlighting them with these terms.

1.2.5.2. Chemistry behind sensory analysis

Consumer acceptance of fluid milk is strongly determined by its sensory characteristics, such as flavour as well as its nutritional value. The development of off-flavours in milk as a result of lipolysis and proteolysis can reduce shelf life and the quality of pasteurized fluid milk (Ma *et al.*, 2000). Several factors may produce off-flavours and/or odours in milk, these include feed and weed flavours, rancid flavours due to the presence of free-fatty acids, malty high acid flavours and odours due to the breakdown of the milk fat component by proteolytic and lipolytic enzymes present in raw milk, oxidized flavours due to exposure to light or oxidizing agents such as rust, copper or chlorine and foreign flavours due to medication, fumigants,

insecticides. Therefore milk processing is designed to provide the consumer with a wholesome, nutritious and quality product, which will be safe for consumption.

1.2.5.3. Lipolytic compounds

Lipolysis corresponds to the hydrolysis (enzymatic) of fat material. The hydrolytic release of free fatty acids from triglycerides causes a flavour defect in fluid milk described as 'rancid' (Shipe *et al.*, 1978). This event known as lipolysis, occurs as milk lipase catalyses the release of free fatty acids from triglycerides. A lipase is defined as an enzyme that is able to hydrolyse the esters from emulsified acyl glycerols at an oil-water interface. Heat stable lipases are of particular concern as they affect products stored for long periods. Cow's milk contains a native lipase (Castberg, 1992) that is characterised as a lipoprotein lipase. Other lipolytic enzymes that may play a role in lipolysis in milk are: lipase from somatic cell origin, bacteria, lipases and other miscellaneous esterases (Azzara and Dimick, 1985). In fresh high quality milk, lipolysis is caused mainly by the activity of milk lipoprotein lipase (Olivecrona *et al.*, 1992). In cow's milk, the majority of lipase is associated with the casein micelles in the skim milk fraction. Ma *et al.*, (2000) reported that high SCC pasteurised milk stored at 5°C developed rancid off-flavours between 14 and 21 days after processing, whereas, low SCC milk did not. They hypothesised that somatic cells may have contributed heat stable lipases that survived pasteurisation.

1.2.5.4. Proteolytic compounds

As far as dairy products are concerned, proteolysis leads to gelation, unclean and bitter off - flavours, but the level of population that causes these defects varies from one species to another. Proteolysis of milk during storage can result in the accumulation of small particles that lead to the development of bitterness (Ma *et al.*, 2000) and astringency (Harwalkar *et al.*, 1993). Plasmin activity increases with increasing milk SCC. The major substrate for proteolysis in milk is casein. Plasmin is one major protease in milk acting mainly on α_{s1} -, α_{s2} - and β -casein (Verdi *et al.*, 1987). In normal milk, plasmin is the enzyme responsible for most of the proteolytic activity (de Rham and Andrews, 1982).

It is well established that heat-treated milk is more readily subject to proteolysis than raw milk, probably owing to the existence of a natural proteinase inhibitors in raw milk (Ozer, 1999). Of the bacteria that can secrete exocellular proteinases, the genus *Pseudomonas* is highly proteolytic and, therefore, most of the studies on thermal stability have concerned the pseudomonads. Most proteinases of *Pseudomonas* can survive heat treatment at 149°C for 10s. *Pseudomonas aeruginosa* is able to produce exocellular proteinases. These can remain active at 2°C for up to one month and can hydrolyse casein at this temperature. Most of the proteinases show optimum activity at pH 6.5 - 8.0.

1.2.5.5. Volatile compounds

Knowledge of the chemical compounds responsible for desirable flavour in milk and milk products is increasing but still incomplete. The flavour of high quality milk is delicate, with no distinct taste. It is both slightly sweet and slightly salty because of the presence of lactose and milk salts respectively. The aroma compounds arise from very low concentrations of a number of volatile compounds, most of which are present in sub threshold concentrations (Badings and Neeter, 1980). Dimethyl sulphide has been identified as an important contributor to milk flavour, compounds making a moderate contribution to flavour include diacetyl, 2-methyl-1-butanol, 4-cis-heptenal, 3-butenyl-isothiocyanate and 2-trans-nonenal. A variety of aldehydes ketones, lactones, nitrogen and sulphur containing compounds, as well as ethyl-butyrate, contribute slightly to flavour. Table 1.3 shows odour compounds that are commonly found in dairy products. A number of these compounds may be transferred from the animals feed to the milk, while others result from the minor conversions of milk constituents by chemical, microbial and enzymatic reactions. If the quantity of these aroma compounds exceeds a certain level, the flavour balance may be upset, and these off-flavours will result.

Only a small fraction of volatile present in milk is odour active. Of the odour active compounds found in various raw milk samples, seven common odour active compounds were identified. These include dimethylsulphone, ethyl butanoate, ethyl hexanoate, heptanol,

Table1.3: Odour descriptors of compounds found in dairy products (Fredrich and Acree, 1998).

Compound	Odour descriptor
Ethyl butanoate	Ethereal fruity, sweet, banana
Ethyl hexanoate	Fruity, pineapple, apple
Heptanal	Green, sweet
Indole	Faecal, putrid, musty, floral in high dilution
Nonanal	Sweet, floral
1-octen-3-ol	Mushroom like
Dimethylsulphone	Sulphurous
2-heptanone	Fruity, spicy, cinnamon
2-undecanone	Floral, rose like
Hexanal	Milky, creamy
2-nonanone	Grassy herbal, green fruity
Benzothiazole	Quinoline, rubbery
δ -decalactone	Coconut
Diacetyl	Buttery, creamy
HDF	Cotton candy
EHMF	Sweet, maple, caramel
1-octen-3-one	Mushroom like
Methional	Boiled potato like
3-methylbutanal	Green, malty
Butyric acid	Sharp, cheesy, sweaty, sour, putrid
Isovaleric acid	Rancid, cheese, sweaty, faecal, putrid
Acetic acid	Vinegar like
Propionic acid	Sour milk like
Acetaldehyde	Ethereal, pungent
Dimethyl sulphide	Intense, boiled cabbage, sulphurous

indole, nonanal and 1-octen-3-ol. Heating milk changes the aroma profile of it (Brudzewski *et al.*, 2004). The volatile chemistry of fresh milk is complex due to the heterogeneous nature of the system (Fredrich and Acree, 1998), and not all analytical measurable volatiles contribute to the aroma of milk. Key odourants found in dairy products are identified by gas chromatography and olfactory methods. Using gas chromatography-olfactometry, Moio *et al.* (1994) listed nine odour active compounds in pasteurised milk. These compounds included heptanal, indole, nonanal, 1-octen-3-ol, dimethylsulphone, hexanal, 2-nonanone, benzothiazole and δ -decalactone. Dimethylsulphone provided the most intense odour followed by hexanal.

These compounds can be subdivided into different groups of compound. They are neutral compounds (alcohols, aldehydes, ketones, esters, lactones, furans), alkaline compounds (nitrogen-containing compounds, pyrazines, sulphur compounds, terpenes) and acidic compounds (phenolic compounds, free fatty acids).

Neutral compounds

a. Alcohols

Many metabolic pathways are involved in the biosynthesis of the alcohols that are encountered in milk products: lactose metabolism, methyl ketone reduction, amino acid metabolism as well as degradation of linoleic and linolenic acids (Molimard and Spinnler, 1996). The most common alcohol, identified as a key odorant is, 1-octen-3-ol. Linoleic and linolenic acids are precursors of eight carbon aroma compounds such as this substance. Of the secondary alcohols, 2-heptanol has been identified as a key odorant. Secondary alcohols are formed by enzymatic reduction of the corresponding methyl ketones, which themselves are derived from fatty acids by β -oxidation or from β -ketoacids (Molimard and Spinnler, 1996).

b. Aldehyde

Aldehydes originate from amino acids either by transamination, leading to an intermediate imide that can be decarboxylated, or by Strecker degradation. This reaction is simple and can occur without enzymatic catalysis during ripening (Keeney and Day, 1957). Branched-chain aldehydes probably originate from amino acid degradation via enzymatic processes (Moio *et*

al., 1993) as well as non-enzymatic reactions (Strecker degradation) (Griffith and Hammond, 1989). Within this group, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal are formed from valine, isoleucine and leucine, respectively. Straight-chain aldehydes, such as n-butanal, n-pentanal, n-hexanal and n-nonanal are also quite common. They are characterised by green-grass and herbaceous aromas. Such compounds may also result from β -oxidation of unsaturated fatty acids (Collomb and Spahni, 1996). Straight-chain aldehydes are very unpleasant when their concentrations exceed certain thresholds. Within this group, the most commonly found odorant seems to be nonanal.

c. Ketones

Ketones are common constituents of most dairy products. Due to their typical odours and their low perception thresholds, ketones, and especially methyl ketones, are primarily known for their contribution to the aroma. Fruity, floral and musty notes are associated with various methyl ketones such as 2-octanone, 2-nonanone, 2-decanone and 2-undecanone. From a sensory point of view, another important odorant is 1-octen-3-one which has been commonly associated with mushroom odours. One of the most important diketones is 2,3-butanedione (diacetyl). This component is obtained from pyruvate stemming from lactose and citrate metabolism. The production of 2,3-butanedione is mainly due to the activity of lactic acid bacteria, especially *Lactococcus lactis* ssp. *Lactis biovar diacetylactis* and *Leuconostoc* spp. (Welsh *et al.*, 1989). This volatile compound is appreciated for its buttery and nut-like notes.

d. Esters

Esters are common volatiles. Esterification reactions occur between short- to medium-chain fatty acids and primary and secondary alcohols derived from lactose fermentation or from amino acid catabolism (Bosset and Liardon, 1984). Most esters encountered are described as having sweet, fruity and floral notes. Phenethyl acetate is one of the most important aromatic esters and this compound imparts a floral odour. (Kubickova and Grosch, 1997).

e. Lactones

Lactones are cyclic esters in which the acid and the alcohol functions belong to the same molecule. In cheese, it is suggested that they are generated by hydrolysis of hydroxy-fatty acid

triglycerides (normal constituents of milk fat), followed by lactonisation (Karahadian *et al.*, 1985a; b). Lactones, in fact, are generally associated with pronounced peach, apricot and coconut odour qualities. One of the most common and important lactones identified in cheese is δ -decalactone. This compound is a key odorant of Camembert and Emmental cheese varieties. The presence of different γ - and δ -lactones might be of importance in the final aroma due to their fruity notes and their low perception thresholds.

f. Furans

Chiral 3(2H)- and 2(5H)-furanones are known to be powerful flavour compounds. Another important furan is 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone (homofuraneol). This compound contributes has a low odour threshold value and its caramel-like, sweet odour qualities.

Alkaline component

a. Nitrogen-containing compounds

Among nitrogen-containing compounds, indole is a main odorant of water buffalo Mozzarella and has a characteristic musty odour, reminiscent of stables (Moio *et al.*, 1993). This volatile is likely to be a degradation product of tryptophan. Yeasts, *Micrococci* and *Brevibacterium* are capable of cleaving the side chain of tryptophan releasing indole (Parliment *et al.*, 1982; Jollivet *et al.*, 1992). Skatole is another important nitrogen-containing compound which seems to contribute significantly to the aroma profile of Emmental cheese (Preininger and Grosch, 1994; Preininger *et al.*, 1994).

b. Pyrazines

Pyrazines have been claimed as important contributors to flavour, This compound originates from l-valine degradation as found in *Pseudomonas taetrolens* (Gallois, 1984). However, in Camembert cheese, this pyrazine is responsible for a rotten soil and raw potato-like aroma defect (Molimard and Spinnler, 1996).

c. Sulphur compounds

Volatile sulphur-compounds play an important role in the flavour of cheese as reviewed by Molimard and Spinnler (1996). Sulphur compounds essentially originate from methionine degradation and result from cleavage of a bond between carbon and sulphur by a methionine-demethio-lase (Yvon and Rijnen, 2001). These components are described as having strong garlic and very ripe cheese odours. The most common S-compound in cheese is methional (3-methylthiopropional). This volatile, which can be produced by Strecker degradation, has a characteristic boiled potato and acrid pungent odour. Dimethylsulphide (DMS), DMDS and DMTS are also thought to be important contributors to cheese flavour. DMS is a product of the metabolism of propionic acid bacteria and formed from methionine.

d. Terpenes

Terpenes are important compounds. They originate from the plants that constitute the forage mixture of the pastures (Mariaca *et al.*, 1997). They are then transferred to the milk of the grazing animals and ultimately to the cheese.

Acidic compounds

a. Phenolic compounds

Phenolic compounds appear to make a positive contribution to cheese flavour at about threshold concentration but tend towards an unpleasant note as their concentration increases. The sensory quality ranges from sharp, medicinal, sweet, aromatic to smoky, charred, caramel, unpleasant and 'sheep-yard'.

b. Free fatty acids (FFAs)

Fatty acids are important, or even predominant, components of the flavour types. Fatty acids are not only aroma compounds by themselves, but also serve as precursors of methyl ketones, alcohols, lactones and esters. In general, long-chain fatty acids (> 12 carbon atoms) play a minor role in the flavour, given their relatively high perception thresholds. Short and moderate-chain, even numbered fatty acids (C4-C12) have much lower perception thresholds and each has a characteristic note. For example, ethanoic (acetic) acid and propanoic

(propionic) acid have a typical vinegar odour. Butanoic (butyric) acid has a rancid cheese-like odour and plays an important role in the flavour of many cheese types. Hexanoic acid is also listed among the major odorants. From a sensory point of view, nonanoic acid provides a major contribution to the goaty flavour of goat cheese (Le Quére *et al.*, 1996). Of the phenolic acids, phenylacetic acid with its honey-like note, seems to contribute significantly to the aroma profile (Rychlik and Bosset, 2001a; b).

1.2.6. Techniques used in odour measurement

1.2.6.1. Odour sensing devices in the food industry

Of all the human senses, smell has historically been the most difficult to define either objectively or accurately. Unlike sound (decibels) or light (lumen), there is no scale for measuring the intensity or quality of aromas other than by subjective description or comparison to a more familiar one. The natural function of the human sense of smell is to assess the quality of food or the immediate environment. It is easy to distinguish between the fragrance of a perfume and the putrid odour of rotting meat, and our reactions towards these odours vary accordingly. However it is not so easy to describe or explain the difference in perception (Persaud and Travers, 1997).

Odour sensing devices are needed because, the chemical species to be sensed are diverse and the complexity of measurement becomes greater when it is considered that the headspace of foods, beverages or odours in the normal human environment may contain many hundreds of compounds all interacting with the chemical senses to allow perception of an infinite number of odour nuances. Evaluation of the organoleptic qualities of cooked foods, or contaminants or off-odours is major preoccupation of many food product manufacturers and involves panels of humans trained in distinguishing subtle variations in odours (Persaud and Travers, 1997). At times the most highly trained human testers are subjective, and illness and other factors can influence their performance (Giese, 2000).

1.2.6.2. The Electronic Nose

An electronic nose is composed of a chemical sensing system (such as a sensor array) and a pattern recognition system (artificial neural network). The sensing system can be an array of several different sensing elements (chemical sensors), where each element measures a different property of the sensed chemical, or it can be a single sensing device (spectrometer), that produces an array of measurements for each chemical, or it can be a combination (Giese, 2000). The electronic nose sensors are simply a series of sensors that responds to volatile components of headspace above a sample.

Current ‘electronic nose’ technologies could perhaps be most accurately defined as odour sensors, but should not be confused with gas chromatography or sensory analysis. Like the human sensory system, these electronic odour detection systems incorporate sensors (which are conceptually analogous to human olfactory receptors), and a data-processing system (which conceptually simulates the brain) (Mielle, 1996). Just as humans do not need to identify consciously each different constituent of an odour in order to recognize it, ‘electronic noses’ operate by recognizing the pattern of components. Gardner and Bartlett (1993) defined an electronic nose as ‘an instrument which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odours’.

In an analytical system, the main component is the sensor or detector. The choice of sensors is quite large, however can be classified into two broad groups: viz

‘Hot sensors’ – mainly the semiconducting metal oxide gas sensors, and

‘Cold sensors’ – conducting polymers, bulk acoustic wave (BAW) and surface acoustic wave (SAW) radio frequency sensors.

Semiconductors – metal oxide gas sensors

The hot semi-conducting gas sensors are the most widely used because they currently offer the best ratio of drift and lifetime to sensitivity. These sensors have a logarithmic transfer function: the electrical response is related to the log of the intensity of the stimulus. This

creates a dynamic compression problem when using a high value baseline (e.g. in the presence of high levels of water vapour, ethanol or CO₂) or a high concentration of the analyte (Mielle, 1996).

The main problem with semi-conducting metal oxide gas sensors is that they exhibit poor selectivity. The sensors react to all of the stimuli, although to different extents. Because of this poor selectivity, the sensors are used in arrays of 4-32 sensors, each with a slightly different selectivity (Aishma, 1991), to obtain a unique overall pattern- or 'fingerprint' – that corresponds to the overlapping responses of the different sensors to the range of compounds within the sample. As well as giving better selectivity the use of such arrays leads to a certain amount of redundant information, which is very useful if one of the sensors fail.

Conducting organic polymers

Conducting organic polymer sensors are obtained by electro-polymerisation of a thin film of polymer across the gap between gold plated electrodes. The electrical conductance will change according to the molecules adsorbed onto its surface. Many polymers with different functional groups are now available, the main one being polypyrroles.

The main advantage is that they operate at room temperature. However the response time is long (20 –40 sec), which is a draw back for rapid analysis. The inherent drift over time or with changes in temperature is very high (Neaves and Hatfield, 1995).

The main limitations of cold sensors for use in the food industry is their high sensitivity to compounds such as ethanol, CO₂, or humidity (VanGeloven *et al.*, 1991), which masks desired responses to aroma compounds. The use of such sensors is thus very limited in the field of alcoholic or sparkling beverages, as it can be very expensive and time consuming to remove CO₂ or ethanol from the volatile flavour component.

1.2.6.3. AromaScan (A32S)

The Aromascan® detector system is an array of 32 organic conducting polymers. These are based on heterocyclic molecules such as derivatives of polypyrrol and polythiophene. These polymers have unique adsorptive surfaces that interact with adsorbed volatile chemicals based on their shape and size. The polymers display reversible changes in electrical resistance when polar volatiles adsorb and desorb. Each polymer in the array has a range of selectivity to different chemical species. Thus the array exhibits a broadband response to many thousands of chemical species (Persaud *et al.*, 2002). The advantage of the electronic nose sensors include rapid, real-time detection of volatiles, less preparation time, greater safety and lower costs (Giese, 2000). Many studies have been carried out using the electronic nose. In a study carried out by Olafsdottir *et al.* (2000), the electronic nose was used as a rapid technique to monitor changes in the headspace gas above capelin (fish found mainly in Alaska) during storage. Results obtained indicate that the electronic nose can be used as a rapid detection technique to predict the total volatile base value of the raw material stored under different conditions. In using the electronic nose for dairy products, Ampuero and co-workers (2003) found that this new technology can potentially be applied to process control and monitoring, acceptance or rejection of raw material, intermediate and final products, as well as assessment of synergistic effects of individual odourants.

Data acquisition

The software provided for the volatile sensing system (electronic nose) consists of three modules: data acquisition and instrument control, data manipulation for extraction of patterns and pattern recognition.

The acquisition software samples the sensor array resistance at regular intervals, storing the resultant data in the computer. As the resistance of the conducting polymers are inversely proportional to temperature, the temperature of the array is controlled and monitored (typically to $35 \pm 0.1^\circ\text{C}$). The sample temperature and sample humidity are also monitored. Individual sensors on the array may be deactivated or activated as required. The responses of the sensors are shown in real time in a strip chart display on screen, as can be seen in Fig. 1.2. The signal

is expressed as the percentage resistance change of each sensor compared to the initial sensor resistance.

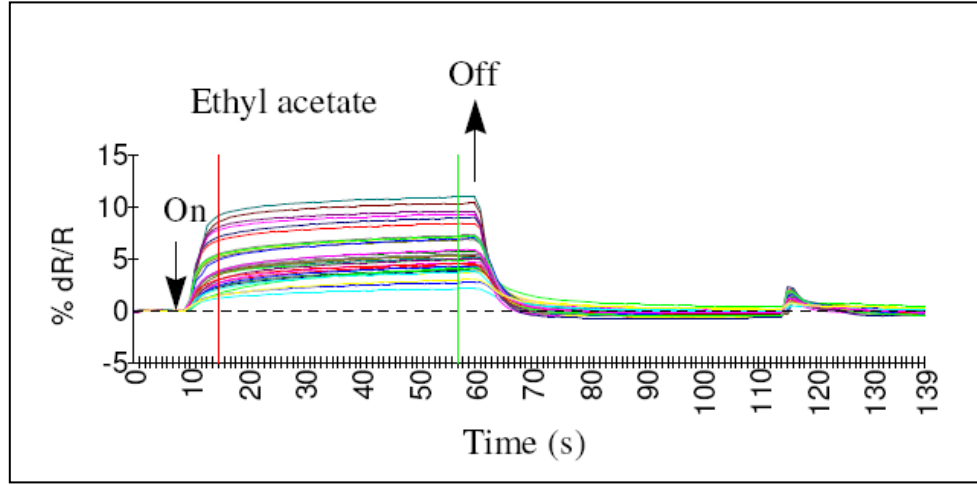


Figure 1.2: Response of a 32 sensor conducting polymer array to ethyl acetate vapour. The percentage change in resistance (dR/R) in response to the vapour is shown (Persaud *et al.*, 2002).

The response to the vapour is then normalised by expressing the fractional change of the individual sensor as a percentage of the fractional changes summed over the whole array. As denoted by the equation below, for an array of n sensors:

$$N_x = 100 \cdot \frac{\Delta r_x / r_x}{\sum_{i=1}^n |\Delta r_i / r_i|}$$

Where, $1 \leq x \leq n$, N_x , is the normalised response of sensor x , Δr_x is the resistance change of the sensor x , r_x is the base (initial) resistance of sensor x , and $\Delta r_i / r_i$ is the fractional change in resistance of the i 'th component of the array. The normalised data then forms a pattern across the sensor array, as shown in Fig. 1.3.

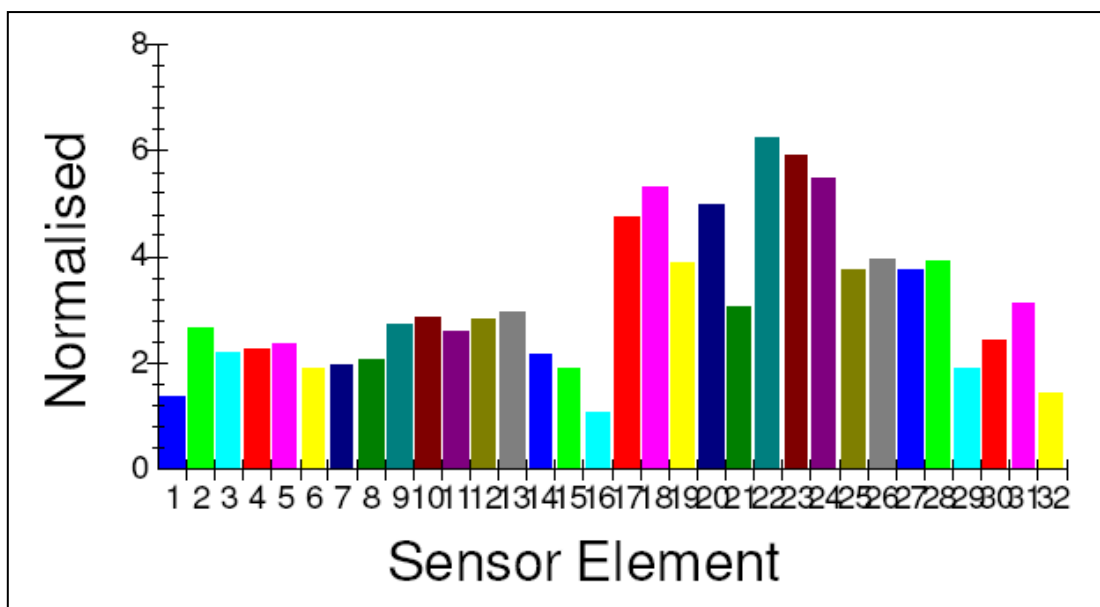


Figure 1.3: Normalised response pattern averaged between the cursors shown in Fig. 1.2.
(Persaud *et al.*, 2002)

Data treatment

Any application will rely heavily on the data processing and pattern recognition software associated with it. The response to a volatile odour of each sensor is proportional to the concentration, and is unique for each type of single or complex odour. With each sensor in an array having a certain response character, an array of sensors with broad but different chemical specificities provide a measurement pattern of broad overlapping selectivity. These response, or signals, are processed to produce a set of descriptors for the input, which can be identified as a ‘fingerprint’ for the odour, and then saved into the database for further manipulation within statistical pattern recognition methods, cluster analysis and artificial neural networks (Persaud *et al.*, 2002).

There exists linear multivariate analysis such as principal component analysis (PCA), discriminant function analysis (DFA), non linear methods such as artificial neural networks (ANN) (Levy and Naidoo, 1999; Hines *et al.*, 1999). A parametric classification is very seldom possible. Usually the inclusion into a given group is determined by the Euclidian

distance or the Mahalanobis distance. The latter takes into account the actual shape of the group whereas the former assumes that the data points belonging to the group are evenly distributed in a sphere around the centre of the group.

a. Principle Component Analysis (PCA)

Principle component analysis (PCA) is a linear combinatorial method which reduces the complexity of the data set, from the initial n-dimensional space (n sensors) to a few dimensions. The inherent structure of the data set is preserved while its resulting variance is maximised. Data points will be scaled along new dimensions, linear combinations of the initial dimensions. The magnitude of the coefficients in the resulting linear combinations, give an indication of the relative importance of the initial dimensions in the data structure. Principle component analysis is performed with no information on the classification of samples. It is based solely on the variance of the data set.

b. Discriminant Function Analysis (DFA)

Discriminant function analysis (DFA) is based on prior data classification. The linear combinations maximise the contribution of those dimensions that generate the largest difference between predetermined groups. With this method, different classifications on the same data set are possible following different properties (e.g. freshness, fruitiness, etc.). Particular care should be taken however to avoid over fitting of errors, i.e., classification based on noise rather than real differences. The resulting DFA classification is highly dependant on the data set used for training.

1.2.6.4. Potentialities of using aromagrams in the valuation of food quality

In production and food quality control, protection is of utmost importance, that is ensuring the health and safety of consumers by taking appropriate action at every stage of production and

food distribution. In order to avoid the sale of contaminated products, expensive inventories are held at the production site whilst samples are tested for microbial contamination, which often may take 2 to 3 days. Since milk has a short shelf life, there is a possibility that these products could be released before microbial results are available. Rapid detection of pathogens, spoilage organisms and other microbial contaminants in dairy products, are critical to ensure the safety of consumers and the quality of foods (Balaban, 2002). For the consumer, in turn, organoleptic quality is equally important and often the deciding factor in most purchases. Thus the presence, contents and composition of volatile substances in food have a substantial influence on its quality. What is unique is that each product has a characteristic composition of volatile components. The aroma of most food products consists of complicated mixtures, sometimes consisting of several hundred compounds. Analysis of odour/s, its identification and quantitative evaluation, can constitute a valuable source of information on the quality of food, which includes both the sensory quality and the consumer's health and safety.

Volatile substances can originate at every production stage from all food components. They are present in raw materials, they come into being intentionally or unintended during the technological process and they are also created during storage of food products. Already thousands of odorous compounds have been detected and still new ones are being identified. Aroma profiles found in food are the result of a huge number of reactions occurring between components of food products. The character of the resulting aroma depends upon a number of factors: availability and structure of the reagents, participation of fat, amino acids and saccharides, reaction conditions (temperature, duration, water activity, pH, oxygen level, etc.). The main sources of volatile substances, also including aromatic ones, are shown in Fig. 1.4.

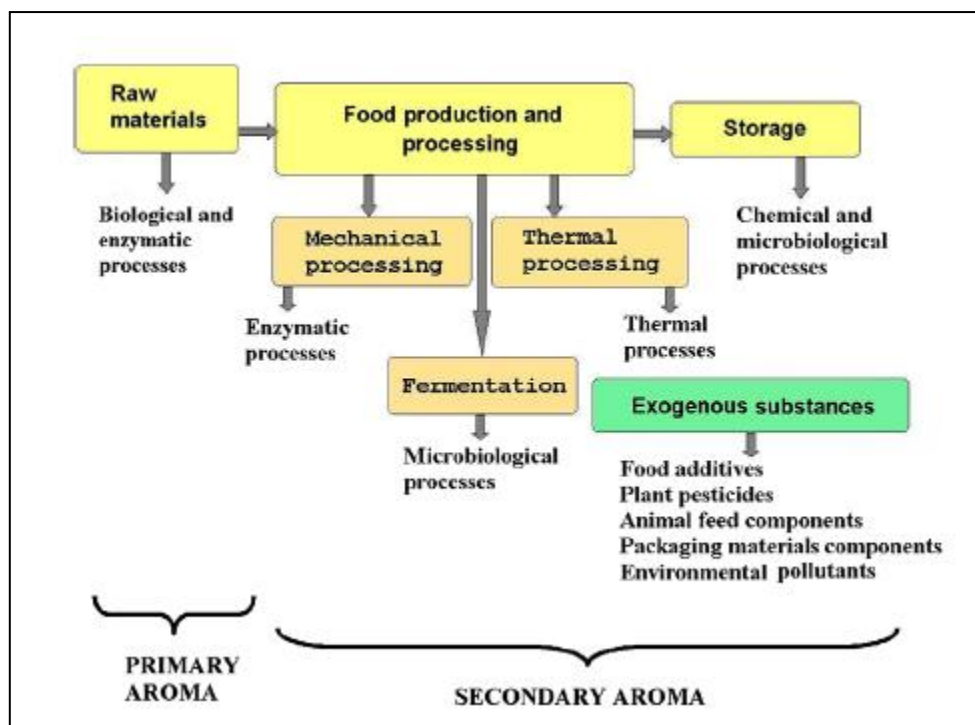


Figure 1.4: Main sources of volatile food components (Plutowska and Wardencki, 2007).

Application of aroma profiles

Numerous volatile compounds originating during production and processing of food may affect the health quality both positively and negatively. The qualitative and quantitative constitution of the originating composition depends strongly on the conditions of conducting the process and it is the result of a huge number of different, often not fully examined, enzymatic, microbiologic and thermal processes taking place in food.

The determination of volatile substances can be thus exploited for the control of technological processes, e.g. to monitor the degree of oxidation of lipids in the production of powdered milk (Ulberth and Roubicek, 1995), in processing of vegetable oils (Stashenko *et al.*, 2000) or production of cooked meats (Brunton *et al.*, 2000). Other example could be the determination of primary and secondary volatile products of saccharides and amino acids conversions. Among these, the reactions of non-enzymatic browning – Maillard reactions – and the degradation of amino acids according to the Strecker mechanism in thermally processed food such as prunes and pumpkin seeds play the main roles (Sabarez *et al.*, 2000; Siegmund and Murkovic, 2004). Monitoring of thermal degradation of sulphur compounds mainly amino

acids is an important indicator of quality in thermally treated meats (Farkaš *et al.*, 1997). Likewise – changes taking place during thermal treatment of milk (Contarini and Povolo, 2002) or fruit and vegetable juices (Jia *et al.*, 1999; Servili *et al.*, 2000) can be monitored using aroma profiles. In turn, determination of changes in the contents of volatile components such as aldehydes, organic acids and their esters, and alcohols in fermented beverages with fermentation performed in different ways and under various conditions can provide information, important for the improvement and optimisation of the process (Apostolopoulou *et al.*, 2005; Bardi *et al.*, 1997; Cortés *et al.*, 2005; Ferreira *et al.*, 1995; Hernández-Gómez *et al.*, 2003; Hernández-Gómez *et al.*, 2005; Madrera *et al.*, 2003; Maicas *et al.*, 1999; Mallouchos *et al.*, 2002; Mallouchos *et al.*, 2003a; Mateo *et al.*, 2001; Nonato *et al.*, 2001; Patel and Shibamoto, 2002; Spranger *et al.*, 2004; Torrea *et al.*, 2003).

An important indicator of quality is volatile substances created during the storage of food. In the simplest case, during storage volatile flavour compounds escape and the gradual decay of flavour occurs. Most often, however, new aromatic substances also come into being, along mechanisms similar to those active in food processing, as the result of chemical or biochemical processes with the participation of microbial enzymes. A group of compounds having a positive effect on the organoleptic quality stands out here, i.e. components of the bouquet originating as the result of maturation of such articles as cheese and wines, but above all components of the bouquet coming into being in spoiled food – overdue, no more fresh and improperly stored. In most cases they are secondary products of lipid oxidation such as aldehydes, ketones, organic acids, hydrocarbons, appearing in a broad range of food products: oils (Cavalli *et al.*, 2004), meat (Andrés *et al.*, 2002), crisps (Sanches-Silva *et al.*, 2005) or milk powder (Fenaille *et al.*, 2003). Particularly disadvantageous, from the point of view of health quality, is the generation of aldehydes (Doleschall *et al.*, 2001). Literature often points to hexanal as a component being an indicator of the degree of oxidation of food products. The relatively high concentration of hexanal and the observed rise in its concentration during storage as well as the low odour threshold indicate its suitability as a marker. Determination of changes in the content of hexanal is usable mainly in the evaluation of the quality of meat products (Andrés *et al.*, 2004; Brunton *et al.*, 2000; Carrasco *et al.*, 2005; Fernando *et al.*, 2003; Morcuende *et al.*, 2003; Nielsen *et al.*, 1997), but for vegetable oils such as olive oil it

has been suggested that rather the determination of nonanal would be advisable, as in products of that type the significant amount of hexanal originates not only from chemical auto-oxidation of lipids but also from the enzymatic “lipoxygenase pathway”.

In the course of a technological process or already during storage, food products can be infected and mould and putrefaction can take place. This causes, of course, significant deterioration of the organoleptic quality and health safety of food, which can be detected on the basis of changes observed in aroma profiles (Lyew *et al.*, 2001; Marsili, 1999; Nakai *et al.*, 1999).

CHAPTER TWO: MATERIALS AND METHODS

2.1 Collection of sample

Raw milk samples were obtained from a local multinational dairy. Milk samples were drawn from the holding tanks prior to pick-up. Samples were stored in a cooler bag during transportation from the dairy to the laboratory. 2l of raw milk was collected for the experiments. The same batch of raw milk was used for all experiments so that comparisons could be made with results obtained for the different techniques used.

2.2. Assessing the effects of heat treatment on milk

2.2.1. Sampling

Raw milk was aliquoted (40 ml) in 12, 100 ml Schott bottles. The bottles were placed into a water bath (Julabo, TW2) and subjected to heat treatment at 63°C for varying time intervals (5, 7, 9, 11, 13, 15 min). Temperature was monitored by placing a thermometer into a sample and timing only started when the temperature reached 63°C. The sample with the thermometer probe was then discarded after the last time reached. The test samples were removed at their respective times and cooled in a cold water bath. Raw milk was used as the control. All experiments were carried out in duplicate.

2.2.2. Microbiological tests (total plate counts and coliform count)

2.2.2.1. Total plate counts

Standard plate counts were carried out using Milk agar (Merck) by the method outlined by Houghtby and co-workers, 1993. To determine the effect of heat treatment at 63°C on milk, the milk that was exposed to varying times (5, 7, 9, 11, 13, 15 min) was serially diluted (10 fold dilutions) in sterile distilled water. One ml of each dilution was transferred to Petri dishes. Approximately 20 ml of molten milk agar at a temperature of 40 °C was then added to

the plates. Plates were then incubated at 37°C for 48 hrs. The plates were read for bacterial colonies using an electronic colony counter and results were recorded as colony forming units (cfu)/ml. Plates that contained 37 or more cfu were counted, provided they did not exceed more than 300 cfu. If they contained more than 300 cfu, they were reported as too numerous to count (TNTC). If they contained less than 30 cfu, then they were reported as too few to count (TFTC). The number of cfu's per ml of micro-organisms was calculated by multiplying the number of cfu's by the dilution factor:

$$\boxed{\text{No. of cfu's per ml} = \text{No. of colonies} \times \text{dilution factor}}$$

2.2.2.2. Total coliform counts

The standard method of Christen and co workers, '1993' was used. Coliform counts were carried out using Violet red bile MUG Agar (Biolab C23). Media was prepared as per label instructions. Using a sterile pipette, 1 ml of sample was transferred to a sterile Petri dish together with ± 12 ml of tempered MUG agar. Plates were then swirled, for even distribution of the culture. After the media had solidified, a further 3-4 ml of medium was overlayed, to completely cover the surface, thereby inhibiting surface colony formation. These plates were then incubated at 30°C for 24 hours.

The results were interpreted as the presence of red colonies, yielding a positive result for coliforms, and represented as the number of colonies per ml.

2.2.3. Chemical analysis

2.2.3.1. Freezing point depression (FPD)

A cryoscope (Fiske Advanced Cryoscope) was used for this analysis. Initially the sample tube from the freeze chamber was removed. Thereafter the probe, stir freeze wire, mandrel and the top of the freezing chamber (in specific order) were cleaned using a damp cloth and distilled water. Care was taken not to bend the probe or the stir freeze wire. A clean dry test tube was

filled up to volume mark, with test sample and placed into the freezing chamber. After a cycle or run was completed, results were obtained from the machine and tabulated.

2.2.3.2. Protein stability testing

Protein stability was determined by the use of specific concentrations of alcohol. Alcohol has dehydrating properties, which destabilises the protein dispersion. If the proteins are already slightly unstable, then the addition of alcohol will result in dehydration of protein resulting protein precipitating out as floccules. For this test 5 ml of sample and 5 ml of ethanol (75 or 80%), were combined in a sterile glass test tube and allowed to mix. If the milk sample coagulated it was reported as a positive. All other samples were reported as negative.

2.2.3.3. Aschaffenburg-Mullen Phosphatase test

This test is based on an enzyme-substrate reaction that detects the presence of the phosphatase enzyme. This enzyme is actively present in raw milk and is destroyed by adequate pasteurisation. By testing for the presence of this enzyme it can be determined whether milk has been pasteurised or not. For the test, 5 ml of buffer-substrate solution (substrate: 1.5 g of disodium p-nitrophenyl phosphate hexahydrate (Merck) was added to 1L of buffer solution: 3.5 g of anhydrous sodium carbonate and 1.5 g of sodium bicarbonate dissolved in 1L of distilled water) was placed in a sterile test tube. One ml of test sample was added to the solution, thoroughly mixed and placed into a water bath set at 37°C for 30 min. After 30 min. samples were removed from the water bath and thoroughly mixed. It was then placed into a Comparator (Lovibond 2000) to determine any colour change. The control sample (pasteurised milk) was placed on the left hand side of the instrument, and the test sample was placed on the right hand side of the instrument. The comparator was then placed under a good source of light, and results matched to glass pieces available. These glass pieces were represented by unit values. Results were reported as follows: after 30 min of pasteurisation 0 units indicates proper pasteurisation, 6 units indicate doubtful results, and ≥ 10 units indicate under pasteurisation. Following these results, the milk samples were further incubated at the same temperature for 90 min. Results obtained after the second incubation period were reported as

follows: 0-10 units indicates proper pasteurisation, 10 \geq 18 slightly under-pasteurised, 10 \geq 42 under-pasteurised, and >42 indicates completely under-pasteurised.

2.2.4. Measurement of quality factor using electronic nose technology (AromaScan™)

The method involved drawing 40 ml of the test sample from each of the pasteurised milk samples into sterile 100 ml Schott bottles. Each bottle was closed with an omnifit cap which has special openings. The tubing attached to the inert carrier gas, in this case nitrogen gas, was inserted through one of the openings to just above the surface of the milk sample in the bottle. The tube which was to carry the volatiles generated in the headspace of the bottle into the AromaScan analyser was attached to the second opening. Once the sample was attached to the AromaScan, it was then ready for analysis. The data acquisition software was opened and the following acquisition parameters, as listed below were entered in the relevant boxes on screen to analyse the samples:

Sampling details

Detection threshold	:	0.7
Integration threshold	:	5 seconds

Valve sequence

Reference	:	40 seconds
Sample	:	120 seconds
Wash	:	10 seconds
Reference	:	120 seconds
Type of wash	:	None
Reference gas	:	Nitrogen

Sampling interval: 5 seconds. This means that although the instrument was analysing the volatiles continuously, the profile generated was only updated every 5 seconds during the analysis time. This was a matter of preference.

Humidities (purge and reference): 70% RH. This value is as per Gammatec (supplier) instructions.

Cycles: 1. This means that the process of referencing (that is when the instrument is equilibrating), sampling, washing and then referencing again occurred only once. Although each sample analysed was subjected to 5 such cycles to get a more significant representation of the volatiles being emitted, leaving this value at 1 allows labelling and identification of individual cycles. If the value had been set at 5, then the referencing, sampling, washing and referencing again would have been continuous for 5 cycles and individual cycles could not have been labelled.

The other parameters used were referencing, which was initially 40 seconds, followed by the all-important sampling interval of 120 seconds during which time the volatiles generated in the headspace of the sample bottle were actually allowed to interact with the sensors. Washing of the sensors using water vapour, which served to eliminate residues from the surfaces of the sensors leaving the sensors clean for subsequent analyses, was set at 10 seconds. This was followed by an additional referencing step of 120 seconds. During the switch from the first referencing to the sampling interval, the valve attached to the gas supply tubing was closed, and the valve attached to the tubing leading to the AromaScan analyser was opened.

Once the acquisition parameters had been set, the gas supply was turned on and the analysis started. Once turned on, the inert carrier gas serves two simultaneous functions during the first referencing step. One of these was the establishment of a baseline resistance by its interaction with the sensor elements in the sensor array i.e. it was regarded as a reference interaction. The other function was the creation of conditions of equilibrium in the atmosphere above the sample in the Schott bottle. Conditions of equilibrium were created by the pressure of the gas within the bottle whereby volatile organic compounds (analytes) diffuse from the sample in the atmosphere above the sample and at the same time diffuse from the atmosphere back into the sample. When the two diffusion rates equal, the concentration of

the analytes remained equal between the sample and the atmosphere above it, i.e. a headspace at equilibrium was created. Once these two functions had been served (after 40 seconds as defined by the value entered for the first reference in the acquisition parameters), the gas supply valve was closed and the valve attached to the tubing leading from the sample to the AromaScan analyser was opened. The volatiles in the headspace were thus allowed to interact with and cause changes in the baseline resistance of the sensors during what was defined as the sampling interval.

Analysis generated profiles, with each of the areas representing referencing, sampling, washing and referencing again as defined by the acquisition parameters. Each sample was analysed 5 times to generate 5 such profiles which were automatically recorded. Once the profiles for 5 cycles had been captured for each of the test and control samples on the test days using the above-mentioned acquisition parameters the data, with the exception of the first of the 5 cycles, was grouped (i.e. only 4 of the 5 cycles were used). This was done using the data reduction software of the AromaScan. This software allows data from each run to be opened at will and permits selection of the portion of the profiles corresponding to the 120-second sampling interval by entering values which corresponded to the start and end of sampling (in seconds) in the respective boxes. It then allowed addition of the sampling data from the 4 cycles for each sample. Grouping of data results in a window appearing automatically on screen. Here comparisons were made between each of the 4 cycles in terms of how the volatiles from the sample interacted with the sensors. This was done by using the superimpose function. Comparisons were made using line graphs or bar graphs (Appendix 1). Grouped data was then manually saved as a group on the computer using appropriate labels. This effectively constituted the database which permitted comparison of data captured from 4 of the 5 analyses of one sample to that of another. Such comparisons were done using 2-dimensional PCA maps to which instrumental odour intensities were implemented and are the final products of analysis.

For any two samples compared on a PCA map, scientific comparisons were made on the basis of quality values which are generated by the AromaScan software. Quality values of greater than 2 for two compared samples, indicates a significant difference between the samples. In

terms of this research, the control sample was compared to each of the varying pasteurisation times and in terms of quality values. For valid comparisons to be made in terms of distribution of clusters etc., PCA Maps were formatted so that there was uniformity in terms of maximum and minimum values for each of the X- and Y-axes.

2.2.5. Olfactory evaluation

Milk was evaluated for overall odour using the descriptive analysis method during all experiments carried out. The sensory panel consisted of 5 panellists chosen from Post Graduate students in the Department of Biotechnology, M. L. Sultan Campus. The evaluation form was designed in-house. The type of olfactory evaluation used for this study was descriptive analysis as described by Zondervan and co-workers, (1999). This method was chosen as only a common odour descriptor was required and this was then correlated to a chemical compound. The panellists were provided with an evaluation score sheet and asked to identify the particular odour/aroma of the sample based on a selection of odour descriptor terms provided to them on the form (Appendix 2).

Forty ml milk samples were placed in 100 ml Schott bottles and stored at 4°C. One hour before sensory evaluation was to commence, the milk samples were removed from the refrigerator and allowed to be brought to room temperature. This was done so that during the evaluation panellists were able to obtain a true odour perception. Olfactory evaluation was carried out on all samples (5, 7, 9, 11, 13, 15 min) in order to assess the effect of pasteurisation, on time.

2.2.6. Gas chromatography

Gas chromatography is a chromatographic technique that uses a gas (namely nitrogen or helium) as its mobile phase and the stationary phase (column) which is made up of fused silica. The sample is introduced into the column in the form of either a liquid or as a vapour.

For our experiments, it was the headspace gas chromatography was used. The headspace of the milk matrix was analysed for volatile compounds present.

2.2.6.1. Preparation of sample

Gas chromatographic analysis was carried out at the Department of Biotechnology, Durban University of Technology. The method that was used was an adaptation of the method as described by Moio *et al.* 1994. The volatile organic compounds (VOCs) present in raw milk samples and all pasteurised milk samples that were subjected to different time intervals (5, 7, 9, 11, 13 15 min) were analysed. Milk samples were placed in 20 ml headspace bottles, with screw top lids. The lids were specially created, with open tops fitted with silicon septa. Ten ml of milk samples were placed into each bottle for experiment. Prior to analysis, these bottles were placed into a convection oven maintained at 60°C for 60 min, so that headspace was generated. Immediately after removal from the oven, using a headspace syringe (SGE – 5MDR), a 5 ml volume of the headspace air was removed and injected into the gas chromatograph for analysis.

2.2.6.2. Standards identification

All standards used for the study was GC grade. Standards used include ethyl-2-butanoate (Aldrich), ethyl-2-hexanoate (Aldrich), indole (Fluka), dimethyl sulphide (Aldrich), octanol (Sigma), nonanal (Sigma), heptanal (Fluka), hexanal (Chemika). 0.1 µl of standard was injected into the gas chromatograph, and retention times were determined using a a Varian CP 3800 Gas chromatograph under the following instrument parameters:

Column	:	Capillary, WCOT fused silica (#CP97721) (Varian)
Column length	:	30 m
Internal diameter	:	0.15 mm
Film thickness	:	0.12 µm
Detector	:	F. I. D.

Carrier Gas : Nitrogen
Flow rate : 1 ml/min
Injector temp. : 160°C
Detector temp. : 200°C
Column oven temp. : 50°C held for 10 min. then ramped to 180°C at 5 °/min

Experimental results were reported, on the basis of peaks being detected within the retention times of any of the standard samples run. Where standards were not available results were compared to the literature cited. These results were reported only as an indicator of whether a compound was present and not as for quantitative purposes.

2.3. The effect of shelf life testing on milk

2.3.1. Preparation of sample for shelf life testing

For shelf life testing the 40 ml of raw milk was transferred into 100 ml Schott bottles, these bottles were then pasteurised at 63°C for 15 min using a water bath (Julabo, TW2). After 15 min all bottles were removed from the water bath and placed in a bath containing cold water to decrease the temperature. Once milk samples attained room temperature they were placed into the refrigerator (4°C) until they were required for further experiments. These samples were left at 4°C (refrigeration temperature) for different time intervals 1, 3, 5, 7, 9, 11, 13, 15 day. After each specified time interval all relevant tests were carried out on the specified sample. Fresh milk was used as the control. All tests were carried out in duplicate.

Total plate counts and coliforms were determined as described in 2.2.2. in each of the samples 1, 3, 5, 7, 9, 11, 13, 15 day intervals. All samples were stored at refrigeration temperatures.

2.3.2. Measurement of shelf life of milk using the electronic nose

The procedure used for electronic nose was the same as described in section 2.2.4. For the effect of shelf life testing, milk samples were analysed using the electronic nose after the specified 1, 3, 5, 7, 9, 11, 13, 15 day interval. The samples were removed from the refrigerator, allowed to reach ambient temperature and then connected to the equipment for analysis. The sampling procedure and sample analysis were the same as outlined in section 2.2.4.

2.3.3. Olfactory analysis

Olfactory evaluation to test the effect of shelf life of milk was the same as outlined in section 2.2.5. However in this set of experiments, the panellists were provided with a sample on each specified day 1, 3, 5, 7, 9, 11, 13, 15 and they were allowed to evaluate the milk samples.

2.3.4. Gas chromatography

Gas chromatographic analysis was carried out on milk samples on days 1, 3, 5, 7, 9, 11, 13, 15 in order to assess the shelf life quality of milk. The methods used for this experiment were the same as outlined in 2.2.6. The analytical conditions and preparation of the sample for analysis remained the same as noted in 2.2.6.

2.4. Statistical analysis

A linear regression between total plate count and time of pasteurisation was used to determine whether there is a linear trend. For this analysis the total count in raw milk was excluded.

The prevalence, sensitivity, specificity, positive and negative predictive values, the accuracy of the electronic nose were determined in a two by two table using hexanal, nonanal and

indole as standards. Statistical analysis carried out in this study was similar to the analysis done by Gardner, 1996.

A description of the terms is given below:

2.4.1. Prevalence

Is the number of samples where a specific compound is identified with sensory evaluation.

2.4.2. Sensitivity

The results of the test are compared to some absolute (volatile profile) for example, for a test to identify a volatile compound using the electronic nose, the sensitivity to test is the probability that if olfactory evaluation of the sample identifies the compound, the test will be positive.

		Volatile profile		
		<i>True</i>	<i>False</i>	
Test outcome	<i>Positive</i>	True Positive	False Positive	→ Positive Predictive Value
	<i>Negative</i>	False Negative	True Negative	→ Negative Predictive Value
		↓ Sensitivity	↓ Specificity	

That is, sensitivity is the probability of making the right decision if using the electronic nose when olfactory evaluation identified the volatile compound. It is the parameter of the test.

$$\text{Sensitivity} = \frac{\text{No. of True Positives}}{\text{No. of True Positives} + \text{No. of False Negatives}}$$

2.4.3. Specificity

The probability of making the right decision is using the electronic nose when sensory evaluation did not identify the volatile compound.

2.4.4. Positive predictive value

The positive predictive value is the probability that the volatile compound is present by olfactory evaluation given that the electronic nose detected the volatile compound.

The positive predictive value (PPV) can be defined as:

$$\text{PPV} = \frac{\text{No. of True Positives}}{\text{No. of True Positives} + \text{No. of False Positives}}$$

or, alternatively

$$\text{PPV} = \frac{\text{sensitivity} \times \text{prevalence}}{\text{sensitivity} \times \text{prevalence} + (1 - \text{specificity}) \times (1 - \text{prevalence})}$$

2.4.5. Negative predictive value

It is the probability that olfactory evaluation will not pick up the compound once the electronic nose has not identified the compound.

The negative predictive value (NPV) can be defined as:

$$\text{NPV} = \frac{\text{No. of True Negatives}}{\text{No. of True Negatives} + \text{No. of False Negatives}}$$

or, alternatively

$$\text{NPV} = \frac{\text{specificity} \times (1 - \text{prevalence})}{\text{specificity} \times (1 - \text{prevalence}) + (1 - \text{sensitivity}) \times \text{prevalence}}$$

2.5.6. Accuracy

Accuracy is also used as a statistical measure of how well a binary classification test correctly identifies or excludes a condition. That is, accuracy is the proportion of true positives and true negatives in the test. It is the parameter of the test.

		Volatile compound		
		<i>True</i>	<i>False</i>	
Test outcome	<i>Positive</i>	True Positive	False Positive	→ Positive Predictive Value
	<i>Negative</i>	False Negative	True Negative	→ Negative Predictive Value
		↓ Sensitivity	↓ Specificity	Accuracy

$$\text{Accuracy} = \frac{\text{No. of True Positives} + \text{No. of True Negatives}}{\text{No. of True Positives} + \text{False Positives} + \text{True Negatives} + \text{False Negatives}}$$

CHAPTER THREE: RESULTS

3.1. Microbiological results of heat treatment at 63°C of raw milk

The relationship between heat treatment and microbial counts of raw milk (Table 3.1) and the effect of varying time on microbial counts using the standard plate is shown in Table. 3.2 Raw milk samples had bacterial counts (35×10^4 cfu/ml) that are within the set guidelines. Heat treated milk counts dropped ten fold to 1.13×10^3 cfu/ml. These counts are indicators of milk that is acceptable to the consumer as set out in the current South African Standards (Table 1.2) (page 6).

The microbial counts obtained at varying time intervals of heat treatment at 63°C applied to raw milk showed a proportionate decrease in total microbial counts with increase in time. After 5 min of exposure to heat treatment, the bacterial load was well within the South African legislated limits. Milk that was exposed to heat treatment for 15 min yielded the lowest microbial load. The linear regression between the time of heat treatment and bacterial counts gave a p value <0.0001 , indicating a strong linear trend (Fig. 3.1). This showed that increasing the time during heat treatment decreased bacterial counts in milk (Table. 3.2). No coliforms were detected in any one of the heat treated milk samples.

Table 3.1: Total plate counts and chemical analysis of raw and pasteurised milk

Sample	Total count* (cfu/ml)	Total coliform count (cfu/ml)	Freeze Point Depression	Alcohol (Protein Stability)	Phosphatase [■]
Raw milk	35×10^4	0	0.52°C	Negative	6/24
Pasteurised milk	1.13×10^2	0	0.52°C	Negative	0/0

* Values represented in the table reflect averages of duplicate counts.

■ Values represented in red indicate 30 min incubation time (0 indicates proper pasteurisation; 6 indicates doubtful results and ≥ 10 indicates under pasteurisation), and values represented in blue indicates 90 min incubation times (0 – 10 indicates proper pasteurisation; $10 \geq 18$ indicates slightly under pasteurised and $19 \geq 42$ under pasteurised)

Chemical analysis was carried out on all heat treated milk samples to establish quality aspects of the milk included: Freeze Point Depression (FPD), protein stability (alcohol) and phosphatase test. Results obtained (Table 3.2) were in keeping with the standards set (Government Gazette, 1997). The results obtained from protein stability, results were negative for all heat treated milk samples. Phosphatase results showed that after 30 min. of incubation of heat treatment, milk samples up to 9 min obtained unacceptable readings, this indicated that the phosphatase enzyme was still active. Milk samples exposed to 11 and 15 min heat treatment gave acceptable readings. After further 90 min incubation time results obtained were similar, with milk samples up to 9 min heat treatment, reflecting negative unacceptable results, and 11 and 15 min heat treatment times obtaining positive acceptable results.

Table 3.2: Effect of time on heat treatment (63°C) using microbiological and chemical analysis

Sample	Total count* (cfu/ml)	Freeze Point Depression (FPD)	Alcohol (Protein Stability)	Phosphatase [■]
Raw milk	35 x 10 ⁴	0.52°C	Negative	6/24
5 min heat treatment	3.64 x 10 ³	0.52°C	Negative	6/24
7 min heat treatment	2.96 x 10 ³	0.518 °C	Negative	6/24
9 min heat treatment	2.65 x 10 ³	0.52°C	Negative	6/10
11 min heat treatment	1.72 x 10 ³	0.52°C	Negative	0/6
15 min heat treatment	1.13 x 10 ³	0.52°C	Negative	0/0

* Values represented in the table reflect average values of results.

■ Values represented in red indicate 30 min incubation time (0 indicates proper pasteurisation; 6 indicates doubtful results and ≥ 10 indicates under pasteurisation), and values represented in blue indicates 90 min incubation times (0 – 10 indicates proper pasteurisation; 10 ≥ 18 indicates slightly under pasteurised and 19 ≥ 42 under pasteurised)

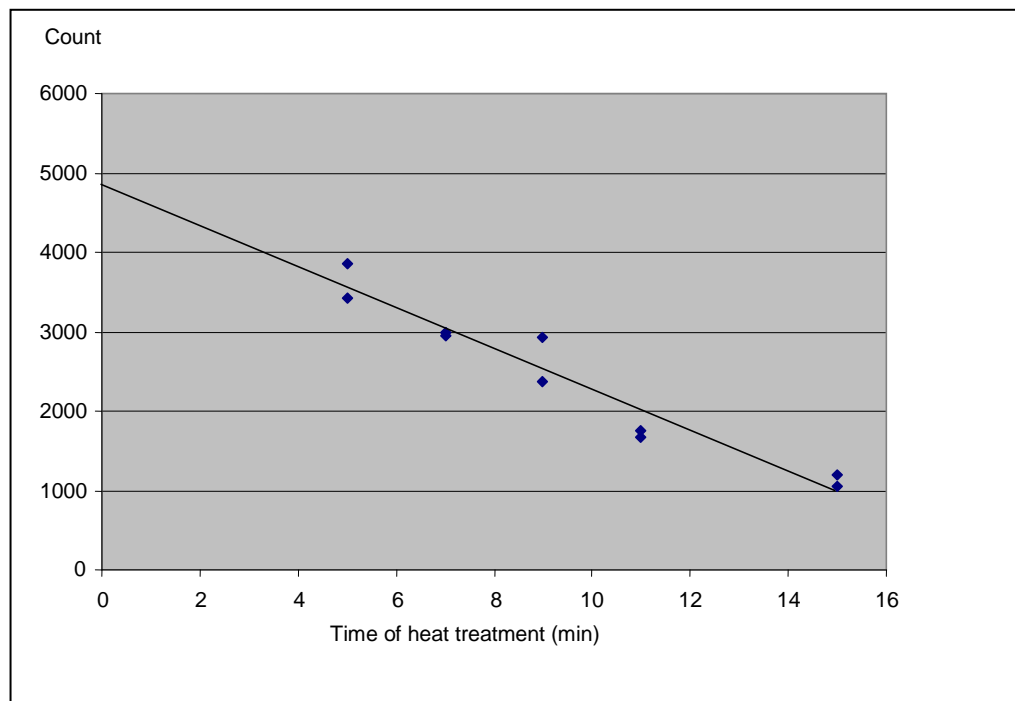


Figure 3.1: Linear regression curve of total plate count (cfu/ml) and time of heat treatment (63°C).

3.2. Microbiological results obtained for determining the shelf life of fresh pasteurised milk

The microbiological counts increased with increasing storage time (Table 3.3). There was a strong linear trend over the 15 day storage period (p-value of <0.001) (Fig. 3.2). The bacterial counts were within South African guidelines for milk except that there were also no coliforms detected while from a visual examination, a curdling effect was observed on the 9th day storage period. This could be due to bacterial enzymatic action on the milk, which resulted in curdling of the milk, since bacterial numbers did not multiply exponentially.

Table 3.3: Effect of shelf life on fresh pasteurised milk with respect to total plate counts (cfu/ml)

Sample	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Total count (cfu/ml)	Visual appearance
Fresh milk	113	15	-	-	-	1.13×10^3	White fluid
Day 1	194	20	2	-	-	1.94×10^3	White fluid
Day 3	216	49	6	-	-	2.16×10^3	White fluid
Day 5	229	35	2	-	-	2.29×10^3	White fluid
Day 7	243	27	3	-	-	2.43×10^3	White fluid
Day 9	268	24	-	-	-	2.68×10^3	Milk ropo-coagulation
Day 11	277	39	2	-	-	2.77×10^3	Milk ropo-coagulation
Day 13	321	30	1	-	-	3.21×10^3	Separation
Day 15	352	27	3	-	-	3.52×10^3	Separation

*Values represented in the table reflect average values of results

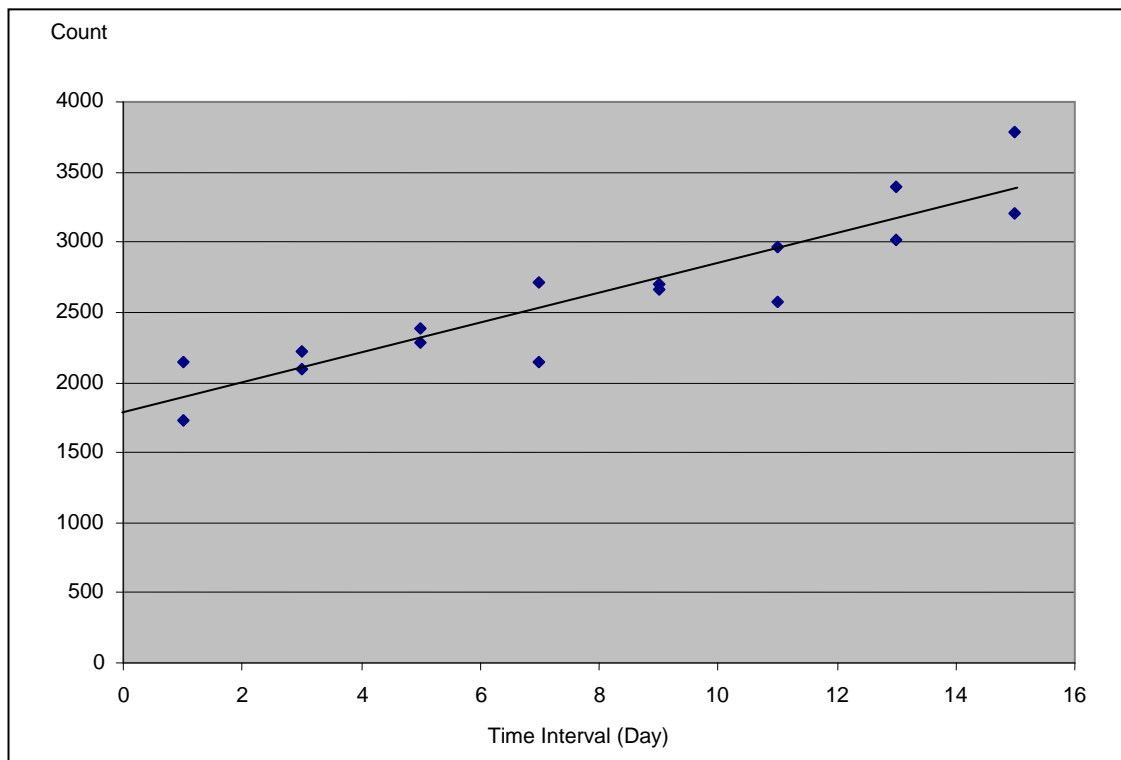


Figure 3.2: Linear regression curve of total plate count (cfu/ml) for assessing shelf life of milk

3.3. Olfactory evaluation of milk

3.3.1 The effect of time on heat treatment at 63°C

The chemical composition of aroma and flavour of milk and other dairy products is complex due to the complicated matrix of milk. Olfactory results obtained from the score sheets are shown in Table 3.4-3.5. The five panellists perceived the odours differently and described the odours of milk as sweet, fruity, mushroom and musty. Two out of five panellists described the odour of raw milk as sweet which was attributed to the presence of ethyl hexanoate (Friedrich and Acree, 1998; Bendall, 2001).

The odour of heat treated milk for 5 min and 7 min was described by the majority of the panellist as milky or creamy which was ascribed to the presence of hexanal. Nine minutes after heat treatment, the milk was described as slightly creamy or fruity, and after 11 and 15 min heat treatment as mainly fruity or sweet. According to literature cited by Ampuero and Booset (2003), this was an odour associated with nonanal, due to nonanal having a human detection threshold of between 0.2 – 7 ppm.

Table 3.4: Olfactory evaluation of raw vs pasteurised milk

Sample	Panellist 1	Panellist 2	Panellist 3	Panellist 4	Panellist 5
Raw milk	Sweet (Ethyl butanoate)	Fruity (Ethyl hexanoate)	Fruity (Ethyl hexanoate)	Mushroom (1-octen-3- ol)	Musty (Indole)
Pasteurised milk	Fruity (Ethyl hexanoate)	Sweet (Nonanal)	Musty (Indole)	Sweet (Nonanal)	Floral (Nonanal)

Table 3.5: Olfactory evaluation of heat treated milk (63°C) at varying time intervals

Sample	Panellist 1	Panellist 2	Panellist 3	Panellist 4	Panellist 5
Raw milk	Sweet (Ethyl butanoate)	Fruity (Ethyl hexanoate)	Fruity (Ethyl hexanoate)	Mushroom (1-octen-3- ol)	Musty (Indole)
5 min	Milky (Hexanal)	Milky (Hexanal)	Creamy (Hexanal)	Sterilized (Hexanal)	Faintly fruity (Ethyl hexanoate)
7 min	Creamy (Hexanal)	Milky (Hexanal)	Fresh milk (Hexanal)	Musty (Indole)	Musty (Indole)
9 min	Slight creamy (Hexanal)	Faintly fruity (Nonanal)	Milky (Hexanal)	Fruity (Nonanal)	Fruity (Nonanal)
11 min	Fruity (Nonanal)	Fruity (Nonanal)	Sweet milky (Nonanal)	Sterilized (Hexanal)	Fruity (Nonanal)
15 min	Fruity (Ethyl hexanoate)	Sweet (Nonanal)	Musty (Indole)	Sweet (Nonanal)	Floral (Nonanal)

3.3.2. The effect of shelf life testing of pasteurised milk using olfactory evaluation

The olfactory attributes of milk assessed for shelf life were sweet, creamy, buttery and sour, which according to Friedrich and Acree (1998) are odour descriptors for: nonanal, hexanal, diacetyl and butanoic acid, respectively. Fresh milk which was used as the control, here the predominant odour descriptor was fruity which was associated with ethyl hexanoate. For day 1 to 5 the most frequently recognised odour was sweet ascribed to nonanal. On day 7 the odour of the milk was perceived as creamy, which was due to the presence of hexanal. From day 9 to 15 milk was frequently identified as having a buttery odour, and this could be due the presence of diacetyl. Butanoic acid which was identified by a sour odour with olfactory evaluation, was noted as the common odour of milk from day 11 to day 15. These results are tabulated in Table 3.6.

Table 3.6: Sensory evaluation of shelf life testing of pasteurised milk

Sample	Panellist 1	Panellist 2	Panellist 3	Panellist 4	Panellist 5
Fresh milk	Sweet (Ethyl butanoate)	Fruity (Ethyl hexanoate)	Fruity (Ethyl hexanoate)	Mushroom (1-octen-3-ol)	Musty (Indole)
Day 1	Sweet (Nonanal)	Sweet (Nonanal)	Musty (Indole)	Sweet (Nonanal)	Floral (Nonanal)
Day 3	Creamy (Hexanal)	Creamy (Hexanal)	Musty (Indole)	Milky (Hexanal)	Grassy (Hexanal)
Day 5	Musty (Indole)	Creamy/bland (Hexanal)	Sweet (Nonanal)	Sweet (Nonanal)	Sweet (Nonanal)
Day 7	Creamy (Hexanal)	Creamy (Hexanal)	Slightly Sour (Propionic acid)	Buttery (Diacetyl)	Creamy (Hexanal)
Day 9	Fruity (2-heptanone)	Creamy (Hexanal)	Buttery (Diacetyl)	Buttery (Diacetyl)	Creamy (Hexanal)
Day 11	Sour (Butanoic acid)	Sour (Butanoic acid)	Sour (Butanoic acid)	Creamy (Diacetyl)	Creamy (Diacetyl)
Day 13	Sour (Butanoic acid)	Sour (Butanoic acid)	Sour (Butanoic acid)	Creamy (Diacetyl)	Creamy (Hexanal)
Day 15	Creamy (Diacetyl)	Sour (Butanoic acid)	Sour (Butanoic acid)	Creamy (Diacetyl)	Creamy (Diacetyl)

3.4. Aroma profiling using the electronic nose

3.4.1. Aroma profiling of varying time of heat treated (63°C) raw milk

It is evident from the results obtained from the aroma profiling of the electronic nose Aromascan plots (Fig. 3.3) that there was a notable differences between the odours picked up from milk samples exposed to varying times of heat treatment. The results obtained from the 2D PCA map were then statistically represented as numerical values, represented as quality factors and are represented in Tables 3.7 and 3.8. Each sample was analysed five times. Results obtained in Table 3.8 indicated that heat treatment from 9 min to 15 min, changed the aroma of the milk, significantly as indicated by the quality factor values being greater than 2. Using aroma profiling it is

possible to predict that at 9 min of heat treatment, the volatile chemistry of the milk changes significantly.

Table 3.7: Quality factors obtained from electronic nose of raw milk and pasteurised milk

Sample	Quality Factor (QF)
Raw milk	Control
Pasteurised milk	3.132

Table 3.8: Effect of time on heat treatment based on the quality factors obtained from the electronic nose

Sample	Quality Factor (QF)
Raw milk	Control
5 min heat treatment	0.188
7 min heat treatment	0.859
9 min heat treatment	2.263
11 min heat treatment	2.403
15 min heat treatment	3.132

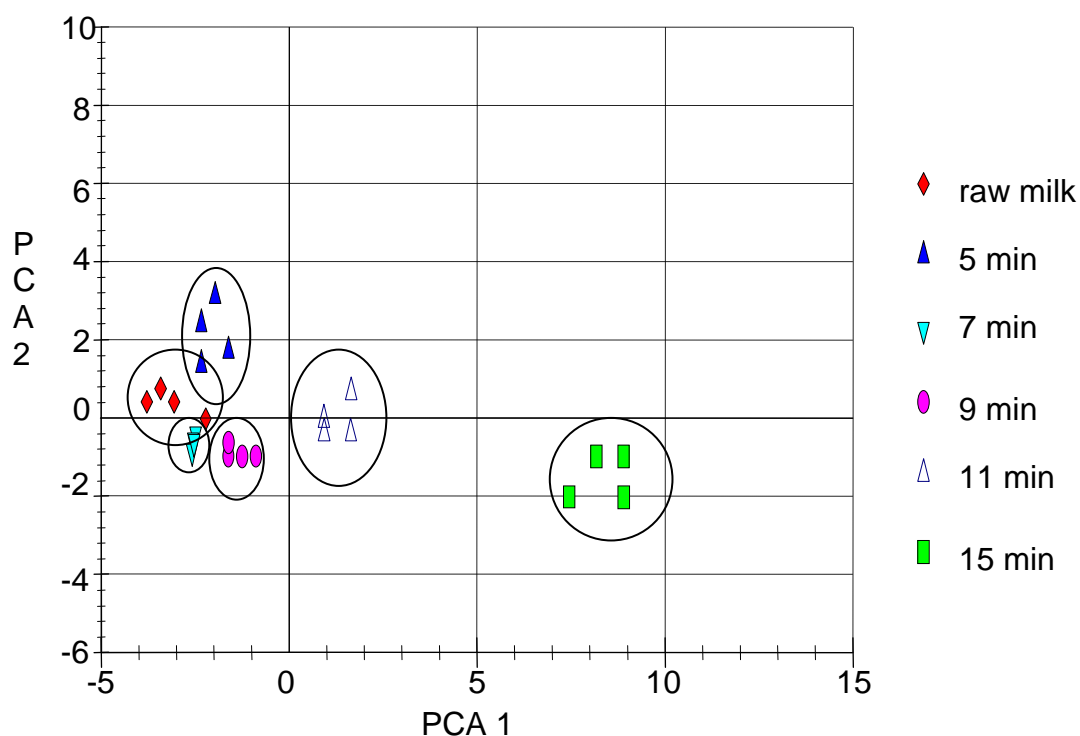


Figure 3.3: 2 D Principle component analysis (PCA) map showing the aroma profile of the effects of heating milk at 63°C over varying time intervals

3.4.2. Aroma profiling using the electronic nose for testing the shelf life of milk

Electronic nose testing used for evaluating the effect of shelf life of milk, showed a significant difference in the aroma profile of milk from day 7 (Table 3.9). This gave an indication that spoilage started from day 7, due to the change in volatiles produced by the milk, as compared to the aroma profile of fresh milk. The quality factor obtained for day 15 was 7.777, which showed the extent of changes in volatile profiles. According to the volatile profile created for milk stored for a 15 day time period (Fig. 3.4), the quality factor obtained indicated milk quality started to deteriorate from day 7 and progressed steadily thereafter as can be seen in Table 3.9.

Table 3.9: Quality factors obtained for shelf life testing of milk

Sample	Quality Factor(QF)
Pasteurised Fresh milk	Control
Day 1	0.806
Day 3	1.072
Day 5	1.677
Day 7	2.463
Day 9	2.984
Day 11	3.612
Day 13	4.654
Day 15	7.777

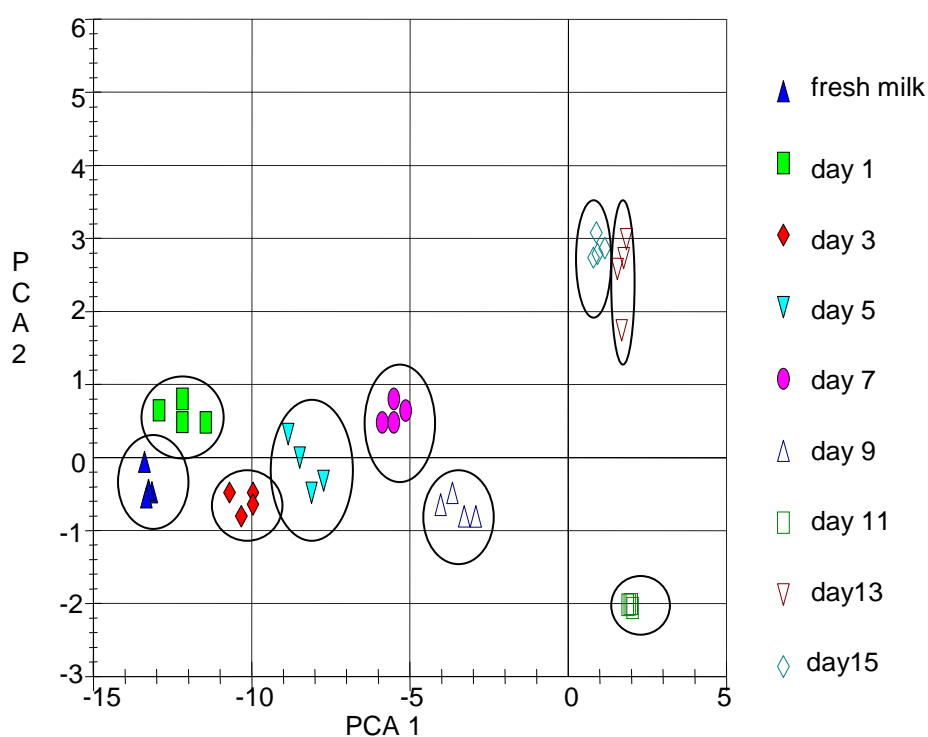


Figure 3.4: 2 D Principle component analysis (PCA) map showing graphically the aroma profiles of fresh milk monitored over day intervals for shelf life determination. Quality values are determined by the distance between each cluster on the graph

3.5. Gas chromatographic analysis of milk

Gas chromatographic analysis in this study was carried out to identify the volatile compounds that were present in the milk samples.

3.5.1 Volatile compounds detected by gas chromatography for heat treated milk at varying time intervals

Many common odourants have been reported in heated milk (Brudzewski *et al.*, 2004). Fig. 3.5 shows the GC chromatograms for the volatile standards, these were selected as they are common volatile compounds that were reported by Friedrich and Acree (1998) to be present in the milk. A number of different volatile compounds were present when milk was exposed to heat treatment at 63°C at different times (Table 3.10). In raw milk samples the most common volatile compounds were, ethyl-2-hexanoate, dimethyl sulphide and nonanal (Fig. 3.6.). In heat treated milk samples the frequently identified compounds included ethyl-2-butanoate, hexanal, nonanal, dimethyl sulphide and ethyl-2-hexanoate (Fig 3.7).

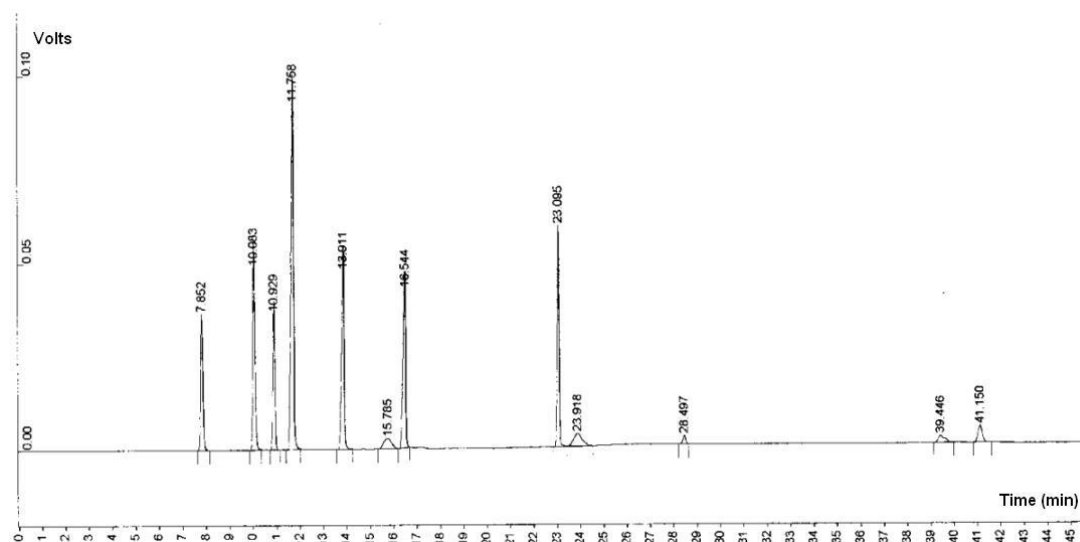


Figure 3.5: GC chromatogram of volatile standards showing retention times for acetone (7.852 min), hexanal (10.093 min), ethyl-2-butanoate (10.929 min), heptanal (11.758 min), octanal (13.911 min), nonanal (16.544 min), dimethyl sulphide (23.918 min), ethyl-2-hexanoate (28.233 min) and indole (41.50 min) respectively

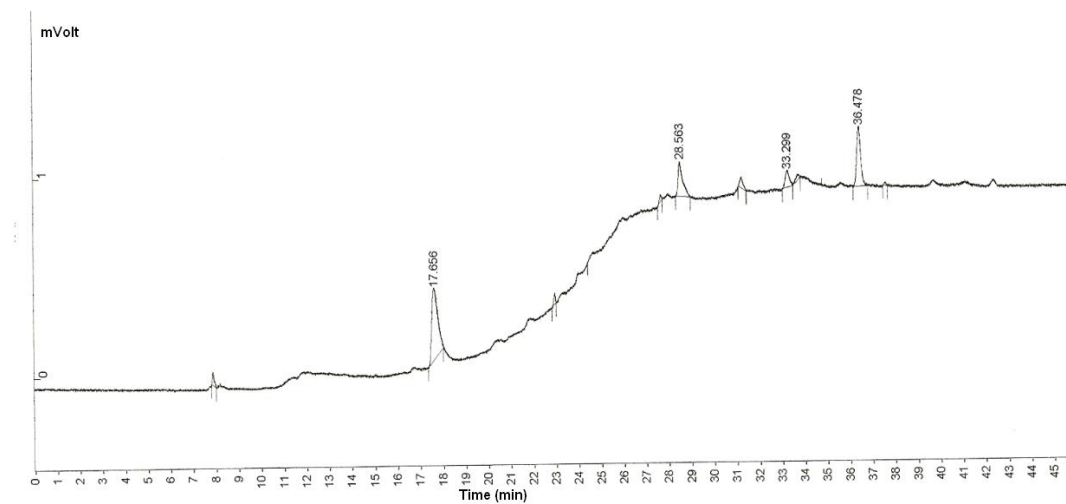


Figure 3.6: GC chromatogram showing retention times of volatiles compounds present in raw milk sample

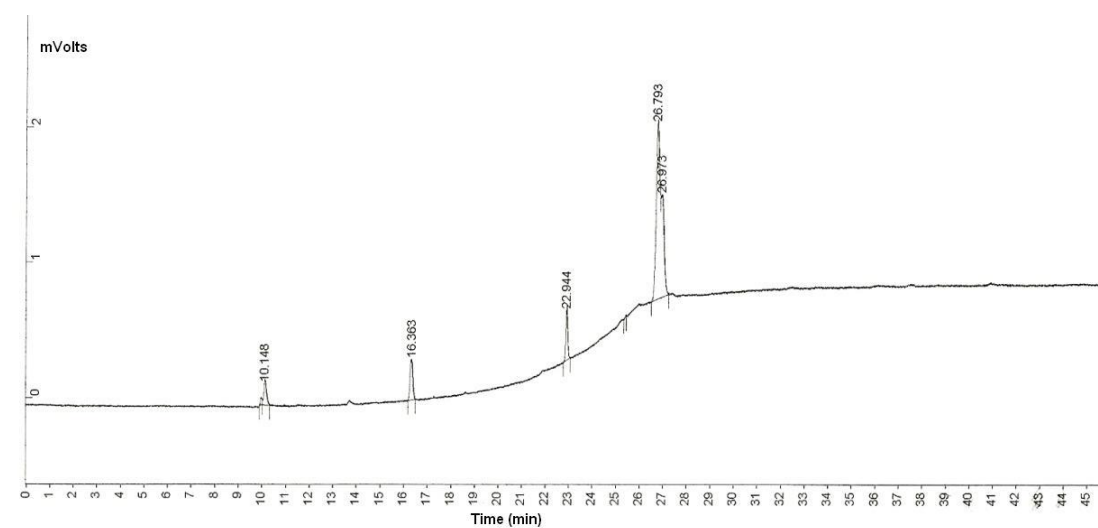


Figure 3.7: GC chromatogram showing retention times of volatile detected in milk sample heat treated for 9 min at 63°C

Table 3.10: Volatile organic compounds identified in heat treated (63°C) milk samples at varying time intervals using gas chromatography

	Ethanol 7.852	Hexanal 10.093	Ethyl-2- butanoate 10.929	Heptanal 11.758	Octanal 13.911	Nonanal 16.544	Dimethyl sulphide 23.918	Ethyl-2- hexanoate 28.233	Indole 41.50
Raw Milk ^a	+					+	+	+	
5 min						+	+	+	
7 min		+	+			+	+		
9 min ^b			+			+		+	
11 min	+	+	+		+	+	+	+	+
15 min ^c	+	+			+	+	+		+

Unknown retention times: ^a30.02, 33.29, 36.47 min ^b26.793 min. ^c35.08, 38.00 min.

3.5.2. Volatile compounds detected by gas chromatography for shelf life testing

The most common volatile compounds present in the samples were identified as ethyl-2- butynoate, ethyl-2-hexanoate, nonanal, and indole. Table 3.11 lists all volatile compounds identified in milk samples over a 15 day period. On day one only ethyl-2-butanoate and ethyl-2-hexanoate were detected. The volatile compounds detected on day 3 and day 5 also included ethyl -2- butanoate and nonanal with day 5 milk sample also containing hexanal, which was further detected in the milk samples for days 7 and 9. From day 7 to day 15 ethyl-2-hexanoate and indole were detected in all milk samples analysed.

Table 3.11: Volatile organic compounds identified in testing shelf life of milk using gas chromatography

Day Interval	Ethanol 7.852	Hexanal 10.093	Ethyl-2-butanoate 10.929	Heptanal 11.758	Octanal 13.911	Nonanal 16.544	Dimethyl sulphide 23.918	Ethyl-2-hexanoate 28.233	Indole 41.50
Day 1 ^a			+					+	
Day 3 ^b			+			+			
Day 5 ^c		+	+			+			
Day 7 ^d	+	+		+	+	+		+	+
Day 9 ^e	+	+						+	+
Day 11 ^f	+		+	+	+	+	+	+	+
Day 13	+		+	+			+	+	+
Day 15	+			+			+		+

unknown retention times: ^a3.5, 4.25, 9.36 min. ^b9.36 min. ^c8.825, 9.2 min. ^d39.5 min. ^e31.79, 35.18, 38.87 min. ^f26.79, 38.07 min.

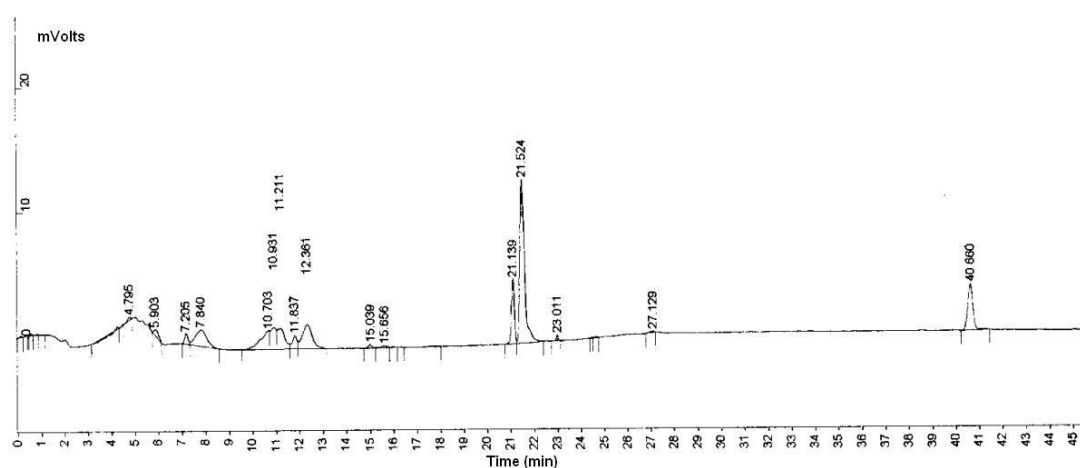


Figure3.8: GC chromatogram showing results obtained for milk samples held for 11 days in assessing for shelf life

Fig. 3.8. represents a GC chromatogram of milk after 11 days of storage at refrigeration temperature (4 °C). These volatiles were ethyl-2-butanoate (10.93 min), heptanal (11.83 min), octanol (12.36 min), nonanal (15.03 min), dimethyl sulphide (23.01 min), ethyl-2-hexanoate (27.12 min) and indole (40.88 min). These volatiles were present in the sample at varying concentration as indicated by varying peak heights. However the most prominent peak at 21.52 min did not correspond to any of the standards but a recent study by Karatapanis *et al*, (2006) identified this peak to be 4-methyl-2-pentanone.

3.6. Evaluation of the electronic nose for detecting pasteurised milk and shelf life of milk

3.6.1. Comparing partially heat treated and pasteurised milk using the electronic nose and with olfactory evaluation

When milk was partially heat treated (samples heat treated for less than or equal to 9 mins) the QF is 2.263. A comparison of the electronic nose to olfactory evaluation had a probability of being correct 60% of the time. It had an accuracy value of 57%. When milk had been completely pasteurized (15 min. heat treatment) the QF is 3.132 and the electronic nose predicted this event 100% of time, and was accurate 67% (Table 3.12).

Table 3.12: Sensitivity, specificity, positive predictive value, and positive predictive value of the electronic nose for pasteurized and partially heat treated milk

	Prevalence ^a (%)	Specificity ^b (%)	Sensitivity ^c (%)	Accuracy ^d (%)	Predictive ^e Value (%)
Raw milk	0	0	0	0	0
Partially heat treated	42	57	60	58	50
Pasteurized	67	0	100	67	67

^a no. of samples where the specific compound is identified with olfactory evaluation when compared to gas chromatography

^b the probability of making the right decision when using the electronic nose when olfactory analysis and gas chromatography identified the compound

^c the probability of making the right decision if using the electronic nose when olfactory evaluation and gas chromatography identified the compound

^d the proportion of times the correct decision was made

^e the probability that the compound is present by olfactory evaluation given that the electronic nose detected the compound

3.6.2. Evaluation for raw and heat treated milk

Comparison of the various methods employed to monitor milk spoilage, results of the microbial counts, olfactory evaluation, aroma profiling using the electronic nose and the gas chromatographic analysis of volatile compounds are summarised in Table 3.13. The aim was to determine whether the electronic nose could distinguish between raw and heat treated milk from volatile aromas expressed as quality factors to standard methods used for assessing milk quality. The main olfactory perceptions described by the panellists for raw and heat treated milk samples were described as fruity for raw milk and fruity or sweet for pasteurized milk. These odours were ascribed to the presence of ethyl-2-hexanoate, nonanal and indole. The electronic nose gave a quality factor of 3.132, when comparing raw milk to pasteurised milk.

Statistical evaluation showed that when the hexanal (milky, creamy) was identified the electronic nose gave a prevalence value of 67%, this indicating that 67% of the time the electronic nose was able to detect hexanal. The sensitivity of the electronic nose in predicting pasteurized milk was 75% indicating that we were correct 75% of time when using the electronic nose. The probability that hexanal was present in milk, when the electronic nose was used, is 75%.

When the compound nonanal (sweet perception) was identified the electronic nose gave a prevalence value of 50% indicating that this olfactory perception can only be picked by the electronic nose 50% of the time. The sensitivity of the electronic nose in predicting pasteurized milk based on the presence of nonanal was 50% indicating that we would be correct 50% of time when using the electronic nose. The predictive value for nonanal was also 50%. When we look at the indole (musty perception) the electronic nose had a prevalence value of only 33% and a sensitivity of 50% (Table 3.14).

For all other samples gas chromatography identified at least one of the compounds identified during olfactory evaluation.

Table 3.13: Sensitivity, specificity, of the electronic nose for testing effect of heat treatment

	Prevalence ^a (%)	Specificity ^b (%)	Sensitivity ^c (%)	Accuracy ^d (%)	Predictive Value ^e (%)
Hexanal	67	50	75	67	75
Nonanal	50	0	100	50	50
Indole	33	75	50	67	50

^a no. of samples where the specific compound is identified with olfactory evaluation and gas chromatography

^b the probability of making the right decision when using the electronic nose when olfactory analysis and gas chromatography identified the compound

^c the probability of making the right decision if using the electronic nose when olfactory evaluation and gas chromatography identified the compound

^d the proportion of times the correct decision was made

^e the probability that the compound is present by olfactory evaluation given that the electronic nose detected the compound

3.6.3. Comparison of the electronic nose with olfactory evaluation for shelf life testing of milk.

In all samples up to Day 9 the gas chromatography identified the same compounds as the olfactory evaluation. On days 11, 13 and 15 the main compounds identified with olfactory evaluation were butanoic acid and diacetyl. These components were not tested for using gas chromatography, however research findings reported by Valero *et al*, (2001) showed the presence of diacetyl and butanoic acid in UHT milk, and reported retention times of 10.430 min and 37.012 min respectively. If we correlate these times to retention times obtained in our study, comparisons can be made.

The accuracy of using the electronic nose was 67% for butanoic acid and 50% for diacetyl, when these compounds were identified using olfactory evaluation. Butanoic acid showed 67% sensitivity when comparing olfactory evaluation to the electronic nose, whilst diacetyl showed 67% sensitivity.

CHAPTER FOUR: DISCUSSION

The effect of heat treatment on pasteurised milk in terms of chemical, physical and organoleptic properties depends on the combination of temperature and time applied at the technological processing stage. The analysis of the aroma of milk is especially complex due to the heterogeneous nature of milk. The effect of heating the milk changes the aroma profile of the milk. Raw milk contains aroma compounds that are not active in processed milk, whilst pasteurised and UHT milks develop aroma compounds that are not present in raw milk. The odourants present in milk can be used as indicators of the aroma quality of heated and raw milk (Brudzewski *et al.*, 2004).

Microbiological tests for testing the effects of varying pasteurisation times using total plate counts (Table 3.2), indicated that although all samples were within the legal standards of the South African industry, the electronic nose was able to detect a significant difference in the milk samples that was pasteurised for 9 min, indicating that the volatile chemistry of the milk had changed significantly at 9 min, after exposure to pasteurisation temperatures. Linear regression between bacterial count and time of pasteurisation showed a strong linear trend.

Studies using sensory evaluation for detecting pasteurisation changes in the aroma of milk, indicated that the predominant sensory perception detected at 5 -7 min was hexanal, however at 9 min of pasteurisation the predominant sensory perception noted by the panellists was nonanal (Table 3.4). The electronic nose was able to detect these differences in pasteurization at 9 min due to the quality factor of the milk sample being 2.263.

Gas chromatographic analysis of pasteurised milk identified the volatile compounds present in the milk samples and also revealed similar results as in sensory analysis. Volatile compounds frequently detected in milk samples included ethyl-2-butanoate, hexanal, nonanal, dimethyl sulphide and ethyl-2-hexanoate. However after 9 min of heat treatment all the compounds were present except ethyl-2-butanoate. These compounds occur in variable ratios at different stages, usually in low concentrations, because of the predominance of other volatiles that evolve from the milk matrix. This has also been

discussed by Ampeuro and Bosset, (2003) who found a complex mixture of organic volatiles (e.g. acetone at overwhelming concentration, hexanal, 2-butanone, toluene, limonene, heptanal, styrene, chloroform, etc) at varying concentrations and with a high percentage of relative humidity. Furthermore the matrix is highly heterogeneous containing different levels of lipids, proteins and carbohydrates.

4.1. Comparison of olfaction to gas chromatography

Comparing olfactory evaluation to gas chromatography it was found that gas chromatography detected at least one of the compounds identified during the olfactory evaluation. There was a common trend for gas chromatography to detect at least one compound identified by olfactory evaluation throughout the series of timed events. This shows that there is a correlation between olfactory analysis and gas chromatography. Olfactory evaluation is highly dependant on the panellist that is trained to detect each compound at varying threshold levels and human sense is subjective.

4.2. Electronic nose profiling for detecting heat treated milk

Results obtained using the electronic nose was reported as quality factors. The quality factor (QF) is a figure of which defines the distance between the centre of the clusters relative to the radius of the cluster. This value can be used as a specification for expressing the quality of a product. Any QF greater than 2 is considered to denote a statistically significant discrimination (Persaud *et al*, 2002). It must be known that the electronic nose has conducting polymer sensors which are non-specific, that is, each of the 32 sensors responds to all volatiles, but with varying degrees. Therefore the electronic nose does not claim to identify and quantify every volatile present, it responds to the overall volatile of the sample (Korel and Balaban, 2002). Results obtained in this study showed that in testing the effects of pasteurisation it was possible to detect changes in the milk samples as early as 9 min, and this was confirmed with chemical testing using the phosphatase test.

4.3. Evaluation of techniques for testing heat treatment of milk

In comparing the different methods used to evaluate the effects of heat treatment in milk, results obtained (Table 3.13) showed that when comparing GC to olfactory evaluation of raw milk, although GC detected ethanol, nonanal, dimethyl sulphide and ethyl-2-hexanoate, olfactory analysis was only able to identify odour profiles for ethyl-2-hexanoate and ethyl-2-butanoate. This could be due to other volatiles being present at very low concentrations and also to odours being fused for compounds resulting in a fused odour perception. For milk samples exposed to 9 min heat treatment time both olfactory analysis and gas chromatography were able to detect the predominant volatile compounds, showing that these compounds were present at values above human threshold concentrations. However, GC also detected ethyl-2-hexanoate and dimethyl sulphide which failed to be identified by olfactory evaluation.

When comparing the results obtained from gas chromatography and olfactory evaluation to the electronic nose, all methods are used in evaluating the volatile compounds, however the electronic nose obtains an overall odour profile, whilst the other two methods are odour sensitive. Also, it has to be noted that when using the electronic nose, changes in volatile compounds of the milk determine its quality factor. Statistical analysis was used to determine how effective the electronic nose was in detecting the predominant volatiles (nonanal, hexanal and indole) detected using gas chromatography and olfactory analysis. Results obtained (Table 3.14) showed that there is a 75% probability that hexanal is present, 50% for nonanal and 50% for indole, based on its positive predictive value. This shows that the electronic nose is an effective tool in evaluating the effects of pasteurisation.

Preferred methods of evaluation for raw milk, include microbiological testing and chemical analysis. These methods give more accurate results, due to milk meeting government standards by adhering to total plate counts and chemical tests, outlined. Olfactory analysis proved to be a valuable assessment method as it was effective in identifying compounds detected using gas chromatography. In determining pasteurisation time, the electronic nose can be effectively used since statistical analysis (Table 3.12) obtained 100% for sensitivity to the instrument.

Table 4.1: Comparison of results for total plate counts, olfactory analysis, quality factors and gas chromatographic analysis of volatile compounds present in raw milk and milk samples subject to varying heat treatment times

Sample	Total Plate Count	Olfactory Analysis		Aroma Profile	Gas Chromatography								
					Ethanol	Hexanal	Ethyl-2-Butanoate	Heptanal	Octanol	Nonanal	Dimethyl Sulphide	Ethyl-2-hexanoate	Indole
Raw milk	2340000	Ethyl hexanoate	Ethyl butanoate	0	+	-	-	-	-	+	+	+	-
Raw milk	2700000												
5 min heat treatment	3860	Hexanal	Ethyl Hexanoate	0.188	-	-	+	-	-	+	+	+	-
5 min heat treatment	3420												
7 min heat treatment	2980	Hexanal	Indole	0.859	-	+	+	-	-	+	+	+	-
7 min heat treatment	2940												
9 min heat treatment	2920	Nonanal	Hexanal	2.263	-	+	-	-	-	+	+	+	-
9 min heat treatment	2380												
11 min heat treatment	1760	Nonanal	Hexanal	2.404	+	+	+	-	+	+	+	+	+
11 min heat treatment	1680												
15 min heat treatment	1200	Nonanal	Indole	3.132	+	+	-	-	+	+	+	-	+
15 min heat treatment	1060												

It can be concluded from results obtained from the study that when testing the effects of heat treatment using the electronic nose as compared to conventional methods, a correlation exists between the electronic nose, olfactory evaluation and gas chromatography, since statistical analysis provided a value of 67% for accuracy. With respect to total plate count, it can be said that comparing results of the electronic nose to this method of assessment, as heat treatment times increased, so too did the quality values of the electronic nose. It can therefore be said that the electronic nose can be effectively used in determining pasteurised from partially heat treated milk and raw milk samples.

4.4. Evaluation of techniques for shelf life testing for milk

Microbiological tests for evaluating the shelf life of milk, total plate counts revealed that there was a steady increase in the number of micro-organisms as the day intervals increased, however these values were still within the allowable limits. Using the electronic nose as a method of detection of spoilage, quality factors obtained indicated that there was a significant difference in the sample on day 7. However, using visual examination milk started curdling on day 9 which was probably due to the action of bacterial enzymes, that survived heat treatment and that were present in the milk.

Barbano *et al.* 2006 reported that raw milk stored at a temperature of 6°C would be expected to have a detectable off-flavour at 28 days, even if the bacterial count was <20 000 cfu/ml, whereas pasteurised milk would not be expected to have an off flavour due to proteolysis until about 55 days. The author's findings demonstrated the importance of milk storage temperatures during shelf-life studies. The reduction in proteolysis with decreasing temperature was not linear and efforts to control milk temperature during storage are important in controlling lipolytic and proteolytic enzyme activity that survives pasteurisation. Our findings, showed that milk stored at 4°C started curdling at 9 days of storage, as indicated by microbiological and enzymatic changes (phosphatase test). Although the results indicate changes within 9 days, which are acceptable for accepting milk as normal, these values are important in the dairy industry as they would identify the risk of milk being unacceptable.

Olfactory evaluation carried out on milk to determine shelf life provided a wide variety of odour descriptors. These were then correlated to the organic compounds: ethyl-2- butanoate, ethyl-2-hexanoate and indole initially; and as the day intervals increased the volatile compounds changed to nonanal and hexanal, and eventually to diacetyl and butanoic acid. This could be due to nonanol and hexanal having human detection thresholds of 0.2-7 ppm (Ampuero and Bosset, 2003) and 5.22-6.48 ppm (Aardt *et al.*, 2005) respectively. Butanoic acid and hexanoic acid have human detection threshold limits of <2.5 ppm. The values listed above could be a possible reason why these compounds have been commonly detected by the panellists.

In assessing the milk for shelf life, gas chromatographic analysis detected a wide variety of volatile compounds. From days 1 to 7, the predominant volatiles detected were ethyl-2-butanoate, hexanal and nonanal, however from day 7 to 15, ethyl-2-hexanoate, indole, dimethyl sulphide and heptanal become predominant volatiles.

Research findings by Valero and co-workers, (2001) on the changes in flavour and volatile components during storage of whole and skimmed UHT milk, reported that the main volatile compounds found in whole milk, were methyl ketones with 3, 4, 5 and 7 carbon atoms. Acetone and butanone are supposed to be derived from bovine metabolism (Bassette *et al.*, 1996; Gordan and Morgan, 1972; Urbach and Milne, 1988). Dimethyl sulphide and dimethyl disulphide were also found, they are probably derived from methionine (Dumont and Adda, 1979) and have a strong contribution (along with methyl ketone) to the aroma of UHT milk (Badings, 1984). These have also been related to the intensity of thermal treatment (Bosset *et al.*, 1994). Acetaldehyde, butanal, hexanal and 2-methyl butanal were the main aldehydes found, while others, such as furfural and benzaldehyde were present in smaller proportions. Acetaldehyde has been reported in heated milk (Jaddou *et al.*, 1978). 3-methylbutanal and hexanal contribute moderately to the UHT flavour (Badings, 1984).

Jeon and colleagues. (1978) and Rerkrai *et al.* (1987) attributed a general increase of aldehydes, particularly hexanal, during storage to oxygen availability and storage temperature. Contarini *et al.* (1997) found a small decrease in hexanal and heptanal content of whole milk and UHT milks stored at room temperature for 90 days. The above-mentioned aldehydes originated from different reactions: hexanal is generated

by the oxidation of unsaturated fatty acids, furfural comes from lactose through the Maillard reaction, 2-methyl-butanal and 3-methyl-butanal originated from isoleucine and leucine by the action of enzymes, whereas benzaldehyde could be generated from phenylalanine. Alcohols could be formed by the reduction from the corresponding aldehydes (Nursten, 1997).

4.5. Comparison of the electronic nose and olfactory evaluation

A comparison of the electronic nose and olfactory evaluation showed that there is a trend for nonanal, indole and hexanal to be the prominent volatiles to be identified in the first few days (up to day 7). From day 7 onwards diacetyl is identified and from day 11 butanoic acid and diacetyl are identified.

4.6. Comparison of the electronic nose to gas chromatography

In all samples up to day 9, gas chromatography identified the same compounds as the sensory evaluation. On days 11, 13 and 15 the main compounds identified with olfactory evaluation were butanoic acid and diacetyl. These components were not tested for using gas chromatography, however they have been referenced from literature by Valero *et al* (2001) and their retention times were used in making comparisons with the gas chromatography.

4.7. Sensitivity and specificity of the electronic nose

The sensitivity of the electronic nose compared to olfactory evaluation was only 44%, 22%, 22%, 0% and 56% for hexanal, nonanal, indole, butanoic acid and diacetyl respectively. However the specificity for hexanal was 100%, nonanal was 71%, indole 29% and for butanoic acid and diacetyl it was 67% and 100% respectively. The accuracy of the electronic nose was 100% for hexanal, 78% for nonanal, 22% for indole, 67% for butanoic acid and 50% for diacetyl. These results are represented in Table 4.2.

Table 4.2: Sensitivity, specificity, positive predictive value, and negative predictive value of the electronic nose relative to olfactory evaluation for shelf life testing

	Prevalence ^a (%)	Specificity (%)	Sensitivity (%)	Accuracy (%)	Predictive Value (%)
Hexanal	44	100	100	100	100
Nonanal	22	100	71	78	50
Indole	22	0	29	22	0
Diacetyl	56	20	100	50	100
Butanoic acid	0	0	67	67	0

^a refer to Table 3.12 for definition of terms

4.8. Comparative evaluation of techniques used in determining shelf life of milk

Olfactory analysis carried out on fresh milk identified ethyl-2-butanoate and ethyl-2-hexanoate as predominant odours detected. Using gas chromatography there was a 100% correlation between the two methods of evaluation. After one week of storage (7 days) microbial counts were still within limits, however milk was most odour active at this time in storage. Gas chromatography detected volatile compounds ethanol, hexanal, heptanal, octanol, nonanal, ethyl-2-hexanoate, indole, butanoic acid and diacetyl. Olfactory evaluation identified hexanal and diacetyl as predominant odours. This once again shows that even though milk has so many volatiles present, using human odour detection only a small fraction of these compounds can be identified. The electronic nose also obtained a quality factor of 2.463 on day 7 showing that the volatile chemistry had changed significantly.

Evaluation of milk on week 2 (14 days) showed results for olfactory evaluation identifying diacetyl and butanoic acid as the predominant odours. Gas chromatography failed to identify any of these volatiles, but detected ethanol, heptanal, dimethyl sulphide and indole. Visual appearance of the milk at this stage was separation and curdling, which is due to the complete breakdown of proteins due

to the presence of ethanol. Electronic nose results showed that milk was significantly different from fresh pasteurised milk and obtained a quality value of 7.777.

The shelf life testing of milk, from a comparative study of all the techniques (Table 4.3) used it can be noted that the electronic nose detected changes in the milk samples as early as day 7, providing a quality value of 2.463. According to electronic nose prediction, it can be said that the milk on day 7, was unacceptable according to its volatile chemistry, which could be due to microbial and enzymatic activity in the milk sample. Visual examination of the milk revealed a white fluid, whilst sensory evaluation obtained a perception that was 'creamy'. All these results reveal that the milk was still wholesome or near to spoilage, however, the electronic nose was able to detect a change. Gas chromatography was used as an identification method, to identify which volatiles were present that could have lead to milk being undesirable.

Table 4.3: Comparison of total plate counts, olfactory analysis, quality factors and volatile compounds identified by gas chromatography present in milk samples tested for shelf life evaluation.

Sample	Total Plate Count	Sensory Analysis		Aroma Profile	Gas Chromatography										
Day Interval					Ethanol	Hexanal	Ethyl-2-Butanoate	Heptanal	Octanol	Nonanal	Dimethyl Sulphide	Ethyl-2-hexanoate	Indole	Butanoic acid*	Diacetyl*
Fresh Milk	2340000	Ethyl Hexanoate	Ethyl butanoate	0			+					+			
Fresh Milk	2700000														
1	1730	Nonanal	Indole	0 (0.806)			+			+					
1	2150														
3	2100	Hexanal	Indole	0 (1.072)		+	+								
3	2220														
5	2280	Nonanal	Hexanal	0 (1.677)	+	+				+					
5	2380														
7	2150	Hexanal	Diacetyl	1 (2.463)	+	+		+	+	+		+	+	+	+
7	2710														
9	2660	Hexanal	Diacetyl	1 (2.984)	+	+						+	+	+	
9	2700														
11	2570	Butanoic acid	Diacetyl	2 (3.612)	+			+	+	+	+	+	+	+	
11	2970														
13	3020	Butanoic acid	Diacetyl	3 (4.654)	+		+	+			+	+	+		
13	3400														
15	3210	Diacetyl	Butanoic acid	3 (7.777)	+			+			+	+	+		
15	3790														

*as reported by Valero *et al.*, (2001)

CHAPTER FIVE: REFERENCES

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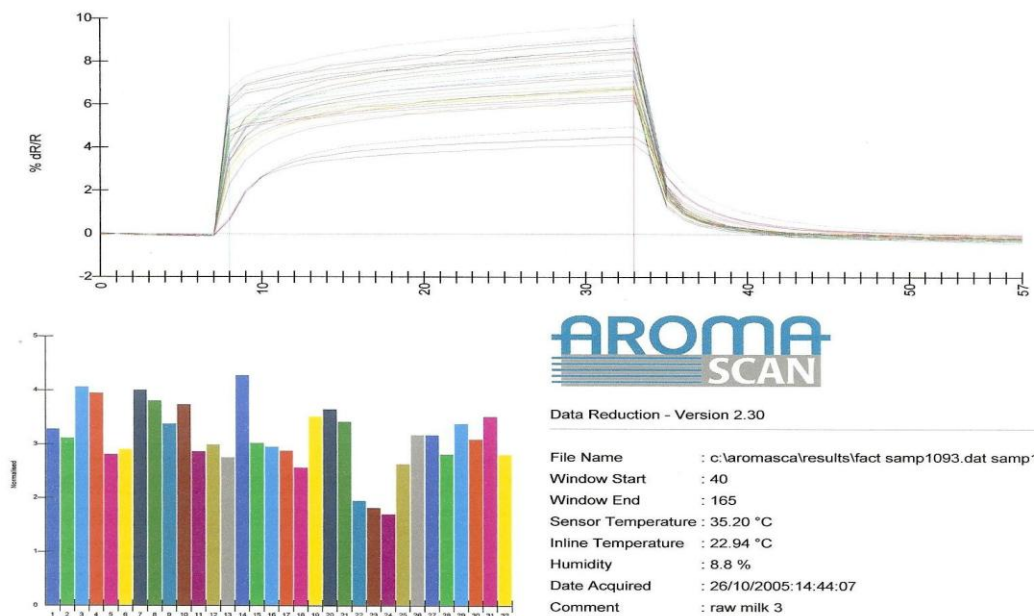
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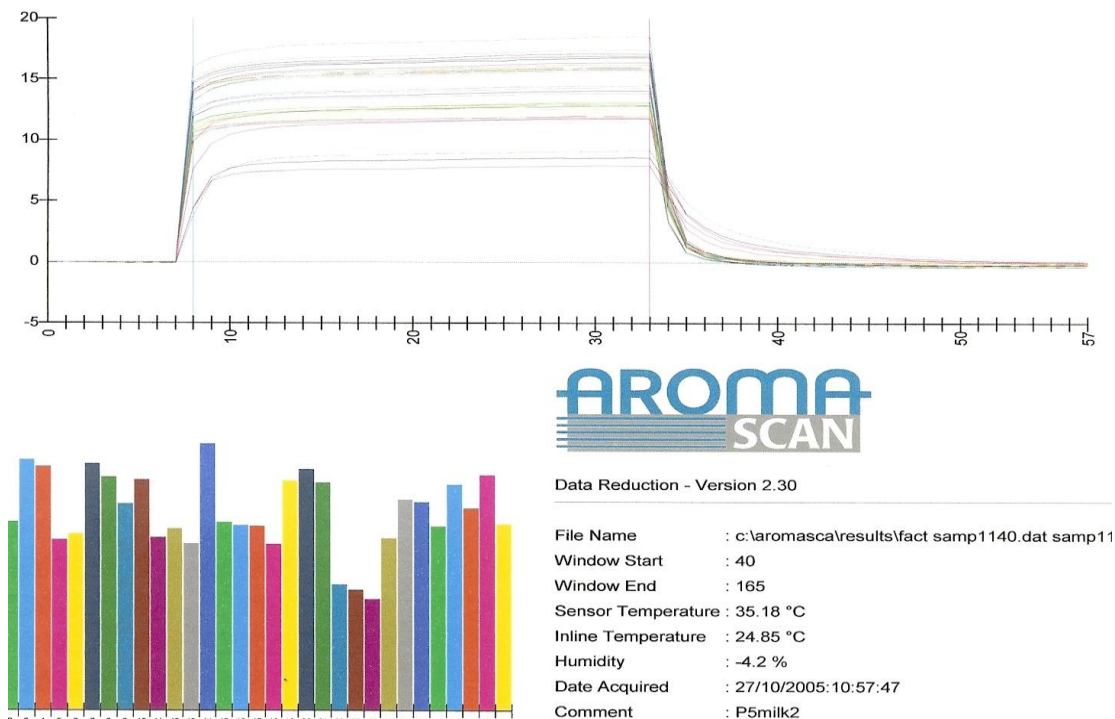
APPENDIX ONE

Data reduction showing a response of the 32 gas sensor array to the odours of milk samples detected.

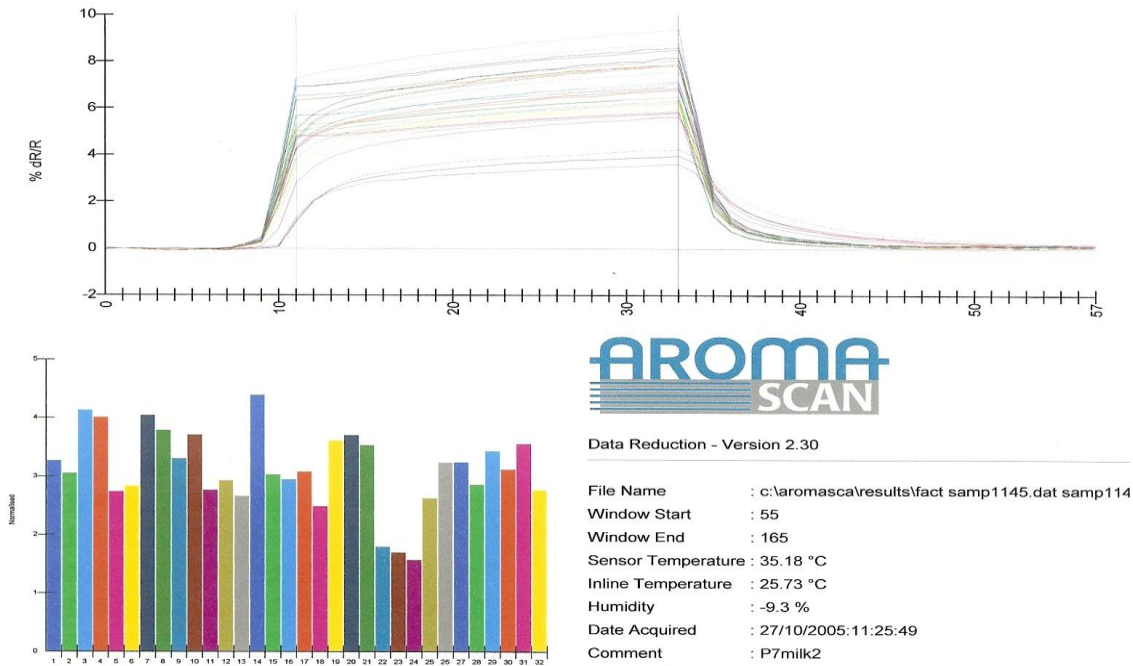
RAW MILK



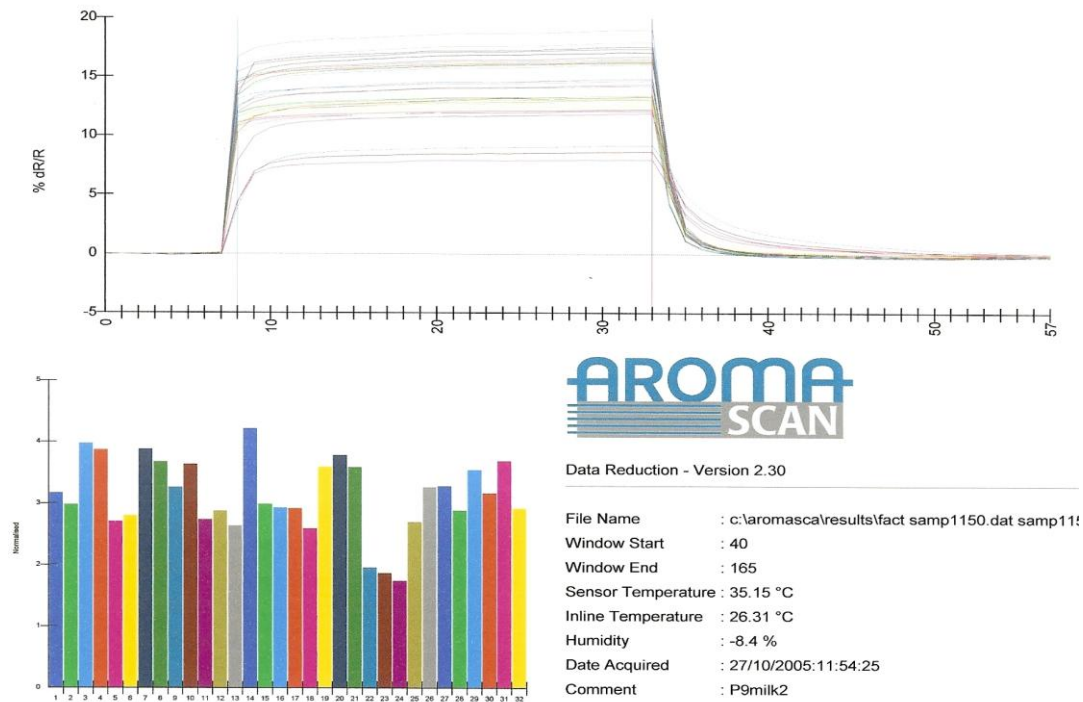
5 min PASTEURISATION



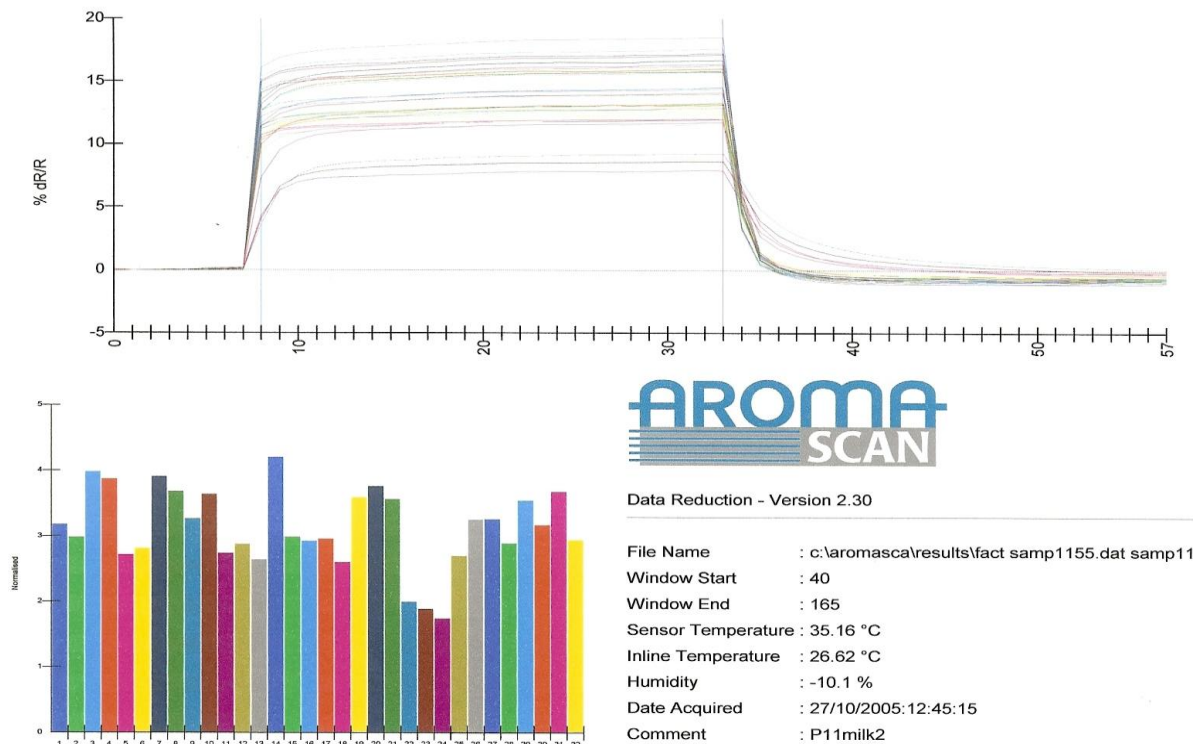
7 min PASTEURISATION



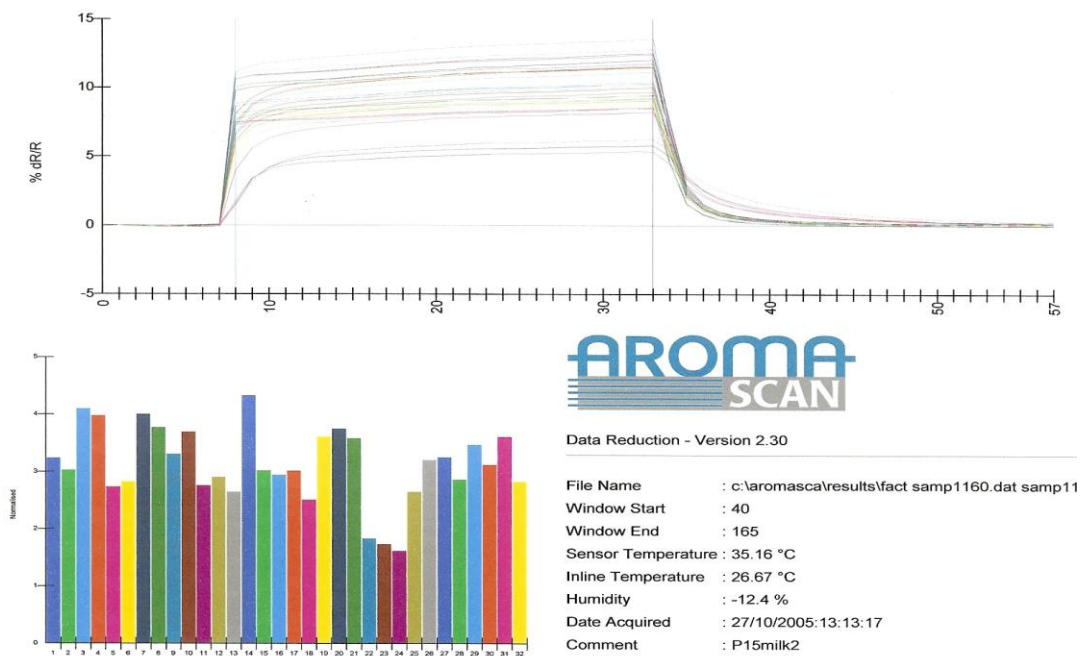
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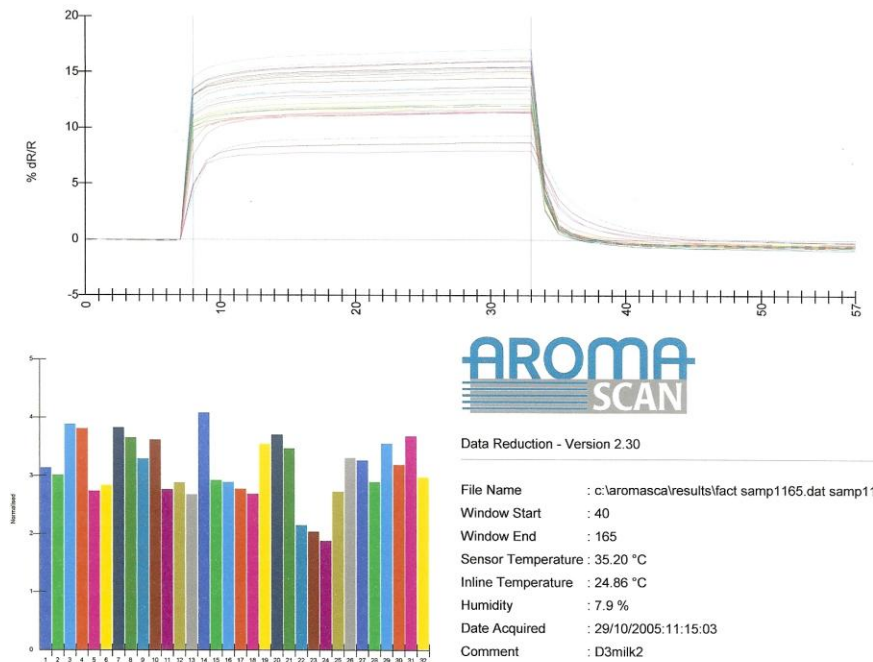
11 min PASTEURISATION



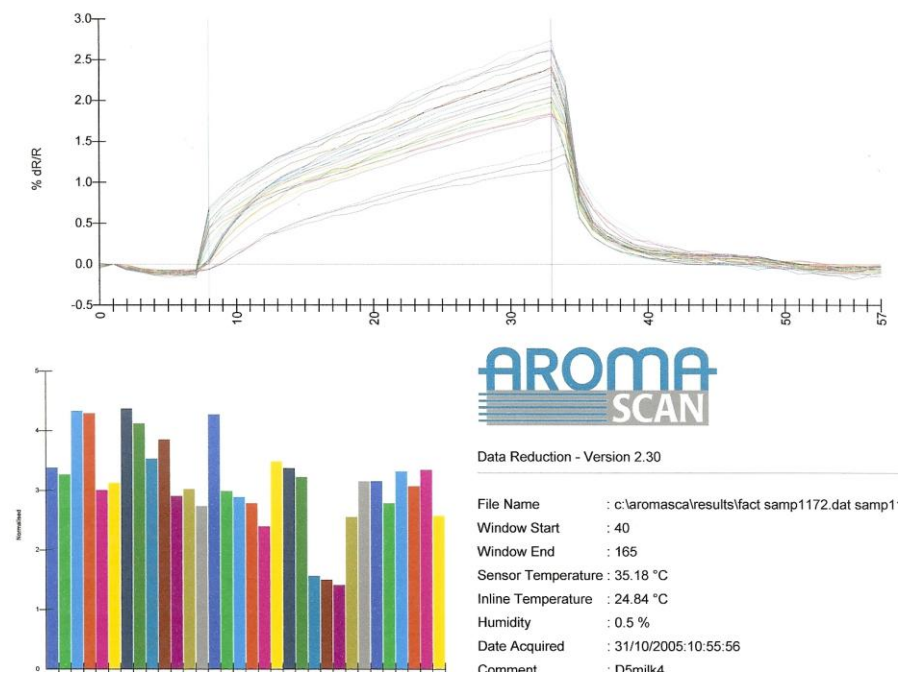
15 min PASTEURISATION



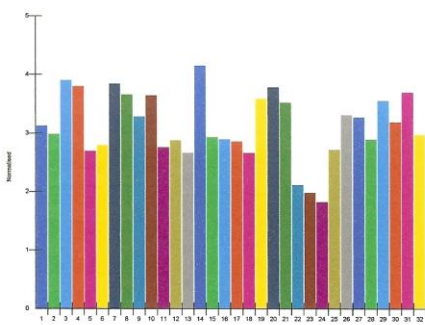
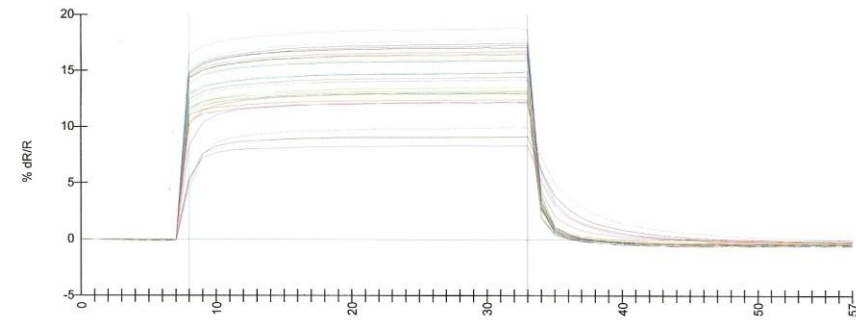
3 DAY MILK SAMPLE



5 DAY MILK SAMPLE



7 DAY MILK SAMPLE

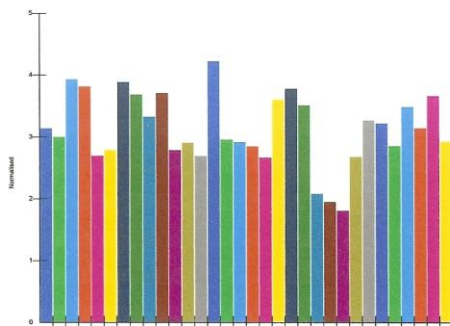
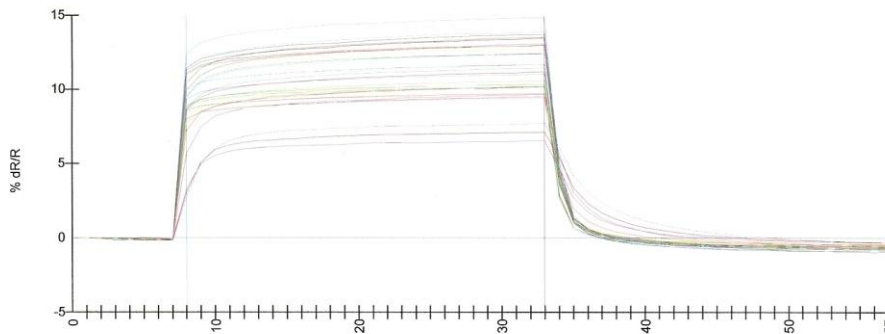


AROMA
SCAN

Data Reduction - Version 2.30

File Name : c:\aromasca\results\fact samp1177.dat samp1
Window Start : 40
Window End : 165
Sensor Temperature : 35.21 °C
Inline Temperature : 21.70 °C
Humidity : 9.5 %
Date Acquired : 03/11/2005:10:39:01
Comment : D7milk2

9 DAY MILK SAMPLE

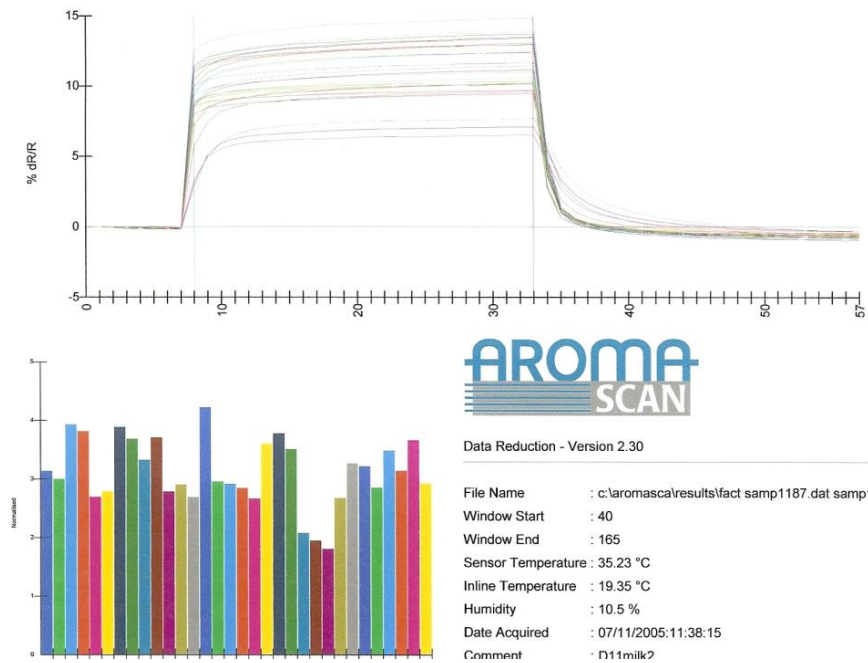


AROMA
SCAN

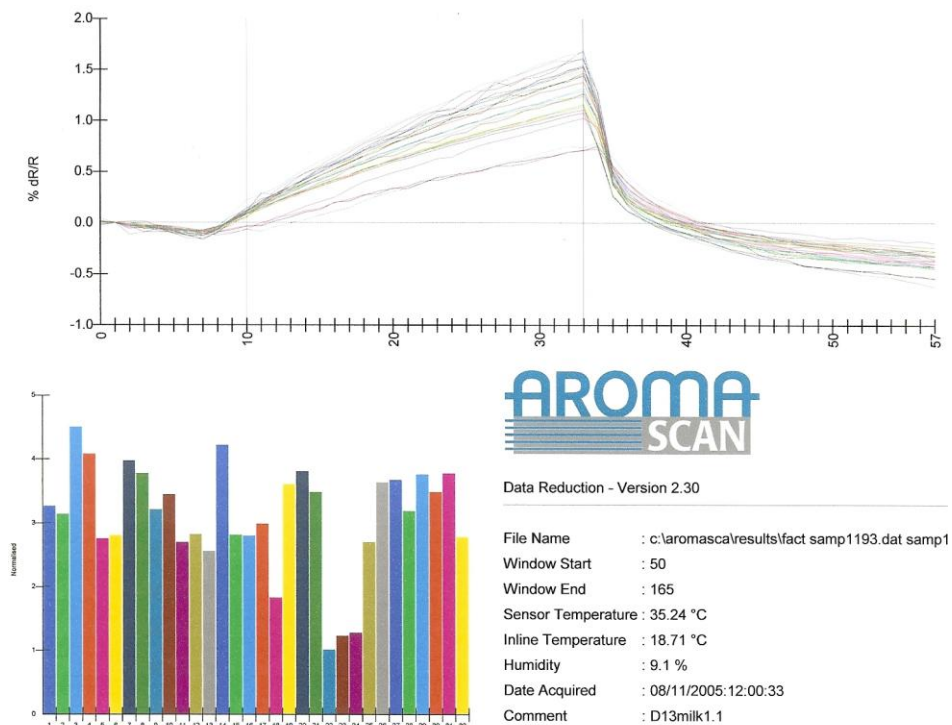
Data Reduction - Version 2.30

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Comment : D11milk2

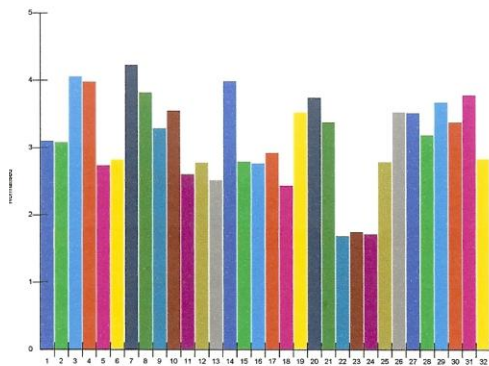
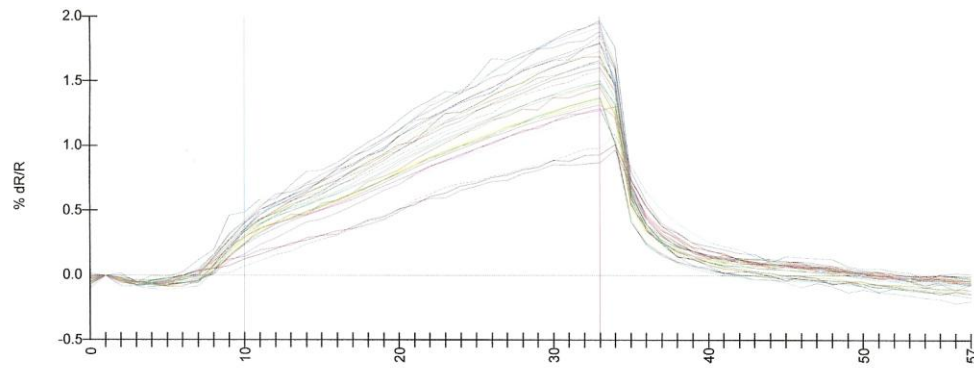
11 DAY MILK SAMPLE



13 DAY MILK SAMPLE



15 DAY MILK SAMPLE



AROMA
SCAN

Data Reduction - Version 2.30

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 Humidity : 0.7 %
 Date Acquired : 11/11/2005:08:34:22
 Comment : D15milk2

APPENDIX TWO

Olfactory Analysis – Aroma Sensing

Name: _____

Date: _____

Instructions:

1. Open sample just before sniffing.
2. Sniff the sample and record the odour that you perceive in the table below. (Choose only one odour)
3. Rate the strength of the odour if necessary.

SAMPLE NO.	DESCRIPTION OF ODOUR: Fruity, Sweet, Green, Fecal, Putrid, Musty, Floral, Mushroom Like, Spicy, Grassy Herbal, Coconut, Buttery, Creamy, Caramel, Boiled Potato Like, Malty, Cheesy, Rancid, Sweaty, Sour, Vinegar like, Sour Milk, Boiled Cabbage, Other _____.	STRENGTH OF ODOUR: Slight, Mild, Strong,