OBJECTIVE MONITORING OF MILK QUALITY USING A DYNAMIC HEADSPACE GAS CHROMATOGRAPH AND COMPUTER-AIDED DATA PROCESSING.

by

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ABSTRACT

This research was undertaken because of the need to develop an objective method for quality control of milk. A major problem in the dairy industry is off-flavour often found in milk. Quality control of milk is heavily dependent upon sensory evaluations supported by microbiological and chemical analyses. The chief purpose of this research was to demonstrate a simple and economical system for quality control of milk using a gas chromatograph and computer-aided data processing.

Two experiments were conducted: one using microbial off-flavours and another using chemically induced off-flavours. First, Ultra High Temperature (UHT)-sterilized milk was inoculated with *Pseudomonas fragi, Psuedomonas fluorescens, Lactococcus lactis, Enterobacter aerogenes, Bacillus subtilis* and a mixed culture (*L. lactis*: *E. aerogenes*: *P. fragi* = 1:1:1) with approximately 10^4 CFU mL^{-1}. The samples were stored at 4°C up to 10 days for *P. fragi* and *P. fluorescens* and at 30°C up to 24 hours for *L. lactis, E. aerogenes, B. subtilis* and the mixed culture. Several multivariate analyses were applied to the standaridized peak areas of GC data. A new multivariate analysis technique, principal component similarity analysis (PCS), was capable of classifying milk samples with regard to bacterial species and storage time. Artificial neural networks (ANN), partial least squares regression analysis and principal component regression

analysis were also applied. ANN provided the most accurate means of classification.

Secondly, pasteurized milk was treated to develop different off-flavours (light-induced, oxidized, cooked and heated) according to the procedures of the American Dairy Science Association. The same pasteurized milk samples as those used for gas chromatographic analysis were used for sensory evaluation. Gas chromatography (GC) combined with PCS was more effective than sensory evaluation as a means of distinguishing milk samples.

It was concluded that a combination of GC and chemometric methods may have great potential in evaluating the chemical and microbial quality of milk.

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CHAPTER I

INTRODUCTION

A. Background of the Study

Milk off-flavours present serious quality control problems in the dairy industry. Off-flavours may develop in milk for many reasons, including bacterial growth, the action of native milk or bacterial enzymes, as well as chemical changes catalyzed by light or metals (Badings and Neeter, 1980). Once these off-flavours develop in raw milk, it is very difficult to remove them and they remain after pasteurization. Heat stable enzymes liberated by gram negative psychrotrophic bacteria also bring about various defects: curdling in UHT or pasteurized milk (Cox, 1993), shortened shelf life (Smithwell and Kailasapathy, 1995), induction of various off-flavours (McKellar, 1981), abnormal cheese texture and reduced cheese yields (Auclair et al., 1981).

Gas chromatography (GC) is a very valuable technique for analyzing aroma compounds in foods. GC has not been applied in industrial quality control because of high purchase and operating costs. Recently a low-cost gas chromatograph has appeared on the market. A portable gas chromatograph (SRI Model 8610, SRI Instruments, Inc., Las Vegas, NV) contains a built-in purge and trap system and uses a computer as its integrator. Its flexibility and low cost make it attractive for quality control purposes.

This model GC has been applied to analyze mango (Vodovotz et al., 1993) and cheese flavour (Arteaga et al., 1994c). This suggested the suitability of the GC for volatile detction of milk samples.

Due to the enormous amount of data produced by automated instrumental analysis, efficient data-processing techniques are an absolute necessity in modern food analysis. The most useful solution for this increasingly critical analytical problem of data processing lies in multivariate analysis techniques. Various multivariate analyses have been applied in Food Science (Aishima and Nakai, 1991; Forina et al., 1987). They are principal component analysis (Aishima, 1979; Cadet et al., 1991; Headley and Hardy, 1989; Heymann and Noble, 1987; Lamberto and Saitta, 1995), cluster analysis (Aishima, 1991; Godwin et al., 1978; Mawatari et al., 1991; Resurreccion et al., 1987; Ulberth and Kneifel, 1992), factor analysis (Li-Chan et al., 1987; Resurreccion and Shewfelt, 1985; Rubico et al., 1988; Wu et al., 1977), multiple linear regression analysis (Biggs and McKenna, 1989; Hall and Andersson, 1985; Pierce and Wehling, 1994), discriminant analyses (Aishima, 1991; Bewig et al., 1994; Leland et al., 1987; Pham and Nakai, 1984; Powers and Keith, 1968; Vallejo-Cordoba and Nakai, 1994b), principal component regression analysis (Vallejo-Cordoba and Nakai, 1994a) and partial least squares regression analysis (Arteaga et al., 1994a; Baardseth et al., 1995; Banks et al., 1992; Defernez and Wilson, 1995; Martens et al., 1983; Servili et al., 1995). Recently artificial neural networks (ANN) have become the focus of interest in many disciplines (Arteaga and Nakai, 1993; Eerikäinen et

al., 1993; Horimoto et al., 1995; Norback, 1994; Sutherland, 1994; Zadow, 1994; Zhou et al., 1994). ANN is a new computer technique which simulates the function of the human brain. ANN is best suited to complicated classification, such as nonlinear classification. A new technique, genetic algorithm, also deserves attention (Hibbert, 1993; Lucasius and Kateman, 1993).

A new multivariate analytical technique, principal component similarity analysis (PCS), has been developed. It combines principal component analysis and pattern similarity computation. It was found useful for preliminary classification using mango (Vodovotz et al., 1993) and cheese (Furtula et al., 1994a; 1994b).

Combining increasingly more powerful computer hardware and software now makes it practical to construct an objective flavour evaluation system for routine quality control of milk.

B. The Problem

There are two major problems in the quality control of flavour in milk. (1) Characterization of the perceived flavour of milk is a complex task. Traditionally, an experienced person smells the milk and decides whether or not it has off-flavours. Although the nose is very sensitive, it has limitations. This method is subject to errors from differences among individual sensory assessments. (2) Microbial aspects of milk are very important in quality control. There are many tests for bacteriological quality

(Heeschen, 1991). The most common in the dairy industry are the Standard Plate Count and Psychrotrophic Bacteria Count. However, a minimum of 2 and 10 days respectively, are required to obtain results (Heeschen, 1991).

C. Need for the Study

The dairy industries have recognized the above problems and have developed more objective scoring systems for sensory evaluation (American Dairy Science Association, 1987). More objective methods have been sought through research on relationships between analytical data and flavour. In particular, gas chromatograms have been used as "fingerprints" of specific flavours or off-flavours. Computers have permitted the application of multivariate analyses for objective evaluation of flavour and classification of off-flavour. However, no systematic objective methods have been established for milk quality.

For microbial aspects of milk, there is a need for more rapid procedures for identifying the cause and extent of milk spoilage, which would facilitate routine quality control.

D. Objectives of the Study

The overall objective of this research was to demonstrate a simple and economical system of gas chromatography and computer-aided data processing for quality control of

milk. The specific objectives were to:

1. Evaluate the capability of a low-cost gas chromatograph for quality control of milk flavours.
2. Classify quality of milk based on a volatile profile measured by gas chromatography and determine possible causes of contamination.
3. Classify milk samples with respect to the presence of particular bacterial species.
4. Apply several multivariate analyses to find the most accurate classification method.

E. Plan of the Study

Two experiments were conducted: one using milk inoculated with various bacterial species and another using milk subjected to such factors as light, oxidation, cooking and heating.

A model system was established using Ultra High Temperature (UHT)-sterilized milk. Bacteria chosen were those which play an important role in the deterioration of milk. UHT milk was inoculated with *P. fragi*, *P. fluorescens*, *L. lactis*, *E. aerogenes*, *B. subtilis* and a mixed culture (*L. lactis*: *E. aerogenes*: *P. fragi* = 1:1:1) to approximately 10^4 CFU mL^{-1} and stored at 4°C for 10 days for *P. fragi* and *P. fluorescens* and 30°C for 24 hours for the remaining bacteria.

Pasteurized milk was treated to develop light-induced, oxidized, cooked and heated flavours according to the procedures recommended by the American Dairy Science Association (Shipe et al., 1978). The same pasteurized milk samples as those used for gas chromatographic analysis were also utilized for the sensory evaluation.

Several multivariate analyses were applied to classify milk samples. In particular, a new technique, principal component similarity analysis (PCS), was evaluated as a means to classify milk samples and predict possible causes of contamination.

F. Basic Assumption

The SRI gas chromatograph (SRI Model 8610, SRI Instruments, Inc., Las Vegas, NV) has been applied to analyze mango (Vodovotz et al., 1993) and cheese flavours (Arteaga et al., 1994c). It was found to give adequate chromatograms for classification. PCS was applied to gas chromatographic data to classify mango samples (Vodovotz et al., 1993) and high pressure liquid chromatographic data of cheddar cheese to identify quality defects (Furtula et al., 1994a; 1994b). This study began with the assumption that a combination of GC and multivariate analyses might be applied to solve problems 1 and 2, as mentioned earlier (p. 3 & 4).

CHAPTER II

REVIEW OF RELATED LITERATURE

A. Off-flavours in Milk

1. Introduction

Extensive research has been published on the causes and prevention of off-flavours in milk, but quality control problems persist in the dairy industry.

The flavour of fresh milk (raw or low-temperature pasteurized) cannot be easily defined in specific terms. It is characterized by the pleasant mouth-feel and a slight salty/sweet taste (Badings, 1984). A great number of odorous compounds in milk have been identified (Badings, 1984; Urbach, 1987; 1990). These belong to many different classes such as carbonyl compounds, alkanols, fatty acids, lactones, esters, sulfur compounds, nitrogen compounds and aliphatic and aromatic hydrocarbons. Most of them are present in sub-threshold concentrations and keep a delicate balance. If the concentration of one constituent varies considerably from its usual average content, off-flavours may occur (Badings and Neeter, 1980).

The source of off-flavours in milk are well described in several review papers (Badings and Neeter, 1980; Bassette et al., 1986; Bradley, 1980; Burton, 1983; Forss, 1971; 1979; Shipe et al., 1978; Thomas, 1981). In 1978, the American Dairy Science

Association's Nomenclature, Standards and Bibliography Committee categorized off-flavours according to the nature of the mechanisms in their production, along with terms generally employed to describe off-flavours in each category. On this basis, off-flavours were divided into seven categories: heat-induced, light-induced, oxidized, lipolyzed, microbial, transmitted and miscellaneous (Shipe et al., 1978). Using this nomenclature, Bassette et al. (1986) reviewed the flavour of both raw and pasteurized milk, including the effects of feeds, processing and storage conditions, the handling of milk and prevention and control of off-flavour development. Burton (1983) classified off-flavours into two major categories: off-flavours not related to storage and those developed during storage. Forss (1979), also reviewed the mechanisms of formation of major off-flavour compounds. In this review, off-flavours of milk will be discussed in relation to several causes: (1) chemical reactions, (2) microorganisms and enzymes and (3) extraneous compounds.

2. Flavour Defects Due to Chemical Reactions

Oxidized, light-induced and heat-induced off-flavours developed from chemical reactions.

Oxidized off-flavour. Oxidized flavour is often described as "cardboard" or "metallic" (Shipe et al., 1978). It develops from increased concentrations of carbonyl compounds resulting from the oxidative breakdown of unsaturated fatty acids in the

absence of light. There is abundant literature on the compounds isolated and identified from oxidized dairy products (Bassette et al., 1986; Nicholson, 1993). Oxidation of polyunsaturated fatty acids of phospholipids from milk fat globular membranes induces the formation of hydroperoxides which leads to the formation of hydrocarbons, alcohols, acids, aldehydes and ketones (Shipe et al., 1978).

Factors affecting development of oxidized flavour are feed, stage of lactation, milk handling and chemical processing (Bassette et al., 1986). Metallic ions, particularly copper, catalyze the reaction. A lower level of ascorbic acid in milk promotes copper-induced oxidation, but higher concentrations prevent the development of oxidized flavours (Shipe et al., 1978).

Light-induced off-flavours. Light-induced off-flavour in milk has been studied by many researchers and several good review articles have been written (Bekbölet, 1990; Hansen et al., 1975; Shipe et al., 1978). Light-induced flavour is divided into two categories. The first category is referred to as a "burnt", "activated" or a "sunlight" flavour. It develops rapidly by degradation of proteins. The second category is similar to an oxidized flavour and is related to lipid oxidation.

Burnt flavour develops initially and then the oxidized flavour becomes discernable after a few days. The major compound is 3-methyl thiopropanol. The intensity of light-induced flavour is dependent on wavelength, intensity, exposure time, container materials and levels of ascorbic acid and riboflavin (Shipe et al., 1978).

Homogenization makes milk more susceptible to degradation of serum proteins. A key component in light-induced flavour is methional resulting from the breakdown of the methionine in the presence of riboflavin (Forss, 1979)

Much research has been conducted on oxidized flavour due to photooxidation of lipids (Bekbölet, 1990; Nicholson, 1993). Factors affecting development of the burnt flavour also influence the oxidized flavour, except for homogenization and ascorbic acid. The compounds responsible for photoxidation of lipids are different from those responsible for oxidized off-flavour development by metal catalysis. It has been postulated that photoxidation involves the monoene fatty acids of triglycerides, while oxidized flavour involves polyenes of the phospholipids (Shipe et al., 1978).

Heat-induced off-flavours. Heat-induced flavours are mostly the result of thermal treatments. Heat treatment completely inactivates enzymes and destroys the most heat-resistant microorganisms. However, it causes heat-induced off-flavours. The heat-induced flavours have been classified into four types: (i) cooked or sulfurous, (ii) heated, (iii) caramelized and (iv) scorched (Bassette et al., 1986; Shipe et al., 1978). The nature and intensity of the off-flavour depend on duration and temperature of heat treatment as well as heating methods used.

Chemical compounds associated with heat-induced flavours are complex. Proteins and lipids are sources of heat-induced off-flavour. Sulfur compounds have been considered major contributors to heat-induced flavours in milks. A number of

sulfur compounds can be readily formed in milk from sulfur-containing amino acids if sufficient heat above the pasteurization temperature is applied. Hydrogen sulfide is responsible for cooked flavour. However, this flavour dissipates after a few days of refrigerated storage. Diacetyl, lactones, methyl-ketones, maltol, vanillin, benzaldehyde and acetophenone are present in milk which has been heated and contribute to the development of the heated flavour (Forss, 1979; Shipe et al., 1978).

3. Flavour Defects due to Microorganisms and Enzymes

Microbial off-flavours. Flavour defects from microbiological metabolism can develop at any stage of production or processing. The descriptive terms include "acid", "malty", "fruity", "unclean", "bitter" and "putrid" (Shipe et al., 1978). Acid, malty and fruity can be recognized by sensory perception alone. The nature and intensity of the microbial defects depend on the types and numbers of contaminating microorganisms and on the by-products associated with the organisms. There are a number of contamination sources: (i) environmental conditions of the dairy farm, (ii) health of the animal, (iii) equipment and (iv) personnel related to the collection, storage and transportation of milk to processing plants (Jeon, 1993). The type and number of organisms associated with processed dairy products are influenced by the time and temperature of processing, and by post-pasteurization contamination from equipment,

containers, environment and personnel, as well as storage temperature and handling of the product by truckers, sales personnel and consumers.

If the milk is not cooled below 4°C, it will develop an acid taste because of *Lactoccocus lactis*. Malty flavour results from the metabolism of *Lactoccocus lactis subsp. maltigenes* (Shipe et al., 1978). The major component of this flavour defect is 3-methyl butanol. Fruity flavour develops as a result of metabolism of *Pseudomonas fragi* (Shipe et al., 1978). The major components are ethyl butyrate and ethylhexanoate (Hosono et al., 1973; Pierami and Stevenson, 1976; Reddy et al., 1968; Toan et al., 1965; Wellnitz-Ruen et al., 1982).

Enzyme-induced off-flavour. Lipolyzed flavour results from hydrolytic cleavage of fatty acids from milk fat triglycerides by lipase enzymes. Lipase may be inherent in milk or produced by psychrotrophic microorganisms. Several terms, used to describe lipolyzed flavour, are "rancid", "goaty", "soapy", "butyric" and "bitter" (Shipe et al., 1978). Since lipolysis is a term to describe lipase catalyzed hydrolysis of triglycerides, it is recommended that "lipolyzed flavour" be used to describe the lipase induced off-flavour (Bassette et al., 1986).

There is a sufficient amount of lipase in milk to cause flavour defects. However, the enzyme known as lipoprotein lipase is not normally active as the milk leaves the cow, because the substrate and enzymes are well partitioned and a multiplicity of factors affect the enzyme activity. The factors that affect the activity of lipase include

(i) the stage of lactation, (ii) temperature manipulation and (iii) handling, storing and processing milk (Jeon, 1993).

Raw milk is usually stored at 4°C or lower in insulated silos for 2 days or longer before processing. The flora of stored milk becomes dominated by psychrotrophic bacteria. Many psychrotrophs produce heat-resistant extracellular enzymes, which can attack both milk fat and proteins and subsequently reduce the quality and shelf life of heat treated milk and dairy products made from such highly contaminated milk (Auclair et al., 1981; Cox, 1993; Deeth, 1993; McKellar, 1981; Smithwell and Kailasapathy, 1995). The effects of bacterial proteinase and lipases on milk and milk products have been documented in several reviews (Bishop and White, 1986; Cousin, 1982; Downey, 1980; Fairbairn and Law, 1986; Law, 1979; Shah, 1994; Stead, 1986).

4. Flavour Defects Due to Extraneous Components

Transmitted off-flavours result from the transfer of substances from the cow's feed or environment into the milk. They can be transmitted either indirectly in the udder via the respiratory and digestive system by way of the blood stream, or directly by contact with the product after milking. These off-flavours are grouped into four flavours: "feed", "weed", "cowy" and "barny" (Shipe et al., 1978).

Feed flavour develops from cows consuming feeds with strong odours of feeds such as silage or green forages in the few hours before milking. Weed flavour results

from the consumption of such things as wild garlic and wild onions. These flavour defects are the result of increased quantities of benzyl mercaptan, methyl mercaptan, dimethyl sulfide, dimethyl disulfide, indole, skatole and trimethylamine (Badings and Neeter, 1980). Urbach (1990) reported the effects of the different feeds on milk flavour. Cows suffering from ketosis or acetonemia produce the cowy flavour (Bassette et al., 1986). Barny flavour develops from cows housed in unventilated areas (Bassette et al., 1986).

Some flavours cannot be attributed to a specific cause or defined in sensory terms. These are termed "miscellaneous off-flavours". Absorbed, astringent, bitter, chalky, chemical, flat, foreign flavours, unfresh and salty flavours belong to this category. These off-flavours are well reviewed elsewhere (Bassette et al., 1986; Shipe et al., 1978).

B. Detection of Off-flavours

1. Introduction

Remarkable progress in analytical instrumentation has benefited flavour research. In the mid-1950's, gas chromatography (GC) was implemented for separating flavour mixtures into individual components. Then in the 1970's, spectrometric instruments were coupled with GC. Mass spectrometry, infrared spectrophotometry and nuclear magnetic resonance spectroscopy were interfaced with GC to identify individual compounds. Among them, gas chromatography-mass spectrometry has become very popular due to its efficiency in the separation and identification of volatile compounds (Aishima and Nakai, 1991).

Since the development of the advanced instrumentation and computer techniques, many researchers have dealt with subjective-objective relationships of flavour. They have used both GC and sensory evaluation to determine relationships and have pointed out that a combination of GC, mass spectrometry and computer data analysis is becoming an efficient system for their areas of research (Kwan and Kowalski, 1980; Lee-Yan et al., 1991; Lin et al., 1993; Rubico et al., 1988).

2. Gas Chromatographic Method

Gas chromatography has been the most suitable method for the separation of flavour concentrates to individual compounds (Aishima and Nakai, 1991). Several

workers have investigated the headspace volatiles from milk. Jennings et al. (1962) were among the first to successfully analyze milk vapour by GC. Off-flavours were characterized by the presence of certain chromatographic peaks. These peaks were detected as off-flavours from oxidation, light induction and transmitted off-flavours. Bassette et al. (1963) also investigated the possibility of headspace gas chromatography for studying off-flavours in milk. Samples of fresh and stored raw milk, with and without off-flavours, were analyzed successfully. Chromatograms were recorded for analyses of rancid, oxidized, sunlight-oxidized and high acid milk. Urbach (1990) investigated headspace volatiles from cold-stored raw milk. Analysis of headspace volatiles was suggested for assessing the quality of cold-stored raw milk. Recently gas chromatography was used for an assessment of UHT-sterilized milk (Lopez-Fandino et al., 1993).

Headspace gas chromatographic analysis is currently the most efficient method for analysis of aroma volatiles (Badings et al., 1985; Lee et al., 1995; Lopez-Fandino et al., 1993; Rizzolo et al., 1992; Urbach, 1987; 1990; Vallejo-Cordoba and Nakai, 1994a). This technique could be useful for investigating off-flavours and the cause of milk spoilage.

The presence or absence of abnormal quantities of microbial metabolites in spoiled food may show whether or not the spoilage was caused by microorganisms. Direct gas chromatographic analysis of biologically produced volatiles in the headspace

vapour of several foods has been used for the characterization and the identification of several bacterial species (Bassette et al., 1967; Eyles and Adams, 1986; Guarino and Kramer, 1969; Lee et al., 1979; Schafer et al., 1982; Zechman et al., 1986).

The feasibility of using GC as a means of studying bacteria in milk has been shown by several researchers. Characterization of bacteria in milk by measuring their volatile metabolites with GC was first employed by Bassette and Claydon (1965). Bawdon and Bassette (1966) studied the effect of growth of *Escherichia coli* and *Aerobacter aerogenes* on milk quality by direct analysis of headspace vapour containing volatile compounds produced during growth. Bassette et al. (1967) also measured volatile compounds produced at different periods of bacterial growth in milk to characterize various microorganisms. They discussed the advantages of gas chromatographic methods in studying bacterial metabolism.

Possible origins of contamination of raw milk are numerous but the main source of psychrotrophic microflora could be attributed to the inadequate disinfection of milking equipment (Cousin, 1982). Growth of psychrotrophic organisms can lead to development the fruity off-flavour in milk. Several researchers have analyzed the fruity off-flavour in milk using headspace gas chromatography (Hosono et al., 1973; Pierami and Stevenson, 1976; Reddy et al., 1968; Toan et al., 1965; Wellnitz-Ruen et al., 1982).

3. Relationship Between Subjective and Objective Methods

Food quality may be directly measured by subjective sensory methods or indirectly through analytical measurements. Traditionally, quality control was dependent on sensory evaluations (Badings and Neeter, 1980). Much research has attempted to develop objective tests for quality evaluation, because sensory evaluation is subject to errors in individual assessments. Objective methods for diagnosing milk flavour will not replace subjective sensory analysis completely, but will be a valuable supplement.

In objective methods for flavour analysis, some characteristics other than flavour have to be measured. Therefore considerable research has focused on correlations between flavour and flavour compounds. This has shown significant correlation between gas-liquid chromatographic patterns and sensory evaluations for flavour. Keller and Kley (1971) showed a 0.93 correlation coefficient between haylike flavour scores and total peak areas of gas chromatograms for milk samples. Jaddou et al. (1978) also reported a high correlation between the total volatile sulfur compounds per mL milk and intensity of "cabbage" off-flavours in heat treated milk. Rubico et al. (1988) evaluated relationships among sensory quality attributes and gas chromatographic peaks obtained from a peanut beverage. The sensory attributes were related to changes in various chromatographic peaks. Aishima (1979) reported that evaluation and discrimination in sensory tests of soy sauce could be performed by

comparing the profiles of aroma substances. Gas chromatographic and sensory analyses were also investigated in the study of black tea (Gianturco et al., 1974), carrot flavours (Simon et al., 1980) and peach (Spencer et al., 1978),

In addition to gas chromatograms, attempts have been made to correlate chemical indices and flavour quality. Woo and Lindsay (1983) developed a method for predicting hydrolytic rancidity off-flavours in butter by correlating quantitative sensory data with individual free fatty acid concentrations using stepwise regression and discriminant analyses. They concluded that their methods should be applicable to indexing hydrolytic rancidity of off-flavours in other dairy products. Collins et al. (1993) investigated the influence of psychrotrophic bacteria counts in raw milk on the sensory acceptance of UHT skim milk. The psychrotrophic counts in the raw milk were strongly correlated with the extent of proteolysis ($r = 0.95$) but not with the extent of lipolysis ($r = 0.18$) in the stored UHT milk. They showed good correlation between extent of proteolysis and bitterness scores in the stored UHT milk. Hankin and Anderson (1968) studied correlations between flavour score, standard plate count and oxidase-positive organisms count in pasteurized milk. They found a significant correlation between flavour scores and oxidase positive organisms count.

Several other papers correlate sensory and chemical data in foods: wines (Kwan and Kowalski, 1980), herb oil (Pino et al., 1995), blueberry-whey beverage (Powers

and Quinlan, 1974), grape jelly (Quinlan et al., 1974), tomato (Resurreccion and Shewfelt, 1985), fish (Sawyer et al., 1984) and oil (Waltking and Goetz, 1983).

Techniques applied in the above papers ranged from relatively simple correlations to multivariate analyses. Simple correlation methods are suitable for some foods, because one or two compounds are related closely with flavour. With multiple objective variables, factor analysis and cluster analysis have been applied to decrease the number of sensory terms and determine the relationships among physicochemical measurements and sensory qualities of several foods (Godwin et al., 1978; Resurreccion et al., 1987). An overview of multivariate analyses for relating sensory to instrumental data can be found in Dijksterhuis (1995).

C. Computer-aided Data Processing

1. Introduction

In most modern chemical analyses, data are collected and stored under computer control. The enormous amounts of information with dozens of parameters require effective techniques for data-processing.

Chemometrics is a mathematical technique for data processing in chemistry. It was developed in 1974 by Kowalski and Wold (Jeon, 1991). Chemometrics is classified into 11 subdivisions according to the American Chemical Society: statistics, optimization, signal processing, factor analysis, resolution, calibration, modelling and parameter estimation, structure-property relationships, pattern recognition, library searching and artificial intelligence (Aishima and Nakai, 1991).

Pattern recognition is defined as the study of data sets to find regularities and similarities inherent to the data. It also refers to the mathematical analysis and modelling of multivariate data to infer or predict properties of a system from indirect measurements (Jeon, 1991; Page, 1986).

Various multivariate analysis techniques have been used for pattern recognition. There are two major classifications, supervised and unsupervised learning methods. The purpose of the unsupervised learning method is to cluster variables or samples into groups that may be mutually related. In supervised learning methods, variables or samples are classified into known groups. Several review papers have been published.

Forina et al. (1987) reviewed the theory and application of chemometrics in food chemistry. Chemometrics for flavour analysis was discussed by Aishima and Nakai (1991). Applications of pattern recognition for quality control can be found in papers by Bailey and Rohrback (1994), Jeon (1991), Page (1986) and Resurreccion (1988).

Principles and theories for each method are well covered in books and review papers (Aishima and Nakai, 1991; Dillon and Goldstein, 1984; Forina et al., 1987; Manly, 1986; Martens and Naes, 1989). In this review, applications for each method will be mainly discussed.

2. Pattern Recognition

a) Supervised Learning Methods

Supervised learning methods assume that the user has information about the groups prior to application of the algorithms. Multiple regression analysis (MRA) (Biggs and McKenna, 1989; Hall and Andersson, 1985; Pierce and Wehling, 1994), canonical correlation analysis (CCA) (Aishima, 1979; Capilla et al., 1988), linear discriminant analysis (LDA) (Aishima, 1991; Bewig et al., 1994; Leland et al., 1987; Pham and Nakai, 1984; Powers and Keith, 1968; Vallejo-Cordoba and Nakai, 1994b), principal component regression analysis (PCR) (Vallejo-Cordoba and Nakai, 1994a), partial least squares regression analysis (PLS) (Arteaga et al., 1994a; Baardseth et al., 1995; Banks et al., 1992; Defernez and Wilson, 1995; Martens et

al., 1983; Servili et al., 1995) and artificial neural networks (ANN) (Arteaga and Nakai, 1993; Eerikäinen et al., 1993; Horimoto et al., 1995; Norback, 1994; Sutherland, 1994; Zadow, 1994; Zhou et al., 1994) have been used for supervised pattern recognition.

In learning the process, a training set is used to develop classification rules which are used to predict properties of unknown samples. The disadvantage of this method is that classification or prediction is dependent on a training data set. If the training set is not representative, an unknown sample may not be classified correctly.

Linear discriminant analysis (LDA). The purpose of this method is to select the variable of greatest discriminatory value and use it for a classification (Aishima and Nakai, 1991). In many instances, stepwise linear discriminant analysis (SLD) is used as a procedure to combine variables which have significant discriminating power for classification purposes. Discriminant analysis techniques have been applied to liquor (Aishima, 1991), vegetable oils (Bewig et al., 1994), oxidized flavour in milk (Leland et al., 1987), cheese (Pham and Nakai, 1984), coffee and potatoes (Powers and Keith, 1968), milk (Smeyers-Verbeke et al., 1977) and shelf life of milk (Vallejo-Cordoba and Nakai, 1994b).

Principal component regression analysis (PCR). PCR is a combination of principal component analysis and linear regression analysis. It provides the possibility of relating blocks of variables and allows an unknown pattern to be

classified and predicted (Aishima and Nakai, 1991). PCR was applied for the prediction of shelf-life for pasteurized milk with a standard error of estimate of 1.3 days within the anticipated shelf-life of 21 days (Vallejo-Cordoba and Nakai, 1994a).

Partial least squares regression analysis (PLS). PLS is one of several multivariate calibration techniques. It is based on double principal component analysis: Y is used to extract latent variables from X, which are in turn used for modelling both X and Y (Aishima and Nakai, 1991). A detailed description of the technique can be found in Martens and Naes (1989).

Martens et al. (1983) applied PLS for determining relationships between different dependent variables and sensory descriptive independent variables of cauliflower. PLS showed that texture preference was predicted by texture descriptors and flavour. Banks et al. (1992) applied PLS to gas chromatographic data from cheddar cheese. An excellent correlation was found between gas chromatographic data and sensory scores of cheese samples with various ages. Ninety-eight percent of the total variation in the maturity scores was accounted for by the model including both volatile and measurements of proteolysis using PLS. On the other hand PCR did not yield reliable predictions for the same data set. Arteaga et al. (1994a) applied PLS to fourth derivative ultraviolet spectrums to determine the composition of protein mixtures. They found a good correlation

between measured and predicted protein composition. The standard error of prediction for 16 test samples were 13.4, 5.5 and 11.9% for α_{s1}-, β- and κ-casein respectively, and the correlation coefficients between measured and predicted composition was 0.91, 0.99 and 0.94 for the three proteins.

PLS has recently been used to study prediction of the quality of carrot chips by chemical composition (Baardseth et al., 1995), prediction of type of fruit used in jam by the fourier transform IR spectra (Defernez and Wilson, 1995) and prediction of sensory and headspace composition of virgin oil (Servili et al., 1995).

Artificial neural networks (ANN). Recently, artificial neural networks have become the focus of interest in many disciplines including Food Science. The ANN are computer techniques which simulate the massive parallel structure of the brain (Lawrence, 1991). There are two principal types of network architecture: feed forward and feed back (Lawrence, 1991). The most popular method is by example and repetition, also called back-propagation network (BPN). BPN has been extensively studied, both theoretically and experimentally and has been by far the most successful system (Jansson, 1991; Lawrence, 1991; Wythoff, 1993).

The BPN technique is one example of supervised learning in feed forward networks, in which the learning rule is a mathematical equation known as the delta rule, or the related least mean squares rule, which minimizes errors between the known values and the network responses (Lawrence, 1991). The BPN is usually

built from three type layers: input, hidden and output. The first layer, called the input layer, takes the input values of a pattern. The last layer, called the output layer, produces the pattern outputs. The layers between are called the hidden layers. Each layer has neurons, which are also called processing elements, units or cells. The strength of a connection between two neurons is called the weight, which determines the magnitude of effect which one neuron can have on the other (Lawrence, 1991). The weighted signals are summed to form a net value. Usually they are simply added together. Total input is run through the activation function, which specifies what the neuron is to do with the signals after the weights have had their effect (Lawrence, 1991). The transfer function is then applied to the activation values to produce output. In a training sequence, the output of the network is compared to known values and errors are back-propagated to the hidden and input layers to adjust the weights and minimize the error. This is repeated many times until the errors between the output and known values are minimized. General reviews and references can be found in Jansson (1991), Lawrence (1991) and Wythoff (1993).

Eerikäinen et al. (1993) reviewed applications for the control of various food processes, ranging from fermentation and extrusion cooking to the drying of cereal grains. Applications of neural networks for the dairy industry can be found in several papers (Norback, 1994; Sutherland, 1994; Zadow, 1994; Zhou et al., 1994).

Several researchers reported comparison of neural networks with other multivariate analyses. A neural network produced better simulation of experimental foam capacity of food proteins than did PCR (Arteaga and Nakai, 1993). Horimoto et al. (1995) also reported better prediction ability of a neural network than PCR for wheat quality for breadmaking.

b) Unsupervised Learning Methods

The unsupervised learning methods do not require information for classification. These methods cluster individual samples based on similarity or distance among their data (Aishima and Nakai, 1991). They are the best approach if the data have been collected over time without any definite experimental designs or any models in mind.

The three major unsupervised learning methods used in food science research are principal component analysis (PCA), factor analysis (FA) and cluster analysis (CA). Recently, a new multivariate analysis technique, principal component similarity analysis (PCS), was developed (Vodovotz et al., 1993).

Principal component analysis (PCA). PCA is a technique to reduce dimensionality of the data. It computes a few linear combinations of the original variables which can be used to summarize the data with minimal loss of information (Manly, 1986). PCA has been applied for soy sauce (Aishima, 1979), sugar cane

(Cadet et al., 1991), whisky (Headley and Hardy, 1989), wines (Heymann and Noble, 1987) and oil (Lamverto and Saitta, 1995).

Factor analysis (FA). This is a technique which is most commonly used for data reduction and simplification in food quality studies. It reduces a large number of variables to a smaller set of new variables called factors. Each factor in their reduced set is highly correlated with a particular subset of interrelated variables. FA has been used in the study of muscle protein (Li-Chan et al., 1987), tomatoes (Resurreccion and Shewfelt, 1985), peanut beverage (Rubico et al., 1988) and wine (Wu et al., 1977).

Cluster analysis (CA). This is a general term for procedures which classify variables or cases according to some measure of similarity (Ennis et al., 1982). The variables within a cluster are highly associated with one another, while those in different clusters are relatively distinct from one another. Several clustering methods are used. Manly (1986) and Dillon and Goldstein (1984) described different algorithms for cluster analysis which differ greatly in theory and practice. CA has been applied to liquor (Aishima, 1991), green beans (Godwin et al., 1978), beer (Mawatari et al., 1991), snap beans (Resurreccion et al., 1987) and yogurt (Ulberth and Kneifel, 1992).

Principal component similarity analysis (PCS). This technique is relatively new. It combines principal component analysis (PCA) and pattern similarity

computation (Aishima et al., 1987). PCS was applied to gas chromatographic data to classify mango samples (Vodovotz et al., 1993). Data from HPLC analysis of cheddar cheese was analyzed by PCS to identify quality defects (Furtula et al., 1994a; 1994b). More detailed descriptions of PCS are in the MATERIALS AND METHODS section (p. 40 & 41).

3. Optimization

Optimization is defined as a method to find the best combination of independent variables to maximize (or minimize) a numerical function (objective formula) of a set of variables (Nakai, 1990).

Optimization methods can be classified into two categories: the group of methods based on curve fitting and the group of evolutionary operations based on sequential process (Nakai, 1990). The curve fitting methods use a search for the points with zero slope on the fitted curves by derivatizing the working equations. Mixture design, linear programming and response surface methodology belong to this category.

Evolutionary operation is a method with many applications, especially in research and development. There is no need for a working theory of the phenomena under study or for descriptive equations to be known in advance. Simplex and random centroid optimization (RCO) belong to this category. RCO was developed by modification of simplex optimization. Simplex optimization incorporating a mapping

process (Nakai et al., 1984) was further extended by applying a constraint (Vazquez-Arteaga and Nakai, 1990) and finally a centroid concept (Aishima and Nakai, 1986). Recently RCO was modified for multifactors and food formulation purposes (Dou et al., 1993). It can deal with up to 20 variables and a constraint.

A variety of optimization techniques have been used for different purposes. In research, optimization techniques have been applied to determine the best conditions for experiments and processing. In processing, the best combinations of processing conditions and ingredients for a product have been optimized (Arteaga et al., 1994b; Dou et al., 1993).

In flavour analysis, simplex optimization was used to optimize blending of ingredients in strawberry juice (Aishima et al., 1987) and wines (Datta and Nakai, 1992). The random centroid optimization technique has been applied to determine the operating conditions for headspace gas chromatography (Girard and Nakai, 1991; Vallejo-Cordoba and Nakai, 1994a; Vodovotz et al., 1993).

CHAPTER III

MATERIALS AND METHODS

The following general steps were taken in the experiments:

Flavour defects due to microorganisms

1. Selection of microorganisms as inocula.
   a. *P. fragi* and *P. fluorescens* as inocula.
   b. *L. lactis, E. aerogenes, B. subtilis* and a mixed culture of the three bacteria as inocula.
2. Determination of incubation time and temperature.
3. Determination of enumeration: medium, temperature and period.
4. Establishment of analytical conditions for the SRI gas chromatograph.
5. Use of various multivariate analyses to classify samples.

Flavour defects due to chemical reactions

1. Selection of off-flavours to be produced in milk.
2. Determination of methods for producing each off-flavour.
3. Use of a gas chromatograph and statistical analyses to classify milk samples.
4. Use of sensory evaluation and multivariate analyses to classify milk samples.

A. Flavour Defects Due to Microorganisms

1. Sample Collection and Preparation

a) Ultra High Temperature (UHT)-sterilized Milk

One case of UHT milk (2% m.f.) in 12 one-liter tetrapak cartons of the same lot number were obtained from a local dairy (Dairyworld Foods, Burnaby, BC) and stored at 4°C. The cartons were opened aseptically with flamed scissors after the carton mouth was wiped with 70% ethanol. The procedures for preparation and sterilization of low density polyethylene carboys fitted with sampling spigots (4 L capacity, Sybron/Nalgene, Rochester, NY) were conducted as described by Skura et al. (1986). Three carboys with magnetic stirring bars were sterilized by filling them with a 200 ppm sodium hypochlorite solution and letting them standing for 2 hours at room temperature. Then they were rinsed throughly with sterile distilled water. Two liters of milk were aseptically transferred to each carboy. In order to mimic the highly oxygenated state of raw milk, air (Pacific Medigas Ltd, Vancouver, BC) continuously overlaid the milk which was stirred at 300 rpm on a VMR mini-stir magnetic table (VMR Scientific, Inc., San Francisco, CA) and equilibrated for 36 hours before the inoculation. The stirring bar remained in the carboy throughout the experiment. Air was sterilized with 0.3 μm bacterial air vent filters (Gelman Sciences, Ann Arbor, MI) and controlled with a flowmeter (400 mL/min) (Series 150, Linde Union Carbide, Somerset, NJ). The dissolved O_2 content was measured

in parts per million (ppm) using a calibrated portable Clark electrode O_2 probe (YSI, Yellow Springs, OH).

b) Microorganisms

The most common spoilage microorganisms of raw milk and pasteurized milk are (1) gram-negative rod shaped bacteria; *Psuedomonas* spp, (2) gram positive spore forming bacteria; *Bacillus* and *Clostridium* spp, (3) lactic acid producing bacteria; *Streptococcus, Lactobacillus, Lactococccus* and *Leuconostoc* spp and (4) yeasts and moulds (Burgess et al., 1994). Therefore, the following cultures were used for this experiment: *Pseudomonas fragi* ATCC 4973, *Pseudomonas fluorescens* biotype A ATCC 17397, *Lactococcus lactis* ATCC 29146, *Enterobactor aerogenes* ATCC 13048 and *Bacillus subtilis* ATCC 6460 (American Type Culture Collection, Rockville, MD). The mixed culture consisted of three bacteria (*L. lactis, E. aerogenes* and *P. fragi*) in a ratio of 1:1:1.

Each freeze-dried culture was rehydrated as described in the manufacturer's instructions (American Type Culture Collection, Rockville, MD). Each culture was transferred to a test tube of the recommended broth (5 mL) and incubated at its optimum temperature for 24 hours in a water-bath shaker at 160 rpm (Lab-Line Melrose Park, IL). An aliquot of 800 µL of each culture was removed and added to sterilized screwcapped polypropylene microcentrifuge tubes (1.5 mL capacity, Ingram & Bell Scientific, Don Mills, ON) including 200 µL presterilized

dimethyl sulfoxide (Fisher Scientific Co., Fairlawn, NJ). Cultures were immediately vortexed and frozen at -86°C (Bio freezer, Forma Scientific, Marietta, OH) for the long-term stock.

For short term storage, a few ice crystals were removed from the microcentrifuge tubes (long term stock) using sterilized wood sticks and streaked out onto the appropriate agar plates and incubated at optimum temperature for 24 hours. Then each culture was streaked on the appropriate medium for isolated colonies. *P. fragi* and *P. fluorescens* were maintained by monthly transfer on trypticase soy agar (TSA) slants (Becton Dickinson Microbiology Systems, Lockeysville, MD) kept at 4°C. *L. lactis* was maintained by weekly transfer on brain heart infusion (BHI) agar slant (Difco, Detroit, MI) kept at room temperature. *E. aerogenes* and *B. subtilis* were also maintained by weekly transfer on nutrient agar slants (Difco, Detroit, MI) kept at room temperature. Short term storage for *L. lactis, E. aerogenes and B. subtilis* was conducted for two weeks before discarding the culture.

c) *P. fragi* and *P. fluorescens* as Inocula

Erlenmeyer flasks (250 mL) containing 25 mL trypticase soy broth (TSB) (Becton Dickinson Microbiology Systems, Lockeysville, MD) were inoculated with a loopful of each bacterium from a slant. Cultures were incubated at 21°C for 18 hours in a water-bath shaker at 160 rpm (Lab-Line, Melrose Park, IL). Ten mL of

the culture was centrifuged at 10000 x g for 15 minutes at 4°C in a RC2-B Sorvall Superspeed centrifuge (Dupont Sorvall, Newtown, CT). The centrifugation was repeated after the supernatant was removed, and the pellet resuspended in 10 mL 0.1 % peptone (Difco, Detroit, MI). The population of the pellet was estimated by measuring optical density at 660 nm with a Shimadzu UV-160 double beam spectrophotometer (Tekscience, Oakville, ON). Two liters of UHT milk were inoculated with *P. fragi* and *P. fluorescens* to a population of approximately 10^4 cfu mL^{-1}. The conditions for UHT-sterilized milk are described in section A.1.a. (p. 32). Samples were collected for plating to provide day 0 cell numbers after 30 minutes. The samples were stored at 4°C up to 10 days and taken at two-day intervals for the determination of bacterial cell numbers. Samples of 30 mL were also collected into amber threaded vials (Fisher Scientific, Ottawa, ON), frozen at -20°C and analyzed by GC within 24 hours. UHT milk without bacteria was included as a control. One trial was conducted.

d) *L. lactis, E. aerogenes, B. subtilis* and a Mixed Culture of *L. lactis*, *E. aerogenes* and *P. fragi* as Inocula

Twenty-five mL of brain heart infusion (BHI) broth was inoculated with a loopful of *L. lactis* and incubated at 30°C for 20 hours in a shaking water bath at 160 rpm (Lab-Line, Melrose Park, IL). Twenty-five mL of each nutrient broth (Difco, Detroit, MI) were inoculated with a loopful of *E. aerogenes* and *B. subtilis,*

respectively and incubated in the same way as for *L. lactis*. The centrifugation and washing procedures were carried out as described in section A.1.c. (*P. fragi* and *P. fluorescens* as inocula, p. 34).

The populations for these bacteria were estimated in the same way as for *P. fragi* and *P. fluorescens*. Since these mesophiles have very long generation times at 4°C, 1.5 L UHT-sterilized milk in a 2 L flask was inoculated with an initial population of approximately 10^4 cfu mL^{-1} and incubated at 30°C. There was no air flow over the milk during this experiment. Instead, milk samples were incubated in a shaking water bath at 140 rpm (SW-20C, Julabo Labortechnik GmbH, Lab Equipment, Seelbach, Germany). Samples, except *B. subtilis* (trial 2), were collected and analyzed every four hours up to 12 hours, once at 24 hours, and then terminated. Milk samples inoculated with *B. subtilis* (trial 2) were terminated at 48 hours. The methodology for GC used with *P. fragi* and *P. fluorescens* was also used for the individual and mixed bacteria. Two trials for each strain and one trial for a mixed culture were conducted.

e) Enumeration

Growth of *P. fragi* and *P. fluorescens* was monitored by dropping 20 µL on TSA plates in quadruplicate. Serial dilution was made with sterile 0.1% peptone. The psychrotrophic count was obtained after incubation of plates at 21°C for 25 hours. Growth of *L. lactis* was monitored by dropping 20 µL on BHI agar plates

and incubated at 30°C for 24 hours. Growth of *E. aerogenes* and *B. subtilis* was monitored by dropping 20 µL on nutrient agar plates and incubated at 30°C for 24 hours. Colony morphology and growth for a mixed culture were observed on selective media under different incubation conditions. These conditions were determined by performing plate counts after various incubation periods and temperatures. The final conditions were: Crystal Violet Tetrazolium Agar (Speck, 1976) for *P. fragi* at 7°C for 5 days, MacConkey Agar (Difco, Detroit, MI) for *E. aerogenes* at 35°C for 24 hours and *Lactobacillus* MRS Agar (Speck, 1976) for *L. lactis* at 30°C for 24 hour.

2. Dynamic Headspace Gas Chromatographic Analysis

A portable gas chromatograph (SRI Model 8610, SRI Instruments, Inc., Las Vegas, NV) equipped with a purge and trap system and a flame ionization detector was used for analysis of volatile components. To obtain the best results after conducting the minimum number of experiments, random centroid optimization (Dou et al., 1993; Nakai, 1990) was used to establish purge conditions. Simplicity of operation, high search efficiency and reduced chance of arriving at local optima make this method an ideal choice for many applications (Girard and Nakai, 1991; Lee et al., 1990; Vallejo-Cordoba and Nakai, 1994a; Vodovotz et al., 1993). The purge conditions which included purge time, purge temperature and sample size were optimized by assessing the number of peaks and their areas. The range for each parameter was determined by

literature search and preliminary experiments (Vallejo-Cordoba and Nakai, 1994a; Vodovotz et al., 1993). The search space for optimum was confined to: sample size ranging from 5 mL to 15 mL; sample temperature varying from 30°C to 55°C and purge time ranging from 5 min to 30 min. The selection for a column, a trap, an internal standard and analytical conditions for GC were determined based on previous analyses of milk (Vallejo-Cordoba and Nakai, 1994a) and the analysis of mango using the SRI gas chromatograph (Vodovotz et al., 1993). A DB-624 megabore column, 75 m long, 0.56 mm diameter and 3.0 μm film thickness (J & W Scientific, Folsom, CA) was installed along with a Tenax Trap (SRI Instruments, Inc., Las Vegas, NV). Ten microliters of a 2.5 ppm solution of 4-methyl-2-pentanone (99.5 % HPLC grade, Sigma, St Louis, MO) was used for the internal standard. Ten milligrams of 1-tetradecanol (Sigma, St. Louis, MO) was used to avoid foaming during the purging. The helium carrier gas (UHP grade, Pacific Medigas Ltd., Vancouver, BC) flow rate was set to 4.62 mL/min. The hydrogen (UHP grade, Pacific Medigas Ltd., Vancouver, BC) and air flow rates for the detector were 20 mL/min and 250 mL/min, respectively. Frozen samples were warmed to 30°C in a water bath (SW-20C, Julabo Labortechnik GmbH, Lab Equipment, Seelbach, Germany) for 15 min and analyzed. Purging conditions were optimized by the random centroid optimization technique. Purging conditions will be discussed in the RESULTS AND DISCUSSION section (p.50). The trap was dry purged for 10 min after purging the milk to eliminate water. Volatiles on the Tenax trap were thermally desorbed at 185°C for 5 min and transferred into the

column. The temperature program was decided by trial and error for the best chromatogram. It began at 30°C for 5 min, and was then ramped at 3°C/min until 180°C and subsequently at 5°C/min up to 220°C. The FID temperature was maintained at 240°C.

3. Data Analysis

a) Data Manipulation

Data for each gas chromatogram were stored in an IBM-PC compatible 386/25MHz personal computer interfaced with the GC system (Peak 2, Version 2.0, SRI Instruments, Inc., Las Vegas, NV). ASCII files were created for peak areas and retention time for each sample. Each gas chromatogram was divided into several windows based on retention time (Arteaga et al., 1994c; Vallejo-Cordoba and Nakai, 1994a). The selection of the numbers were based on the chromatogram which had the maximum peak numbers. Standardization of data was performed by dividing peak areas by the area of an internal standard. Each window was used as an independent variable. An automated peak recognition procedure was applied by using a program written in Basic for a personal computer (Vallejo-Cordoba and Nakai, 1994a) (Appendix A). These data were transferred to a SYSTAT file. All statistical computations were made with an IBM-PC compatible 486/66MHz personal computer.

The data set for *P. fragi* and *P. fluorescens* consisted of 30 milk samples. Each gas chromatogram was divided into 24 windows. In the case of *L. lactis, E. aerogenes, B. subtilis* and a mixed culture, the data set consisted of 104 milk samples. Each gas chromatogram was divided into 38 windows.

b) Statistical Analysis

(1) Unsupervised Learning Methods

(a) Principal Component Analysis (PCA)

PCA was performed by a SYSTAT statistical software (FACTOR) program (Wilkinson, 1990) on the correlation matrix of the sample with no rotation.

(b) Principal Component Similarity Analysis (PCS)

Figure 1 shows the PCS procedure. At first, PCA was calculated in a SYSTAT (FACTOR) program on the correlation matrix of the sample with no rotation. Then principal component scores (eigen value ≥ 1) were transferred to ASCII files. Linear regression analysis was then carried out to compute the coefficient of determination and slope of principal component scores of samples against a reference in the PCS program written in Quick Basic (Vodovotz et al., 1993) (Appendix B).

Step 1. Apply PCA to the original data for k variables and n samples to derive k PC scores and k eigenvalues E and then steps 2-5 are followed for each sample.

$$Pi = Ei / \Sigma Ek$$

Step 2. Compute independent variables V as follows:

$$Vi = 100 (1 - \Sigma Pi)$$

Where i is the principal component numbers. (According to the rule of thumb, Ei lower than 1.0 can be discarded.)

Step 3. Compute dependent variables Y as follows:

$$Yi = Vi + M (\mathrm{PC}i - \mathrm{PC}q)$$

where q is the reference and M is percentage of variance of each principal component factor.

Step 4. Carry out linear regression analysis for Y versus V to compute correlation coefficient (r^2) and slope (S).

Step 5. Plot S versus r^2.

Figure 1. Procedure for principal component similarity analysis (PCS) (Vodovotz et al., 1993).

(2) Supervised Learning Methods

Three supervised multivariate analyses were applied: artificial neural networks (ANN), partial least squares regression analysis (PLS) and principal component regression analysis (PCR). To estimate the true predictive ability of each method, cross-validation was used (Borggard and Thodberg, 1992). The data set is divided into two groups: training and testing data. The model is fitted to the training data set. Predictions are calculated by fitting the model to the testing data set.

For the experiments with *P. fragi* and *P. fluorescens*, 30 samples were divided into training data (24 samples) and testing data (6 samples). For the experiments with *L. lactis*, *E. aerogenes*, *B. subtilis* and a mixed culture, 104 samples were divided into training data (89 samples) and testing data (15 samples). First, random numbers were generated for the samples with Lotus 123 software (version 3.0, Que Corporation, Carmel, IN). Then samples were arranged in ascending order according to the generated random numbers. The testing data were picked up from the top. The remaining data were used as the training data. After a model was calculated using training data, each class of samples in the testing data was predicted. The statistical parameters of coefficient of determination (r^2) and standard error of prediction (SEP) for the known and predicted values were employed to estimate predictive ability of each method. This procedure was repeated five times.

(a) Artificial Neural Networks (ANN)

The neural network software program "Brainmaker" (California Scientific Software, Nevada, CA) was used. A three-layer neural network was used to predict classes using the back-propagation algorithm. A sigmoid function was used as a transfer function, because the sigmoid function is particularly useful for a non-linear relationship (Lawrence and Peterson, 1992). As input neurons for networks, 24 variables for *P. fragi* and *P. fluorescens* and 38 variables for *L. lactis, E. aerogenes, B. subtilis* and the mixed culture were used. The number of output neurons was one, which represents each group. Since output values were groups, each sample was expressed with a two-digit number, the first digit indicating bacterial species and the second storage time. Arbitrary ranges for each class were used. As the first digits, 1, 2 and 3 were assigned to negative control, milk inoculated with *P. fragi* and milk inoculated with *P. fluorescens*, respectively. The second digits, 1, 2, 3, 4 and 5 were assigned to storage days 0, 2, 4, 6, 8 and 10, respectively. In the case of *L. lactis, E. aerogenes, B. subtilis* and a mixed culture, 1, 2, 3, 4 and 5 as the first digit were assigned to negative control, milk inoculated with *L. lactis*, *E. aerogenes*, *B. subtilis* and a mixed culture, respectively. Storage time 0, 4, 8, 12 and 24 hours were assigned 1, 2, 3, 4 and 5 as the second digit, respectively.

The number of hidden neurons is an important factor for the effectiveness of a network. Network performance may vary with the number of the hidden

neurons (Lawrence and Peterson, 1992). With too many neurons, a network may not learn but instead memorize patterns, or it may train and run more slowly. On the other hand, without enough hidden neurons, a network may not be trainable (Lawrence and Peterson, 1992). Therefore the number of hidden neurons was diminished starting from a default number, which is the average of the number of input neurons and the output neurons (Lawrence and Peterson, 1992). The default parameters were used for the learning rate (1.00) and momentum factor (0.9).

(b) Partial Least Squares Regression Analysis (PLS) and Principal Component Regression Analysis (PCR)

PLS and PCR were performed using the commercial software "PLSplus Version 2.1", and add-on software to the spectroscopic/chromatographic software system "LabCalc™" (Galactic Industries Co., Salem, NH). The optimum number of factors for PCR and PLS was determined using cross-validation procedures as described in Martens and Naes (1989). The input and output variables were the same as those used for ANN.

B. Flavour Defects Due to Chemical Reactions

1. Sample Collection and Preparation

Milk was obtained in one-liter gable top paper cartons from a local dairy (Avalon, Vancouver, BC) on the day of pasteurization. The milk was then treated to develop several off-flavours (light-induced, oxidized, cooked and heated) according to the procedures recommended by the American Dairy Science Association's Nomenclature, Standards and Bibliography Committee (Shipe et al., 1978).

Light-induced flavour was produced by exposing 200 mL of milk in a 250 mL glass Erlenmeyer flask to 40-Watt cool white fluorescent lamps for 12 hours at 7°C. The illumination was on average 1080 lux perpendicular to the light source at the midpoint of the exposed vertical surface. Illumination was measured with a foot candle meter (Gossen, GmbH Postsach, Erlangen, Germany) (Schlegel et al., 1969).

Oxidized flavour was produced by adding 1 ppm of cupric chloride (Fisher Certified Grade, Fisher Scientific Co., Fairlawn, NJ) and 20 ppm L-ascorbic acid (Fisher Reagent ACS Grade, Fisher Scientific Co., Fairlawn, NJ) to milk. The oxidized flavour developed when the milk was held for 12 hours at 4°C in the dark.

Cooked flavour was produced by heating 200 mL of milk in a 250 mL flask at 75°C for 1 minute on an electric stove top element (Standard Appliance, model 510, MFG Co., Ltd., Toronto, ON). The milk temperature was measured with a thermometer inside of the flask of milk which was in a pan of water. Temperature was regulated with a thermostat. In the same manner, heated flavour was produced by

heating 200 mL of milk in a 250 mL flask for 15 min at 95°C on the electric stove top element. The cooked and heated samples were immediately cooled in water and held at 4°C for 12 hours in the dark. Samples for chromatographic analysis were placed into an amber threaded vial and frozen at -20°C and analyzed within 24 hours. Pasteurized milk samples of each flavour were used for both gas chromatographic analysis and sensory analysis.

2. Dynamic Headspace Gas Chromatographic Analysis

Dynamic headspace gas chromatographic analysis was carried out as described in section A (Flavour Defects Due to Microorganisms, p. 37). The frozen milk samples were warmed to 30°C in a water bath (SW-20C, Julabo Labortechnik GmbH, Lab Equipment, Seelbach, Germany) for 15 min and analyzed by using a SRI gas chromatograph.

3. Sensory Evaluation

a) Sensory Panel

Sensory analysis was carried out in the sensory evaluation room of the Department of Food Science at the University of British Columbia. The ten panellists (five women and five men, aged between 22 and 55 years) were selected from among students and staff members of the Department of Food Science at the University of British Columbia. All had experience in sensory evaluation panels.

None disliked tasting milk.

b) Judging Procedure

The procedure for training and testing was adapted from the American Dairy Science Association (ADSA) scoring guide (American Dairy Science Association, 1987). In the training period the experienced panellists were reminded of how to prepare for the evaluation according to the standard procedure described by Poste et al. (1991). In that session the panellists agreed upon descriptive terms to characterize the off-flavours to be detected in the sensory evaluation. After the panel was instructed, three testing sessions were conducted. Because of the success of panellists in distinguishing samples, preparations for the evaluation were apparently appropriate.

At each session, the panellists evaluated the samples in individually partitioned booths at room temperature (about 22°C) under red lights. The panellists were divided into two groups. The first group evaluated samples from 10:00 to 11:00 a.m. and the second group from 11:00 a.m. to 12:00 noon.

A table of random numbers was used to assign a three digit code number to each sample. Milk samples were warmed to 30°C in a water bath (SW-20C) for 30 min. Four 30 mL samples were presented in coded, clear 59.1 mL polystyrene plastic cups (Solo Cup Company, Urbana, IL) on a white plate. Thirty mL

untreated milk was also provided as a reference. Distilled water and unsalted crackers were provided to rinse residual particulate matter from the mouth.

Each panellist was given four samples and one reference for the second time with new code numbers. Panellists were asked to detect each off-flavour by smelling and tasting for flavour attributes and overall acceptability. The panellists were asked to smell the reference and then score the intensity of each sample. They were instructed to swirl the contents and immediately place the nose directly over the container. After evaluation of flavour by smell, the panellists were asked to taste each sample. They took a spoonful of milk and rolled it about the mouth. Then all samples were spat out. The intensity of each attribute was scored on a structured 10 point scale (American Dairy Science Association, 1987) (Appendix C). Each panellist also rated overall acceptability on a structured 10 point scale (American Dairy Science Association, 1987) (Appendix C). Four samples, in replicate, were tested by each panellist during a session.

4. Data Analysis

a) Data Manipulation

Data collected from the SRI gas chromatograph were manipulated as described in section A.3.a in the MATERIALS AND METHODS section (p. 39). The data set consisted of 45 milk samples. Each gas chromatogram was divided into 12 windows.

b) Statistical Analysis

(1) Dynamic Headspace Gas Chromatographic Analysis

Principal component analysis (PCA) and principal component similarity analysis (PCS) were conducted as described in section A.3.b.1.a and b in the MATERIALS AND METHODS section to classify the samples (p. 40).

(2) Sensory Evaluation

Scores of each attribute were standardized within each panellist (Wilkinson, 1990). Analyses of variance (ANOVA) were used to test for significant differences among the groups in terms of panellist and treatment. PCA and PCS were carried out as described in section A.3.b.1.a and b in the MATERIALS AND METHODS section to classify the samples (p. 40).

(3) Principal Component Similarity Analysis with Sensory Scores

Acceptability scores by smelling and tasting were incorporated into the principal component similarity analysis plot. This contoured scatter plot was produced by a SYSTAT statistical software (CONTOUR) program (Wilkinson, 1990). Contours show a third dimension with contours on a two dimensional scatterplot. The contour variable (Z axis) was the acceptability scores from smelling and tasting. For smoothing, a quadratic option was used. The detailed procedure was described by Willkinson (1990).

CHAPTER IV

RESULTS AND DISCUSSION

This chapter, discussing the data collected in the study, is divided into three sections: (A) optimization of the analytical conditions for a dynamic headspace gas chromatograph, (B) analysis of data concerning flavour defects due to microorganisms and (C) analysis of data regarding flavour defects due to chemical reactions.

A. Dynamic Headspace Gas Chromatographic Analysis

Parameters for purge were optimized by using random centroid optimization (RCO) and the results are summarized in Table 1. The RCO consists of three steps in a cycle. They are random search, centroid search and mapping process. If the optimum point is not obtained, the cycle is repeated until the optimum point is obtained. Total area of peaks of gas chromatograms was calculated as a response value. The response value was maximized. The results of the first 13 experiments were used to generate the two centroid experiments. The centroid is the average of the parameters for n (number of experiments) experiments excluding the worst response in n+1 experiments (Nakai, 1990; Dou et al., 1993). Then all these data are mapped to aid narrowing the search space for the optimum (Figure 2). The mapping process aids in visualization of the experimental response surface indicating the trend of the data (Nakai et al., 1984). The optimum is located by

Table 1. Summary data for random centroid optimization for SRI gas chromatograph.

Experiments No.	Sample volume (mL)	Purge temp (°C)	Purge time (mins)	Response
1	8.3	35	28.29	2226.66
2	8.3	44	8.06	125.67
3	11.4	31	11.77	77.92
4	10.9	36	18.11	221.52
5	11.4	47	15.87	387.16
6	6.1	56	5.44	85.77
7	11.4	56	25.90	354.37
8	7.9	57	18.86	681.06
9	14.4	39	10.83	244.69
10	11.9	51	7.37	349.97
11	14.4	48	26.49	753.96
12	7.2	44	23.55	298.07
13	7.4	30	22.56	185.44
14[a]	11.4	52	18.90	3132.00
15[a]	11.0	52	21.21	2711.94

[a] Centroid points

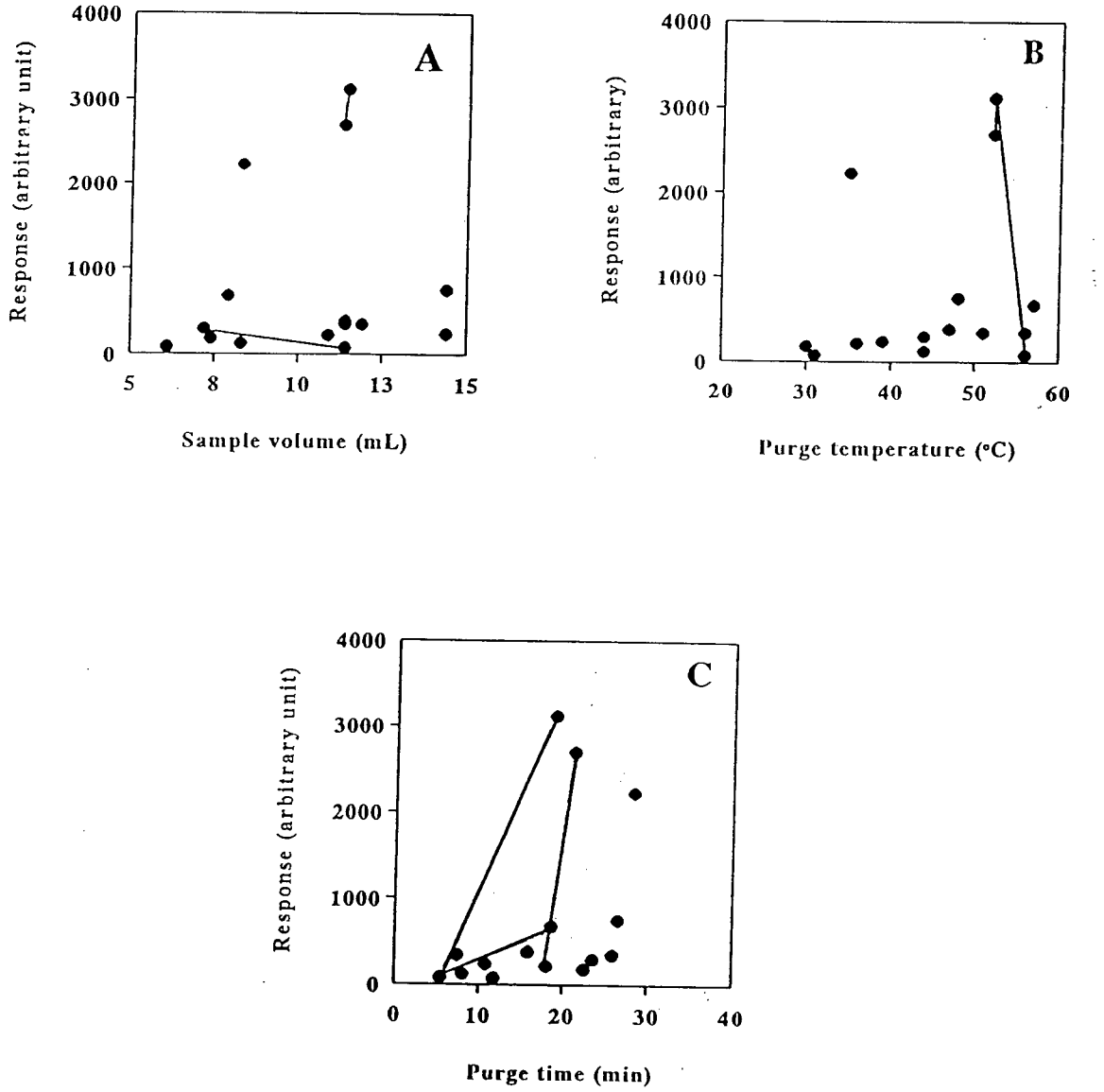

Figure 2. Mapping result of the first cycle of experiments generated by random centroid optimization technique.

applying a curve fitting program to link data points. From Figure 2, the optimum was reached for each parameter after 15 experiments. The parameters were sample size 11.4 mL, purging time 18 min and 54 sec and purging temperature 52°C.

Coefficients of variation (CV) were calculated to investigate the conditions for repeatability (Table 2). Four chromatograms were run with the optimum conditions obtained from the RCO. Six major peaks were picked up and compared for their repeatability. Peaks 1 to 4 showed good repeatability (CV < 10%). When the retention time was longer (peak 5 and 6), the CV increased.

Table 2. Repeatability of analytical conditions for SRI gas chromatograph.

Peak No.	Retention time (mins)	Mean (n=4)	SD [b]	CV (%) [c]
1	53.5	1.161[a]	0.034	2.92
2	57.3	0.143	0.003	1.84
3	57.8	0.109	0.002	1.71
4	60.2	0.054	0.001	2.72
5	67.4	0.057	0.007	12.32
6	75.4	0.061	0.016	26.34

[a] Peak area/peak area of internal standard.
[b] Standard deviation.
[c] Coefficient of variation.

B. Flavour Defects Due to Microorganisms

1. Growth of Microorganisms

The dissolved oxygen tension of milk was 6.59 ppm before the inoculation. *P. fragi* and *P. fluorescens* showed the fastest growth rate within the first four days (Figure 3). By day 6, the population of *P. fragi* reached the stationary phase but the *P. fluorescens* continued to grow until day 10. *P. fragi* had a shorter generation time than *P. fluorescens*. Populations of both bacteria reached 8 Log_{10}CFU mL^{-1} after 4 days, which is the generally accepted numbers for spoilage (Griffiths and Phillips, 1986).

Growth curves for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture are shown in Figure 4. In each trial, the initial population of each strain was approximately 4 Log_{10}CFU mL^{-1}. *L. lactis* grew to a high density, exceeding 8 Log_{10}CFU mL^{-1} after 12 hours. *E. aerogenes* reached the stationary phase after 12 hours. The generation time of *B. subtilis* in trial 1 was slower than *L. lactis* and *E. aerogenes* and reached 8 Log_{10}CFU mL^{-1} at 48 hours. The population of *B. subtilis* in trial 2 began to decline after 12 hours. In the mixed culture, population densities of *E. aerogenes* and *L. lactis* were similar for the first 12 hours. After 12 hours the populations of both bacteria began to decrease. *P. fragi* did not increase as much at 30°C as at 4°C. Consequently, the dilution scheme used for the 30°C at 24 hours sampling was too high to enumerate the population density of *P. fragi*. The total populations, by the end of the experiment, were lower when the organisms were grown in the mixed culture.

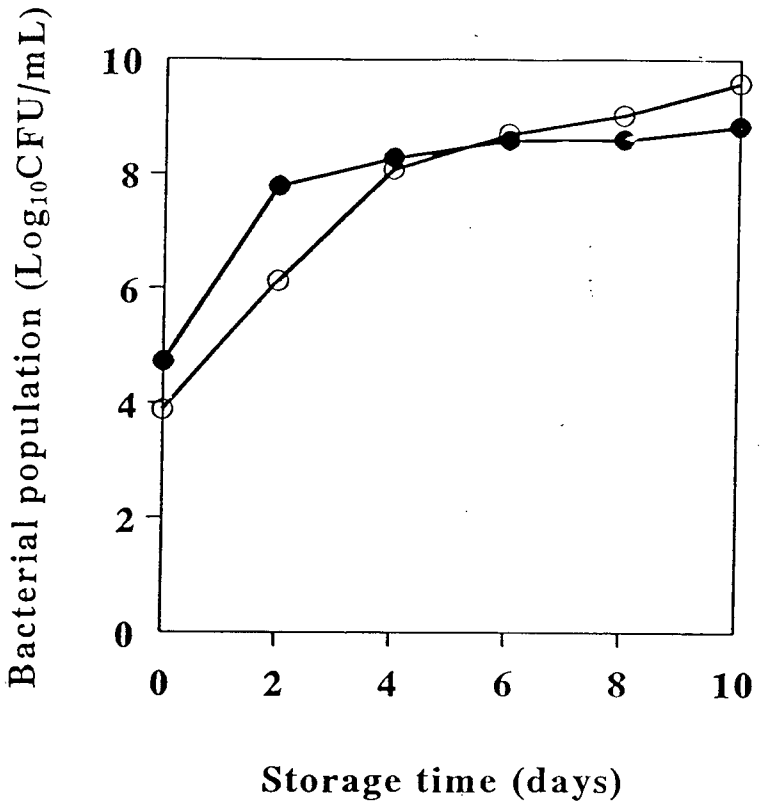

Figure 3. Growth of *P. fragi* and *P. fluorescens* in UHT-sterilized milk. ● = *P. fragi*. ○ = *P. fluorescens*. Incubation temperature was 4°C. One trial was conducted.

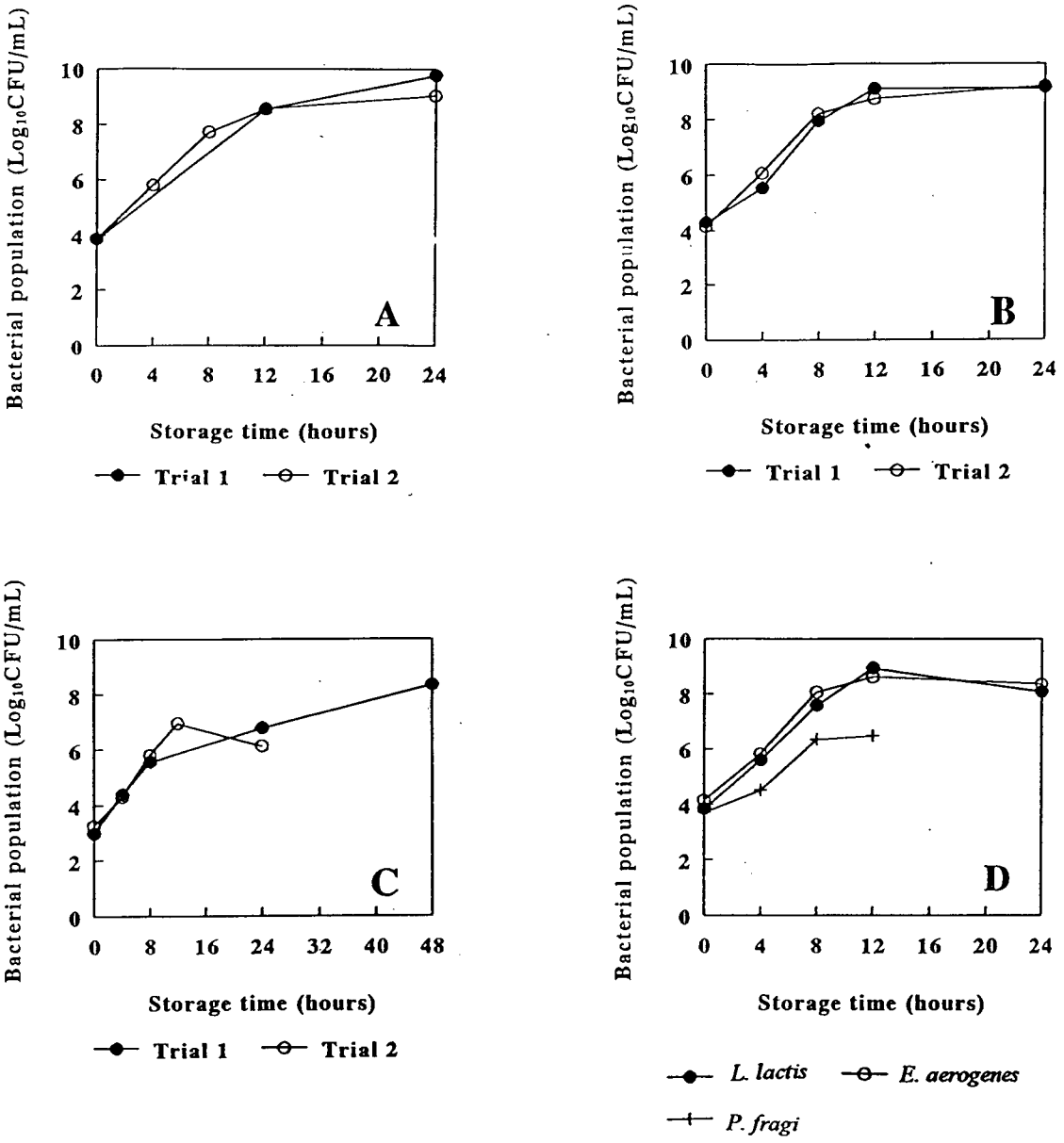

Figure 4. Growth of *L. lactis* (A), *E. aerogenes* (B), *B. subtilis* (C) and a mixed culture (*L. lactis*: *E. aerogenes*: *P. fragi* = 1:1:1) (D) in UHT-sterilized milk. Incubation temperature was 30°C.

2. Principal Component Analysis (PCA)

Effects of inoculating milk with *P. fragi* and *P. fluorescens*. Principal component (PC) scores were extracted among 24 peaks. Figure 5 shows eigen values and their cumulative proportion. The eigen value for a principal component indicates the variance that it accounts for out of the total variances (Manly, 1986). The cumulative proportion up to the fourth PC (eigen value $\geq$ 1.0) accounted for approximately 87%. Therefore, the information was extracted into four principal components with about 13% loss of the information. Figure 6 shows a plot of the values for the first two PC, which account for 74% of the variation in the data. All samples until day 4 were not clearly classified. *P. fragi* had a larger PC1 with more storage days. On the other hand, *P. fluorescens* had a smaller PC2. Figure 7 shows a three dimensional PCA plot. The first three PC account for approximately 82%. When PC scores were plotted in three dimensions, there was no clear difference between the negative control (UHT-sterilized milk without inoculation) and *P. fluorescens*. The milk samples inoculated with *P. fragi* (after 8 days) were plotted far from the rest of the samples. Two and three dimension scattergrams are frequently examined for unsupervised classification. However, ignoring other PC scores by using only two or three PC scores may result in ignoring portions of the variation present in the original data; this may be a reason why PCA is not usually categorized as a classification method (Vodovodz et al., 1993).

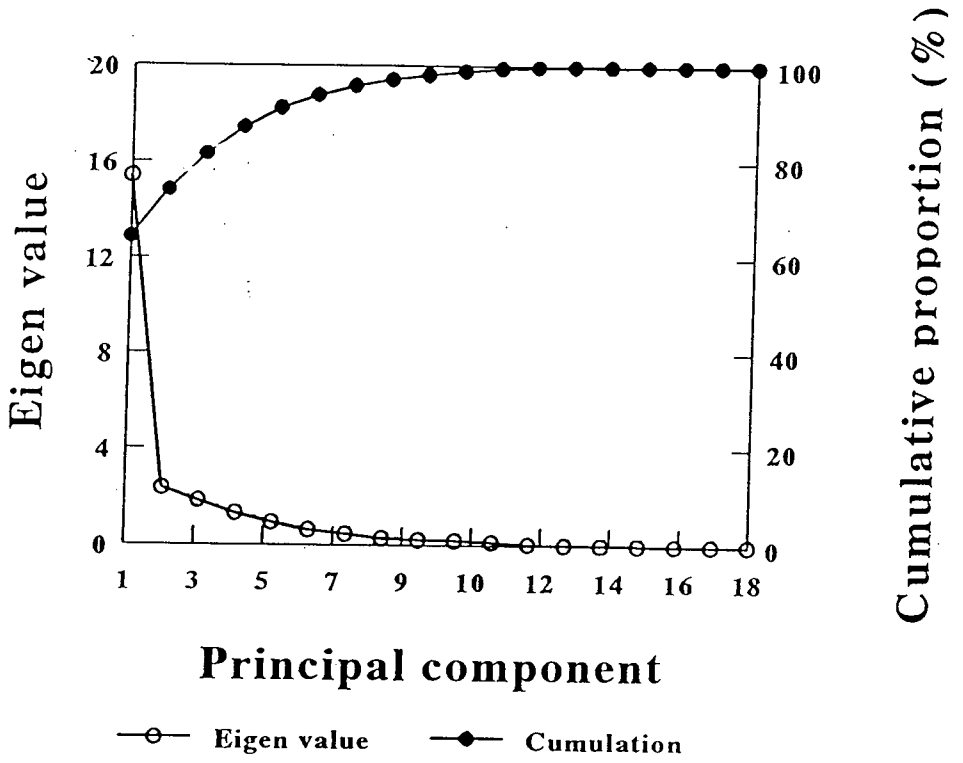

Figure 5. Eigen values and cumulative proportion in total variance on the basis of gas chromatographic peak areas for UHT milk inoculated with *P. fragi* and *P. fluorescens*.

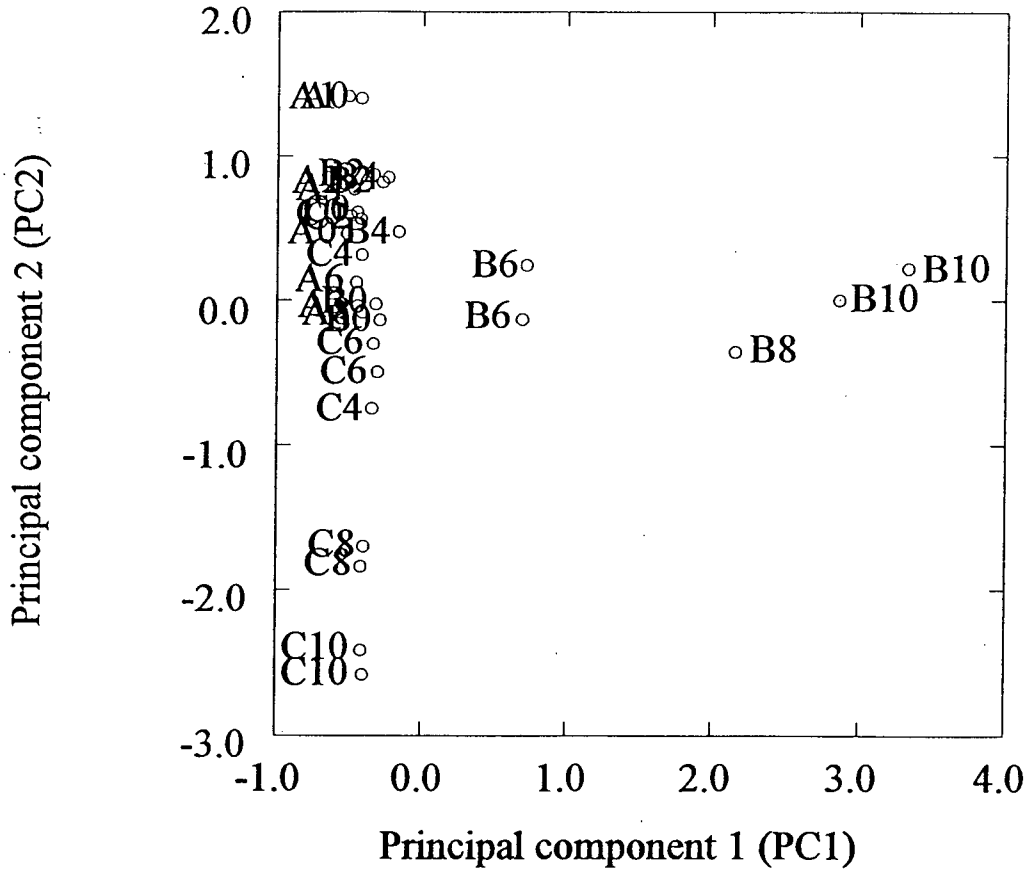

Figure 6. Principal component analysis plot (two dimensional) from gas chromatographic data for *P. fragi* and *P. fluorescens*. A = Negative control. B = *P. fragi*. C = *P. fluorescens*. Numbers after each letter represent storage time (days).

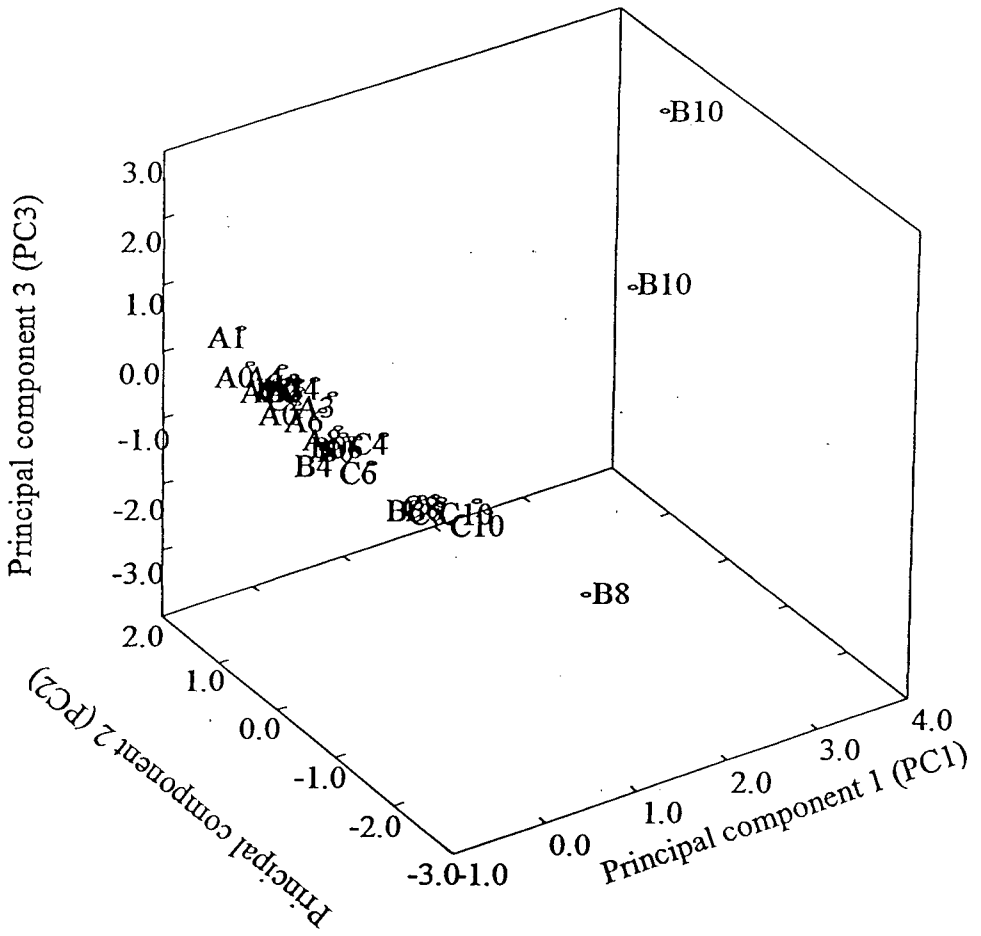

Figure 7. Principal component analysis plot (three dimensional) from gas chromatographic data for *P. fragi* and *P. fluorescens*. A = Negative control. B = *P. fragi*. C = *P. fluorescens*. Numbers after each letter represent storage time (days).

Effects of inoculating milk with *L. lactis, E. aerogenes, B. subtilis* and a mixed culture. Eigen values and their cumulative proportion are shown in Figure 8. The first nine PC had eigen values greater than one and accounted for about 83% of the total variance. The first two PC, which account for about 40% of the total variance, are plotted in Figure 9. Before 12 hours all bacteria were not clearly differentiated from the negative control. PC1 for *L. lactis* increased after 12 hours. PC2 for *E. aerogenes, B. subtilis* and the mixed culture increased after 12 hours. The first three PC scores are plotted in Figure 10. Compared to Figure 9, differences among *E. aerogenes, B. subtilis* and the mixed culture are clear. PC3 scores for *E. aerogenes* decreased in value after 12 hours. PC3 scores for the mixed culture were larger after 12 hours. PC3 scores for *B. subtilis* were close to that of the negative control. PC3 scores for the mixed culture were large.

3. Principal Component Similarity Analysis (PCS)

Effects of inoculating milk with *P. fragi* and *P. fluorescens*. In PCS analysis, PCA is conducted first and then PC scores are used to compute the coefficient of determination and slopes between adjusted PC scores of samples and those of a reference. According to the rule of thumb, an eigen value which is lower than 1.0 can be discarded (Vodovotz et al., 1993). PCA yielded four PC with eigen values greater than 1.0, which accounted for approximately 87% of the total variation (Table 3). Thus the first four PC scores were used to conduct PCS. Figure 11 shows the PCS

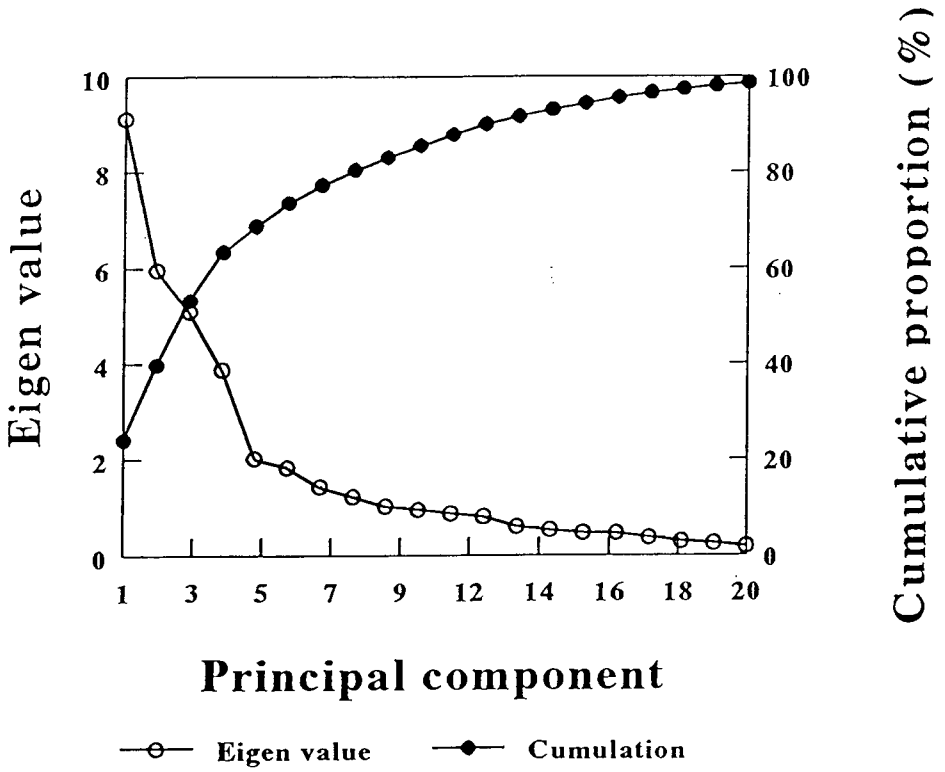

Figure 8. Eigen values and cumulative proportion in total variance on the basis of gas chromatographic peak areas from UHT milk inoculated with *L. lactis*, *E. aerogenes*, *B. subtilis* and a mixed culture and incubated at 30°C.

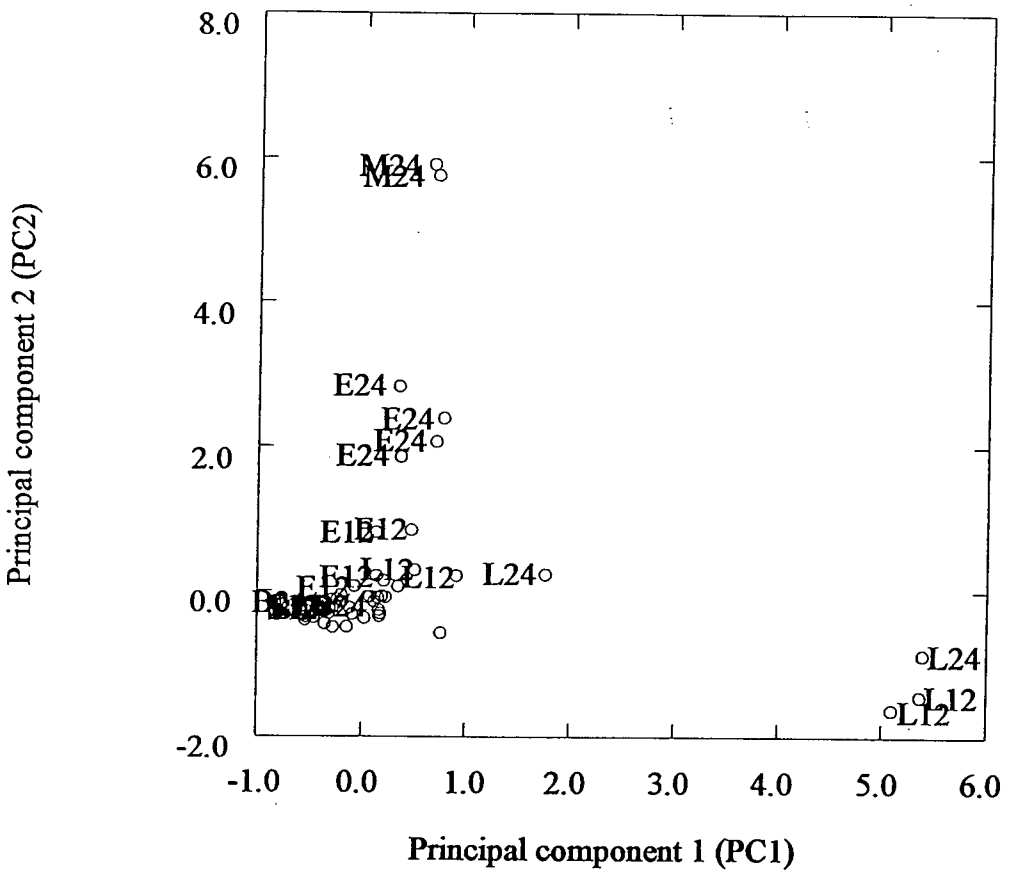

Figure 9. Principal component analysis plot (two dimensional) from gas chromatographic data for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture. S = Negative control. L = *L. lactis*. E = *E. aerogenes*. B = *B. subtilis*. M = a mixed culture. Numbers after each letter represent storage time (hours).

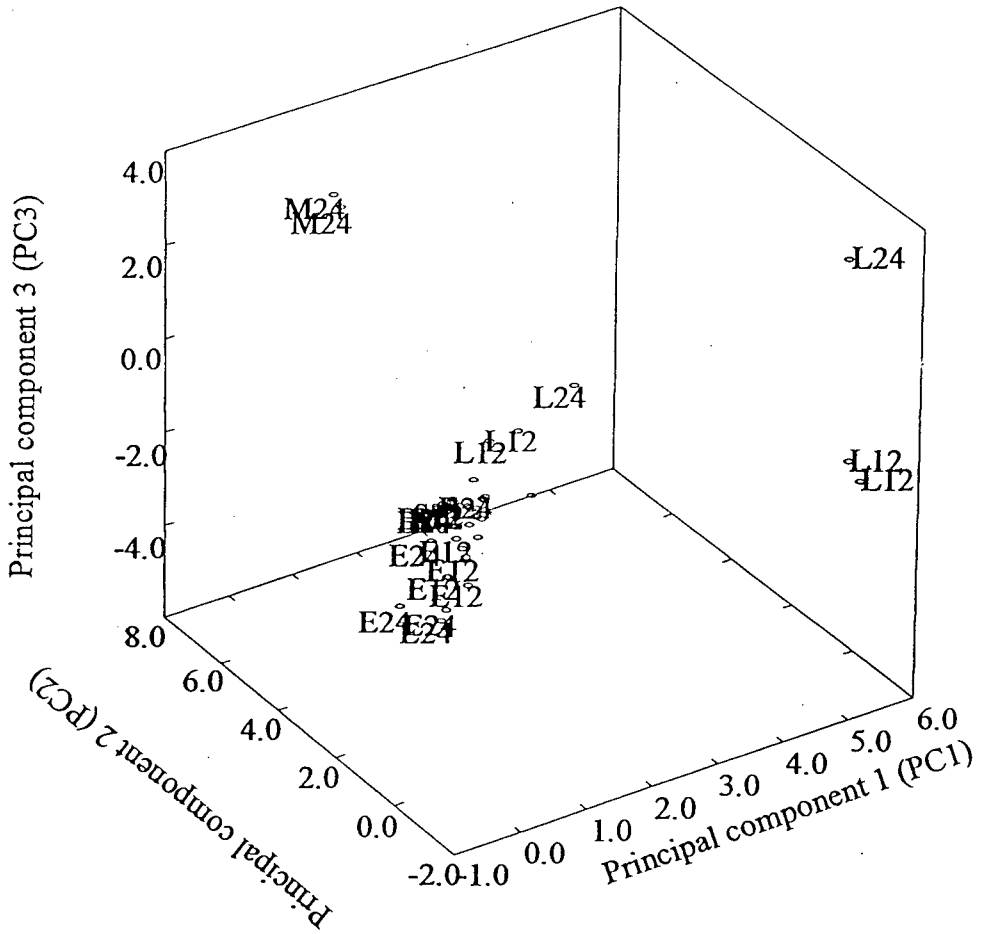

Figure 10. Principal component analysis plot (three dimensional) from gas chromatographic data for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture. S = Negative control. L = *L. lactis*. E = *E. aerogenes*. B = *B. subtilis*. M = a mixed culture. Numbers after each letter represent storage time (hours).

Table 3. Results of cumulative eigen values for *P. fragi* and *P. fluorescens*.

Factor	Eigen value	Variance	Cumulation (%)
1	15.422	64.257	64.257
2	2.364	9.848	74.105
3	1.847	7.697	81.802
4	1.326	5.527	87.329

Table 4. The curve fitting equations for each bacterial species and a mixed culture.

Bacterial species	Curve fitting equation [a]	R^{2} [b]
P. fragi	$y = 0.001 - 0.2327x + 5558.42x^2$	0.81
P. fluorescens	$y = 436.49 - 1414.44x + 1529.76x^2 - 550.70x^3$	0.86
L. lactis	$y = -41.64 + 189.18x - 256.72x^2 + 110.27x^3$	0.94
E. aerogenes	$y = 18.65 - 62.79x + 75.57x^2 - 30.51x^3$	0.60
Mixed culture	$y = 9.43 - 18.18x + 9.75x^2$	0.99

[a] y = slope x = coefficient of determination
[b] Coefficient of determination.

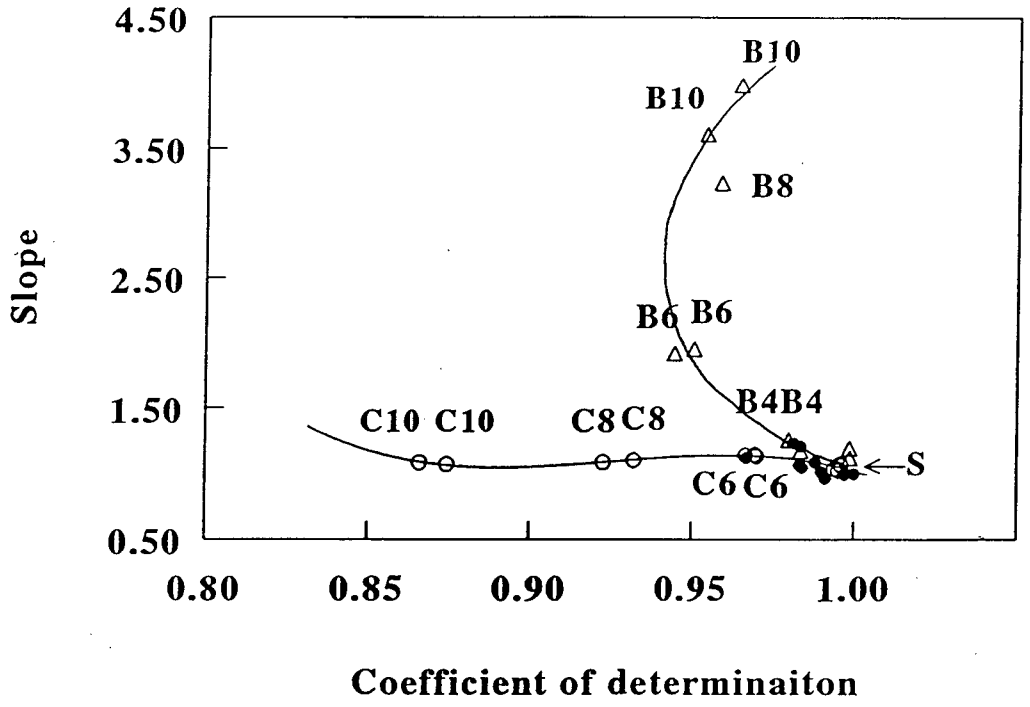

Figure 11. Principal component similarity analysis plot of gas chromatographic data for *P. fragi* and *P. fluorescens* using negative control (day 0) as a reference. ● = Negative control. B = *P. fragi* (Δ). C = *P. fluorescens* (○). Number after B and C represents storage time (days). S = Reference. The coordinates for the reference are (1, 1).

plot of gas chromatographic peak areas. UHT-sterilized milk without inoculation (day 0) was used as a reference. For both *P. fragi* and *P. fluorescens*, there was little difference in the chromatograms between the reference and the inoculated samples until day 4. The chromatograms for *P. fragi* and *P. fluorescens* showed a substantial difference after day 6. The slope of *P. fragi* became greater with longer storage time while the coefficient of determination stayed between 0.95 and 1.0. On the other hand, the slope of *P. fluorescens* was approximately 1.0, but the coefficient of determination became smaller with longer storage time.

The best curve fitting equation was examined to predict the relationship between a quantitative response and explanatory variables (slope and coefficient of determination) for samples with *P. fragi* and *P. fluorescens.* SlideWrite software (Curve Fitter) (SlideWrite Plus, 1995) was used for this purpose. Among several functions, linear and polynomial groups were used. The equations which gave the best coefficient of determination for each bacterium and a mixed culture are shown in Table 4 (p. 66). The coefficient of determination for each equation was 0.81 for *P. fragi* and 0.86 for *P. fluorescens.* These equations can be used to predict the direction of each species. When the plot of an unknown sample is close to one of these lines, the bacterial species can be classified.

To display the difference between the sample inoculated with bacteria and the reference, adjusted PC scores of the sample (day 8) were plotted against the adjusted PC scores of reference (day 0) in Figure 12. As the sample approaches the reference,

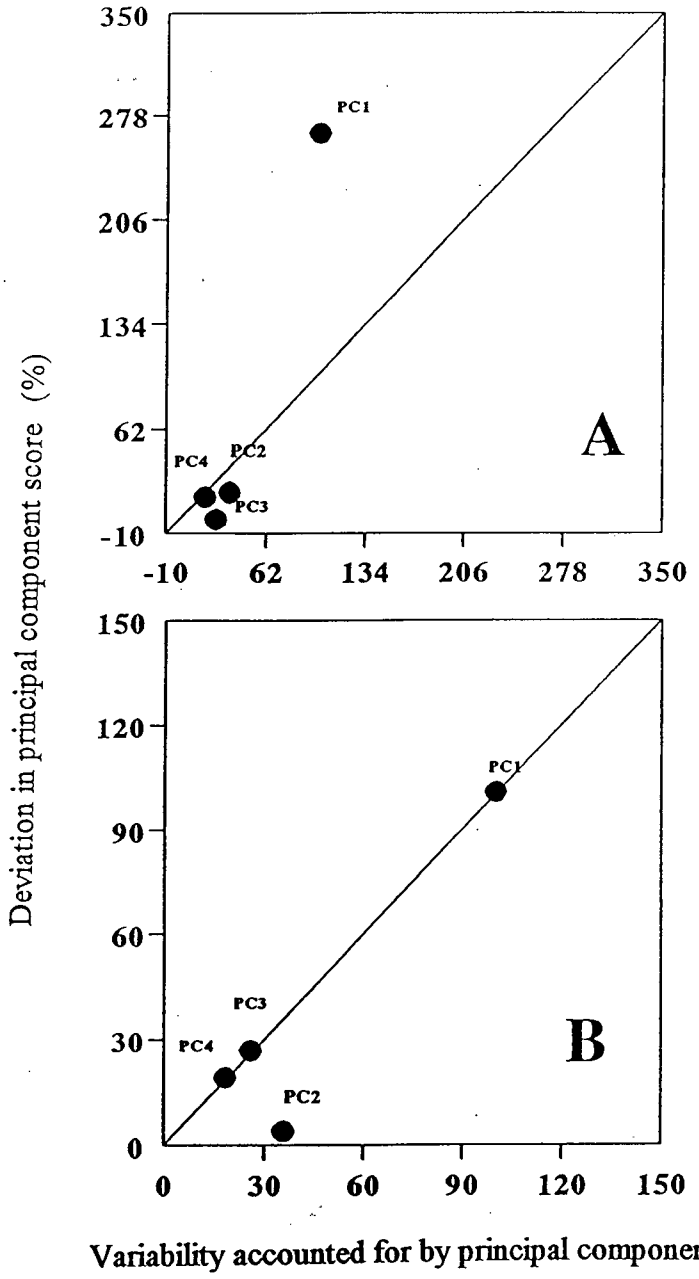

Figure 12. Adjusted principal component scores of UHT-sterilized milk inoculated with *P. fragi* after 8 days (A) and *P. fluorescens* after 8 days (B). Adjusted principal component scores were computed according to steps 1-3 in Figure 1 (p. 41). The 45° line represents the match with PC scores of the reference.

the deviation decreases thus approaching the diagonal line. PC1 of *P. fragi* deviated greatly from PC1 of the reference. The component loadings of the first four PC are shown in Table 5. Peaks 3, 11, 17, 19 and 23 were found to have high loadings (≥ 0.98) in PC1. They may be used for classifying milk samples possibly contaminated with *P. fragi*. While the PC1 of *P. fluorescens* was similar to PC1 of the reference, PC2 showed a clear difference (Figure 12). Since PC2 explained only 9.8% of total variance, little difference was observed between the chromatogram of the *P. fluorescens* inoculated sample and the reference (Figure 13).

Effects of inoculating milk with *L. lactis, E. aerogenes, B. subtilis* and a mixed culture. PCS was carried out using the first nine PC, which accounted for 83% of the total variance (Table 6). PCS plot is shown in Figure 14. PCS plots for each bacterium and the mixed culture are shown individually in Figure 15. A clearer direction for each bacterium can be found in Figures 14 and 15. The chromatograms of milk inoculated with bacteria deviated from those of the negative control during prolonged storage time. In the cases of *E. aerogenes* and *L. lactis*, the samples with longer storage times (after 12 hours) appeared farther away from the position of the reference (coefficient of determination=1, slope=1). All plots for *B. subtilis* were close to the reference. This suggests that a milk sample inoculated with *B. subtilis* would not produce detectable flavour defects. The mixed culture showed differences from milk

Table 5. Factor loadings (pattern) from principal component analysis of data for *P. fragi* and *P. fluorescens*.

	Principal Component (PC)			
Peak No.	1	2	3	4
1	-0.242	0.477	0.400	0.628
2	0.950	0.004	-0.197	0.030
3	0.991	0.010	0.074	-0.012
4	-0.522	0.594	0.217	-0.447
5	0.976	0.034	-0.128	0.103
6	0.881	0.002	0.207	-0.056
7	0.977	0.031	0.178	-0.005
8	0.932	-0.014	-0.022	-0.089
9	0.820	0.173	-0.205	0.336
10	0.963	-0.143	0.144	0.052
11	0.988	0.046	-0.059	0.061
12	0.819	0.046	0.364	0.081
13	0.927	0.027	0.335	-0.009
14	0.375	0.074	-0.826	-0.027
15	0.158	0.731	-0.140	0.035
16	0.803	0.045	0.153	-0.296
17	0.983	0.033	-0.106	0.088
18	0.921	-0.010	0.018	-0.081
19	0.986	0.018	0.124	0.002
20	0.689	-0.038	-0.575	0.026
21	-0.327	0.644	-0.057	0.450
22	0.637	0.298	0.242	-0.375
23	0.981	0.078	0.019	0.094
24	0.003	-0.819	0.236	0.358

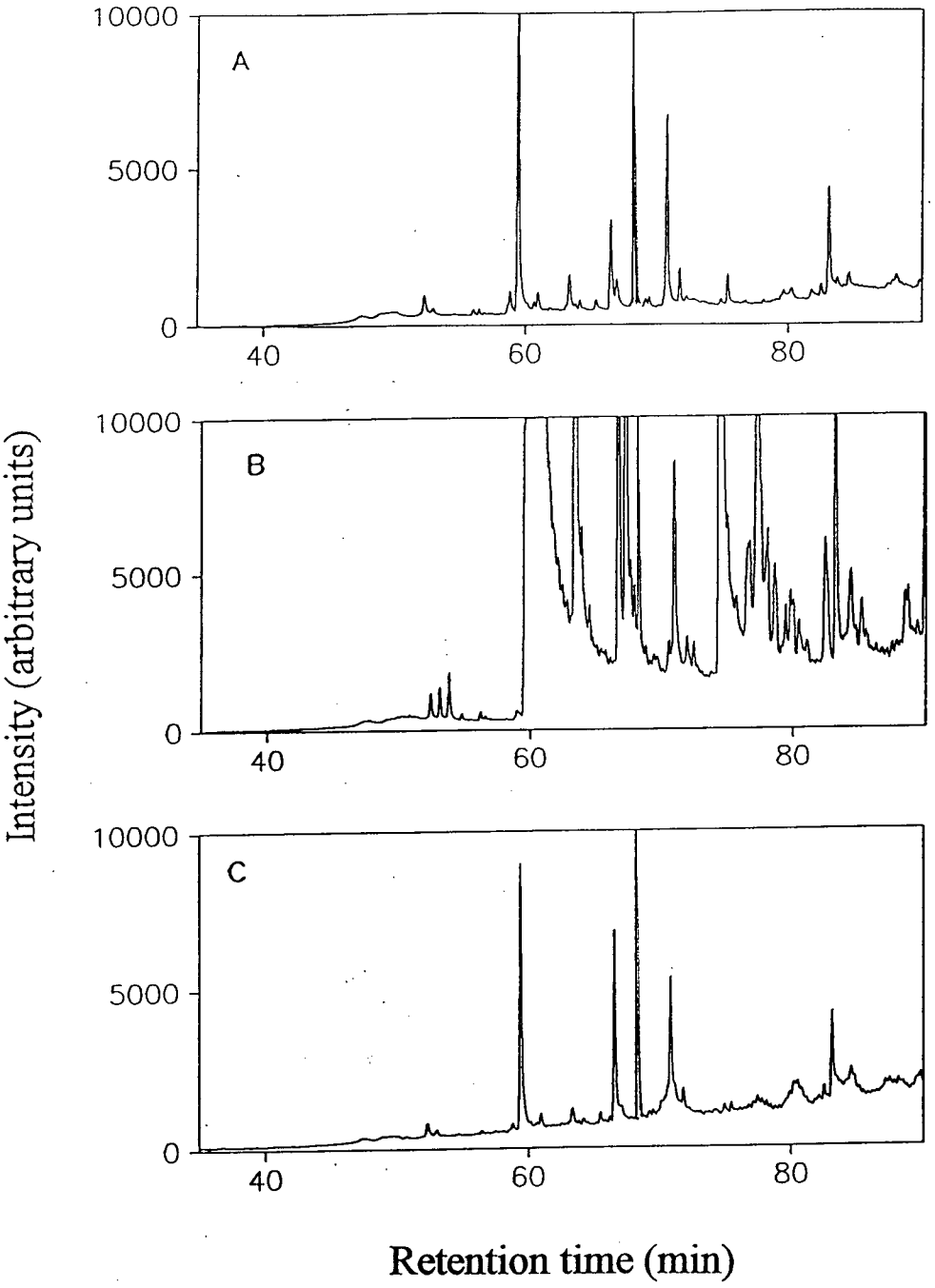

Figure 13. Gas chromatograms of UHT-sterilized milk (negative control, day 0) (A), UHT-sterilized milk inoculated with *P. fragi* after 8 days (B) and UHT-sterilized milk inoculated with *P. fluorescens* after 8 days (C).

Table 6. Results of cumulative eigen values for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture.

Factor	Eigen value	Variance	Cumulation (%)
1	9.138	24.047	24.047
2	5.961	15.687	39.734
3	5.089	13.392	53.126
4	3.877	10.202	63.328
5	2.023	5.324	68.562
6	1.839	4.840	73.402
7	1.434	3.773	77.175
8	1.230	3.237	80.412
9	1.031	2.713	83.125

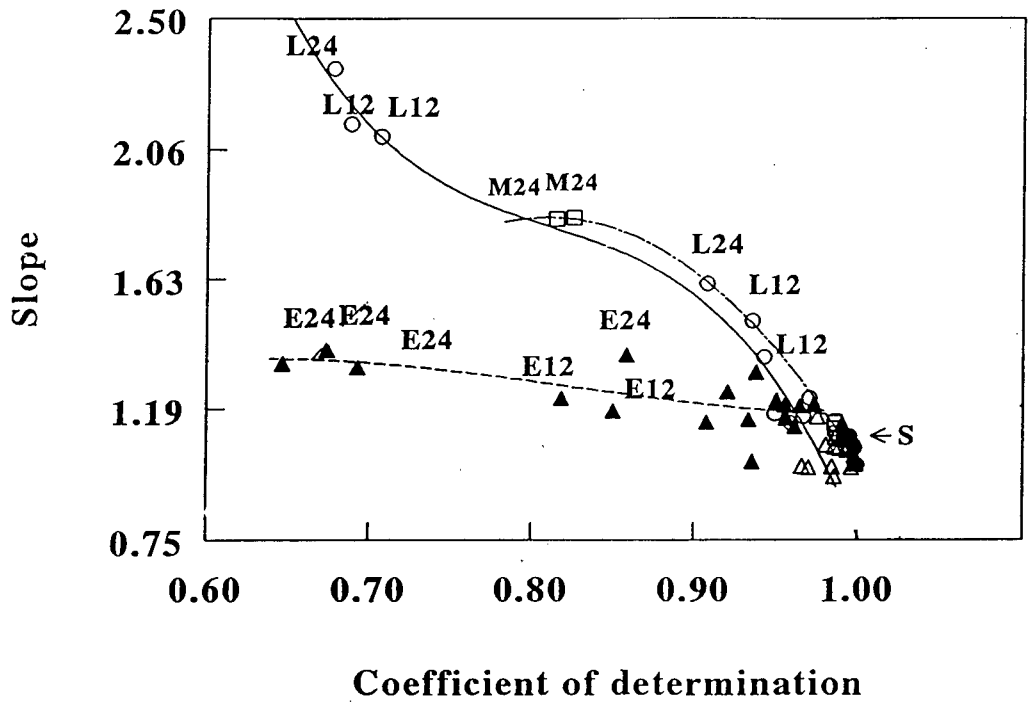

Figure 14. Principal component similarity analysis plot of gas chromatographic data for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture using a negative control (0 hour) as a reference. ● = Negative control. L = *L. lactis* (○). E = *E. aerogenes* (▲). B = *B. subtilis* (Δ). M = a mixed culture (*L. lactis:E. aerogenes:P. fragi* = 1:1:1) (□). Numbers after L, E and M represent storage time (hours). S = Reference. The coordinates for the reference are (1, 1).

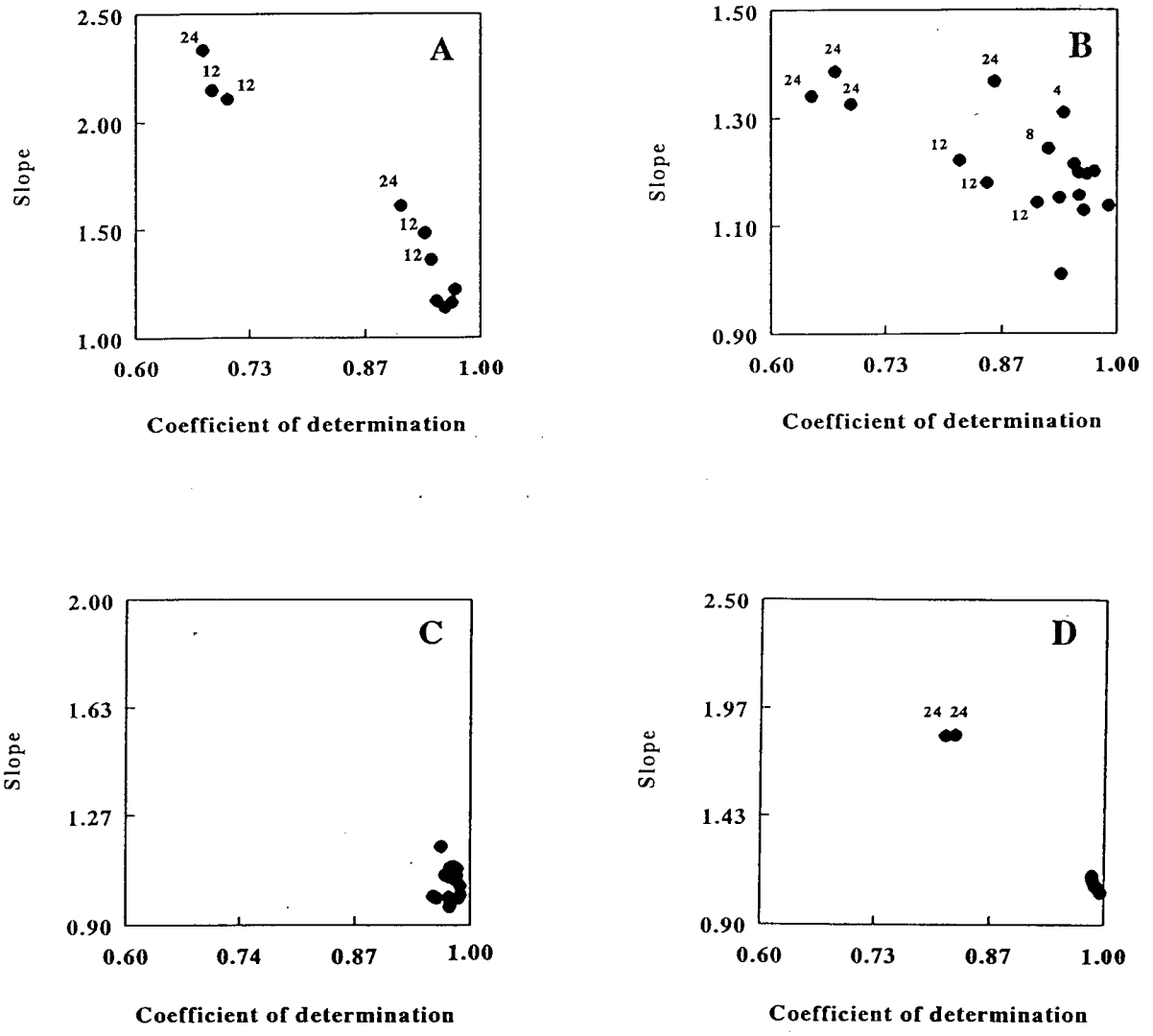

Figure 15. Individual principal component similarity analysis plots of gas chromatographic data for *L. lactis* (A), *E. aerogenes* (B), *B. subtilis* (C) and a mixed culture (D) using a negative control (0 hour) as a reference. Numbers in plots represent storage time (hours). The coordinates for the reference are (1,1).

samples inoculated with a single strain. Most plots of the mixed culture before 12 hours were scattered around the reference. Between 12 hours and 24 hours milk inoculated with the mixed culture produced different chromatograms indicating off-flavours.

The best curve fitting equation was examined for *L. lactis*, *E. aerogenes* and the mixed culture. The method was conduced as described for *P. fragi* and *P. fluorescens*. It was difficult to find the best curve fitting equation. For example, there was little difference between coefficients of determination in linear and polynomial equations in the case of the mixed culture. The equations which gave the best coefficient of determination are shown in Table 4 (p. 66). The coefficients of determination from equations for *L. lactis* and the mixed culture were 0.94 and 0.99, respectively. The coefficient of determination for *E. aerogenes* was 0.60. This indicates that it would be difficult to determine the direction of *E. aerogenes* with respect to off-flavour development.

The adjusted PC scores of each bacterium and the mixed culture are plotted in Figure 16. The behavior of PC1 of *L. lactis* was quite different from PC1 of the reference. PC3 of *E. aerogenes* behaved differently from those PC scores of the reference. The adjusted PC scores of *B. subtilis* and the mixed culture were similar to the reference. The component loadings of the first nine PC are shown in Table 7. Peaks 1, 15, 34 and 37 had high loadings (≥ 0.8) in PC1. These peaks are important in classifying milk samples. Figure 17 shows gas chromatograms of UHT-sterilized milk

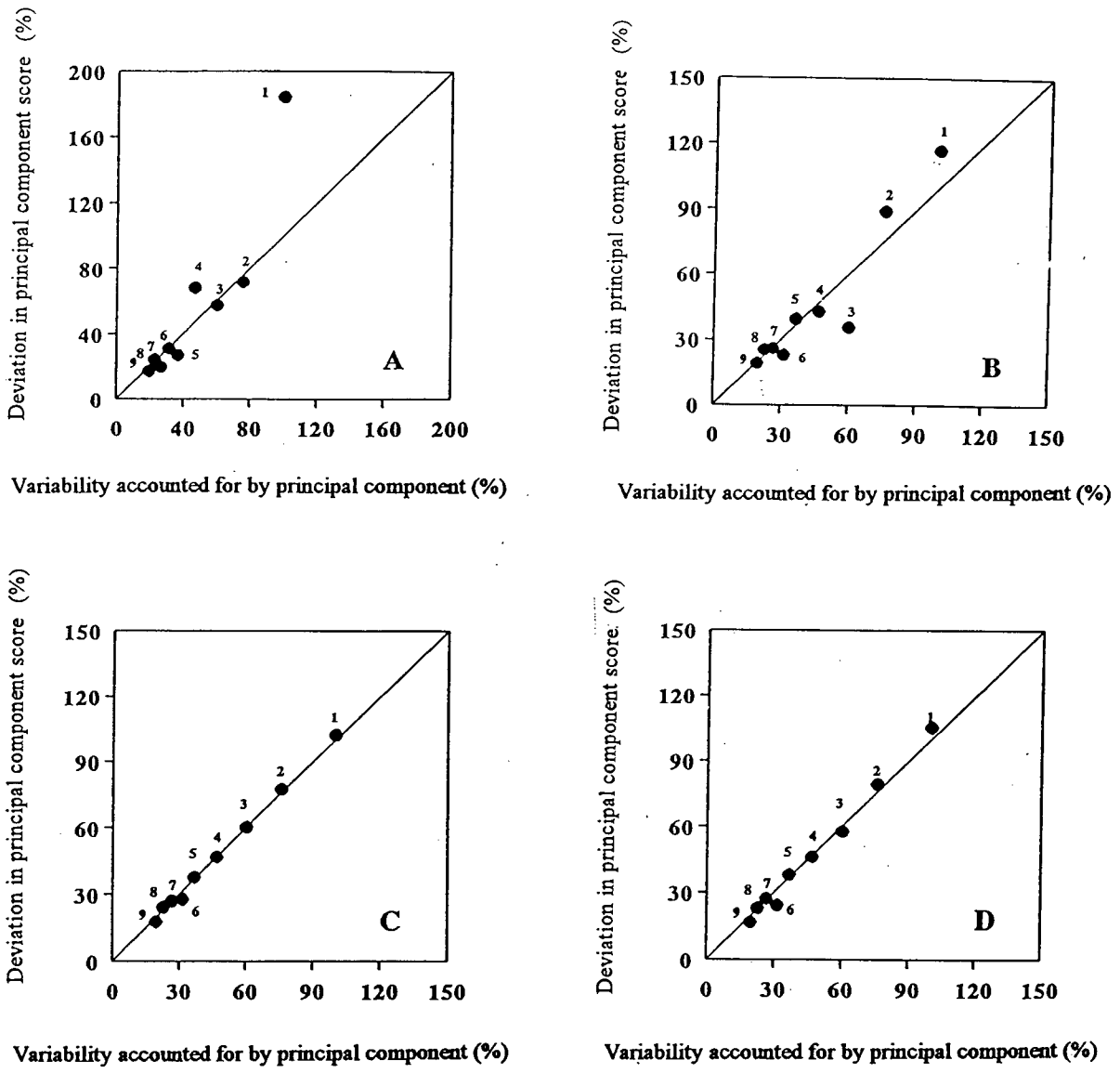

Figure 16. Adjusted principal component scores of UHT-sterilized milk inoculated with *L. lactis* (A), *E. aerogenes* (B), *B. subtilis* (C) and a mixed culture (D) after 12 hours. Adjusted principal component scores were computed according to steps 1-3 in Figure 1 (p. 41). The 45° line represents the match with principal component scores of the reference.

Table 7. Factor loadings (pattern) from principal component analysis of data for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture.

	Principal Component (PC)								
Peak	1	2	3	4	5	6	7	8	9
1	0.831	-0.149	0.155	0.042	-0.180	0.167	-0.360	0.027	-0.004
2	0.113	0.330	-0.593	-0.205	-0.083	0.089	0.203	0.014	0.203
3	0.094	0.423	-0.657	-0.248	0.049	0.253	-0.133	-0.112	-0.121
4	0.122	0.461	-0.732	-0.259	0.059	0.147	-0.139	-0.011	-0.093
5	0.116	0.478	-0.790	-0.260	0.083	0.108	-0.113	0.010	-0.020
6	-0.029	-0.058	0.214	-0.058	-0.469	0.081	-0.312	0.136	0.362
7	0.379	0.750	0.383	0.135	-0.126	0.112	-0.138	0.037	0.010
8	0.114	0.574	-0.553	-0.209	0.104	0.197	-0.231	-0.072	-0.246
9	0.079	0.835	0.405	0.230	0.140	-0.110	0.083	0.054	0.105
10	0.645	-0.093	0.263	-0.674	0.124	-0.027	0.006	-0.083	0.003
11	0.724	-0.113	-0.162	0.379	-0.374	0.243	0.005	-0.001	-0.072
12	0.112	0.037	0.122	0.021	-0.580	0.163	-0.571	0.165	-0.042
13	0.087	0.932	-0.055	0.040	0.138	0.183	0.054	-0.034	0.001
14	0.529	0.537	0.579	-0.269	-0.001	0.043	-0.045	0.000	0.067
15	0.800	-0.135	0.251	-0.376	0.111	0.101	-0.065	-0.053	0.008
16	0.765	-0.127	-0.191	0.556	0.135	-0.005	-0.064	-0.023	0.095
17	0.524	0.071	-0.081	-0.028	-0.651	-0.070	0.301	0.114	-0.234
18	0.421	-0.109	-0.081	-0.160	-0.287	-0.184	0.039	-0.251	0.035
19	-0.363	-0.253	0.041	0.008	0.011	0.522	0.164	0.176	0.381
20	0.091	0.635	0.451	0.293	-0.237	0.088	0.116	-0.026	0.026
21	0.115	0.895	0.337	0.196	0.112	-0.012	0.087	0.006	0.067
22	0.563	-0.069	0.346	-0.723	0.103	0.038	0.007	-0.068	0.006
23	0.740	-0.189	-0.172	0.568	0.123	0.081	-0.045	-0.061	0.070
24	0.552	-0.088	0.322	-0.698	0.130	-0.008	0.066	-0.099	0.009
25	0.699	0.056	0.031	-0.491	-0.254	-0.231	0.191	0.125	0.040
26	0.394	-0.155	-0.076	0.391	0.116	-0.026	0.042	-0.248	-0.179
27	0.094	0.818	0.453	0.243	0.111	-0.041	0.113	0.015	0.105
28	0.016	0.048	-0.137	-0.031	-0.487	0.098	0.546	-0.335	-0.036
29	0.428	0.132	-0.584	0.142	-0.174	-0.117	0.269	0.077	0.179
30	0.742	-0.124	-0.322	0.499	0.147	-0.005	-0.030	0.014	0.088
31	0.208	0.232	0.124	0.246	-0.074	-0.593	-0.103	0.117	-0.451
32	0.184	0.299	-0.722	-0.194	-0.050	-0.217	0.035	0.235	0.188
33	0.331	0.018	-0.292	-0.102	0.106	-0.670	-0.070	0.414	0.257
34	0.917	-0.204	-0.023	0.126	0.200	0.062	0.016	-0.087	0.084
35	0.715	-0.204	0.043	0.074	0.210	0.013	0.095	0.110	-0.079
36	0.488	-0.219	0.048	0.159	0.055	0.285	0.207	0.362	-0.121
37	0.911	-0.227	0.042	0.131	0.189	0.068	-0.001	-0.116	0.071
38	0.039	-0.109	0.156	-0.133	0.155	0.401	0.245	0.650	-0.329

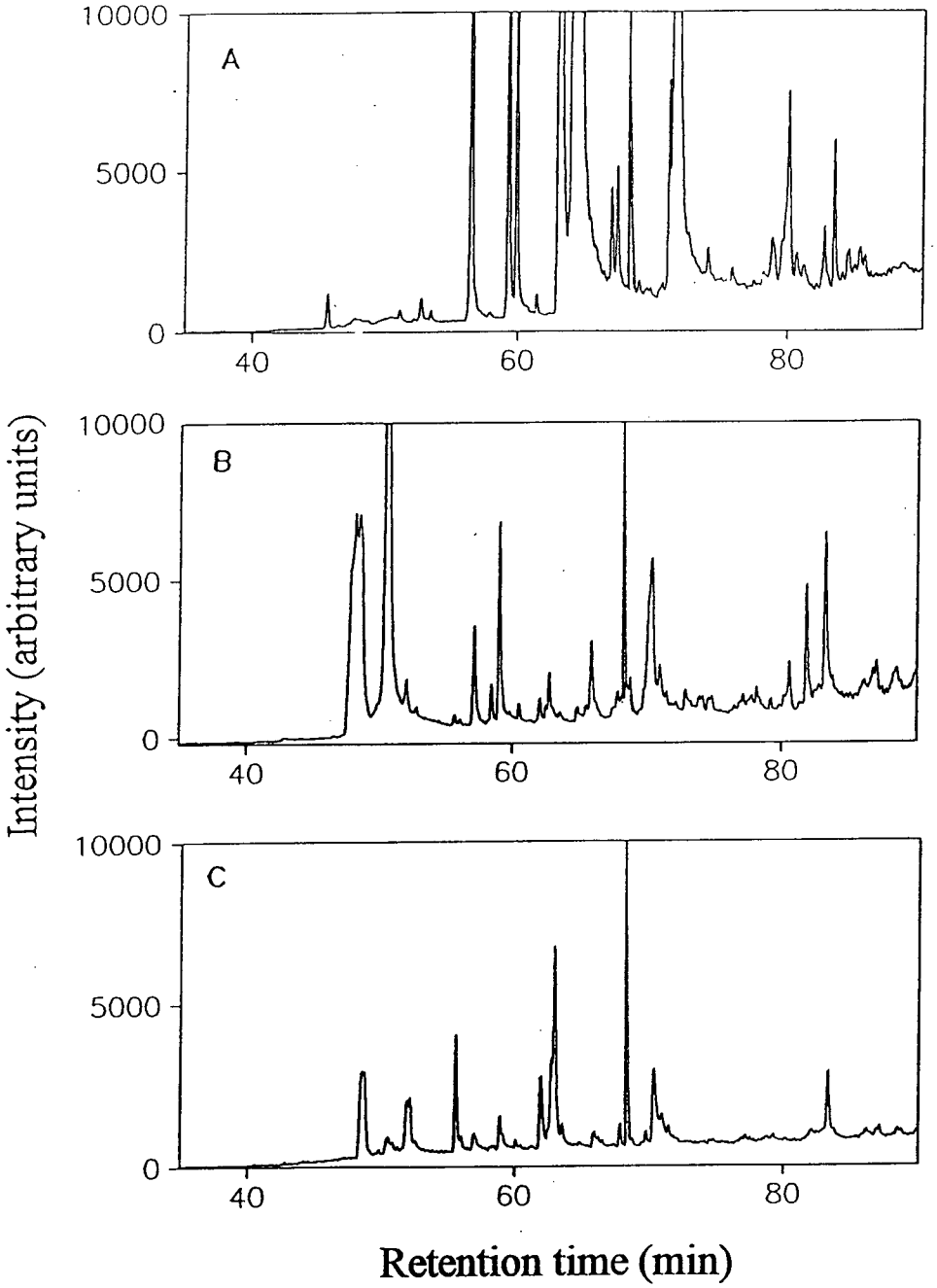

Figure 17. Gas chromatograms of UHT-sterilized milk inoculated with *L. lactis* after 12 hours (A), *E. aerogenes* after 12 hours (B) and a mixed culture after 12 hours (C).

inoculated with differnt bacteria.

These results suggest that the GC-PCS system has potential for partially replacing slow and expensive microbiological assays for identification of causative microbes. Since PCS is an unsupervised multivariate method, it may be useful in identifying causes or even preventing the incidence of accidental quality problems possibly critical to food safety. If this system were established, it could provide valuable assistance in determining the quality not only of milk and dairy products but also of other food products.

4. Comparison Among Supervised Methods

Artificial neural networks (ANN), partial least squares regression analysis (PLS) and principal component regression analysis (PCR) were applied to the gas chromatographic data. Before being compared, each method was optimized regarding the number of hidden neurons of ANN and the number of factors of PLS and PCR.

Effects of inoculating milk with *P. fragi* and *P. fluorescens*. The number of hidden neurons was optimized for the effectiveness of the ANN. With too many hidden neurons, a network may not learn but instead memorize patterns, or it may train and run too slowly. On the other hand, without enough hidden neurons, a network may not be trained (Lawrence and Peterson, 1992). Thus the number of hidden neurons was decreased starting from the default number. The default number was the sum of the numbers of input

and output divided by two (Lawrence and Peterson, 1992). ANN with too few hidden neurons cannot be completely trained. Therefore, the training time was set for half an hour at maximum.

Figure 18 shows results using all data for training ANN. The standard error of prediction (SEP) varied depending on the content of testing data. The pattern of trial 5 was different from the other trials. The difference of SEP within each trial was small (2 ~ 3%). There was no trend with different numbers of hidden neurons. Generally, only a small difference in SEP was found with various numbers of hidden neurons. This indicates the number of hidden neurons did not influence the system. The difference was presumed to come from another parameter, random weight for each connection. The strength of a connection between two neurons is called the weight. It determines the amount of effect that one neuron can have on the other (Lawrence and Peterson, 1992). This weight is randomly assigned. The first assigned weight might influence a network system.

Figure 19 shows prediction ability of cross validated ANN. Compared with Figure 18, the SEP was much greater. The SEP in trial 5 was consistently greater than that of other trials, while the SEP in trial 2 was smaller. This indicates that the SEP varied depending on the training and testing data. SEP was not greatly different within each trial. These results suggest that the prediction ability is dependent on the content of training and testing data. There was no trend with different numbers of hidden neurons except trial 2 where the SEP became smaller with increasing hidden neurons. Finally, the number of

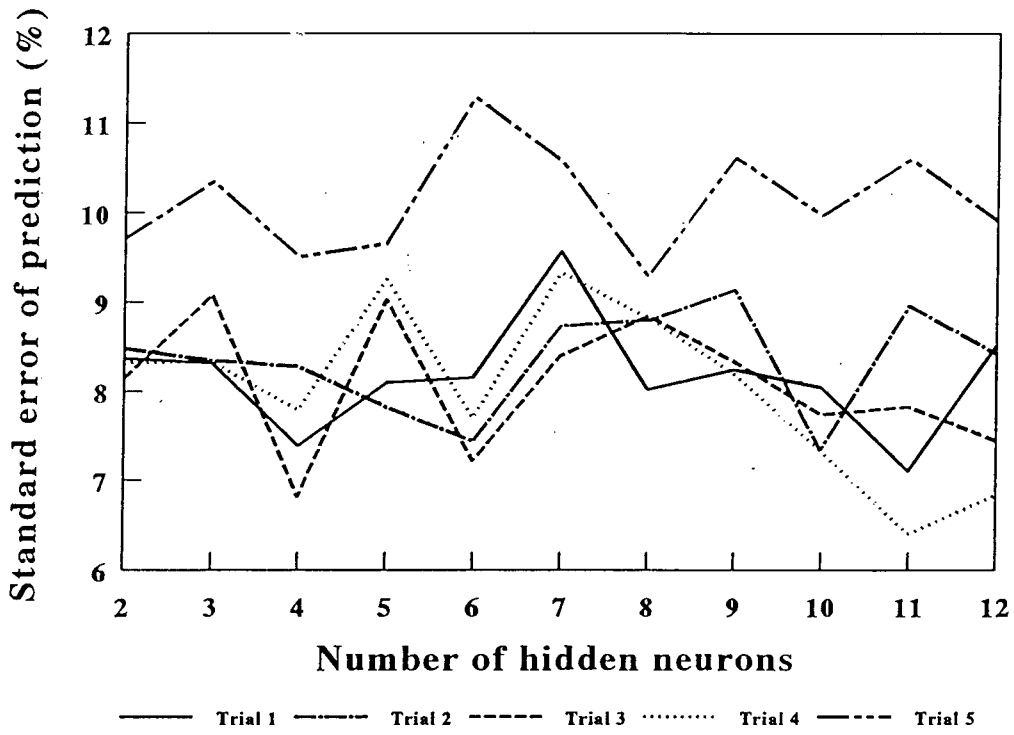

Figure 18. Standard error of prediction (%) by artificial neural networks (ANN) using different hidden neurons for *P. fragi* and *P. fluorescens*: training data include testing data.

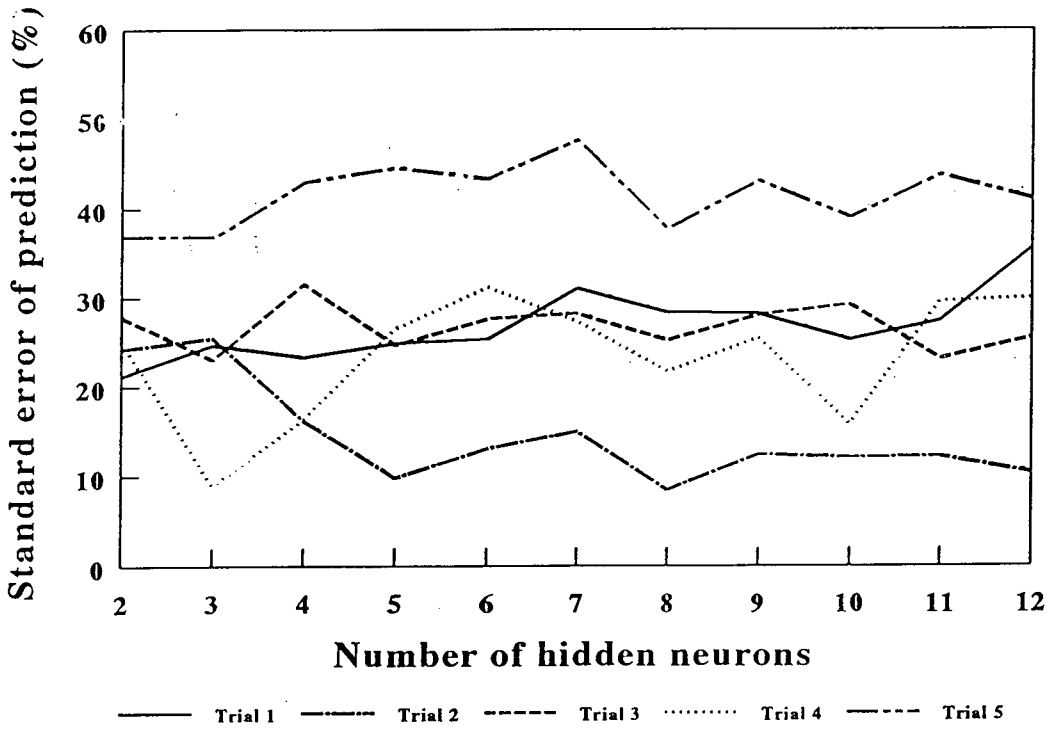

Figure 19. Standard error of prediction (%) by artificial neural networks (ANN) using different hidden neurons for *P. fragi* and *P. fluorescens*: training data do not include testing data.

hidden neurons which had the smallest SEP in the ANN in each trial was used for the comparison with PCR and PLS.

Table 8 presents a comparison of the prediction ability of ANN, PLS and PCR. A model for each method was trained with all data. The number of factors for PLS and PCR were optimized automatically while running the software (PLSplus, 1992). PLS used three factors and PCR used two factors for the optimum model.

The mean standard errors of prediction (SEP) were 7.4%, 17.0% and 23.4% for ANN, PLS and PCR, respectively. The SEP among methods was significantly different ($P < 0.01$) in the one-way ANOVA test. In order to determine which of the methods is significantly different from each other, a Turkey HSD test was applied. The result is also shown in Table 8 using a letter (A and B). "A" or "B" indicate significant differences at $P < 0.01$ or $P < 0.05$, respectively. The SEP of the ANN was significantly different from those of PLS ($P < 0.05$) and PCR ($P < 0.01$). The difference between PLS and PCR was not significant. Coefficients of variation for the SEP were 15.3%, 28.7% and 26.9% for the ANN, PLS and PCR, respectively. The ANN consistently gave better prediction ability.

The mean coefficients of determination (r^2) were 0.98, 0.84 and 0.74 for the ANN, PLS and PCR, respectively. There were significant differences among the methods ($P < 0.05$). From a Turkey HSD test, the r^2 of the ANN was significantly different from that of PCR ($P < 0.01$). Coefficient of variation for r^2 of the ANN was much smaller than those of both PLS and PCR.

Table 8. Comparison of prediction ability of artificial neural networks (ANN), partial least squares regression analysis (PLS) and principal component regression analysis (PCR) for *P. fragi* and *P. fluorescens*: training data include testing data.

Trial	SEP (%) [a]			r^{2} [b]		
	ANN	PLS	PCR	ANN	PLS	PCR
1	7.1	21.9	23.9	0.98	0.73	0.67
2	7.3	17.7	19.8	0.99	0.88	0.96
3	6.8	9.0	26.1	0.98	0.98	0.75
4	6.4	16.8	15.4	0.98	0.83	0.89
5	9.3	19.5	32.0	0.96	0.78	0.43
Mean	7.4**[A1.B]	17.0**[A1]	23.4**[B]	0.98*[A2]	0.84*	0.74*[A2]
SD [c]	1.1	4.9	6.3	0.01	0.10	0.21
CV (%) [d]	15.3	28.7	26.9	1.1	11.4	28.0

* Significant ($P < 0.05$), ** significant ($P < 0.01$) in one-way ANOVA test.
[A1, A2] Significant ($P < 0.01$), [B] significant ($P < 0.05$) in a Turkey HSD test.
[a] Standard error of prediction was divided by range of experimental values in testing.
[b] Coefficient of determination.
[c] Standard deviation.
[d] Coefficient of variation.

Table 9 shows the prediction ability of each method using cross validation. The mean SEP were 21.7%, 29.5% and 33.3% for the ANN, PLS and PCR, respectively. The mean r^2 between actual and predicted values were 0.80, 0.69 and 0.59 for the ANN, PLS and PCR, respectively. The SEP and r^2 among each method were not significantly different from the one-way ANOVA test ($P > 0.05$). The coefficient of variation of the ANN was much larger than that of PLS and PCR. In ANN, trial 2 gave the smallest SEP. The difference between the smallest and largest SEP for the ANN was about 28%. Even though the ANN generally had smaller SEP than PLS and PCR, the content of training and testing data have great influence on prediction ability in the ANN.

Generally, the ANN was able to make better predictions than PCR and PLS. This indicates that the relationship between dependent and independent values may be non-linear. The ANN was also faster and easier to use than PCR and PLS. The result using all data was better than cross-validated results. This suggests that a larger data set increases prediction ability for the ANN.

Effects of inoculating milk with *L. lactis*, *E. aerogenes*, *B. subtilis* and a mixed culture. Figure 20 presents the performance of the ANN with differing numbers of hidden neurons using all data for training. The SEP tended to be smaller with more hidden neurons. Small variations of the SEP were observed within each trial (2~3%).

Table 9. Comparison of prediction ability of artificial neural networks (ANN), partial least squares regression analysis (PLS) and principal component regression analysis (PCR) for *P. fragi* and *P. fluorescens*: training data do not include testing data.

	SEP (%) [a]			r^{2} [b]		
Trial	ANN	PLS	PCR	ANN	PLS	PCR
1	21.2	20.3	26.0	0.75	0.78	0.70
2	8.5	28.9	35.7	0.96	0.89	0.81
3	23.1	43.7	45.3	0.93	0.61	0.52
4	9.0	20.2	21.8	0.95	0.85	0.79
5	36.8	13.5	37.5	0.41	0.30	0.13
Mean	21.7	29.5	33.3	0.80	0.69	0.59
SD [c]	10.0	10.0	9.4	0.23	0.24	0.28
CV (%) [d]	46.0	33.8	28.2	29.3	35.1	47.7

[a] Standard error of prediction was divided by range of experimental values in testing.
[b] Coefficient of determination.
[c] Standard deviation.
[d] Coefficient of variation.

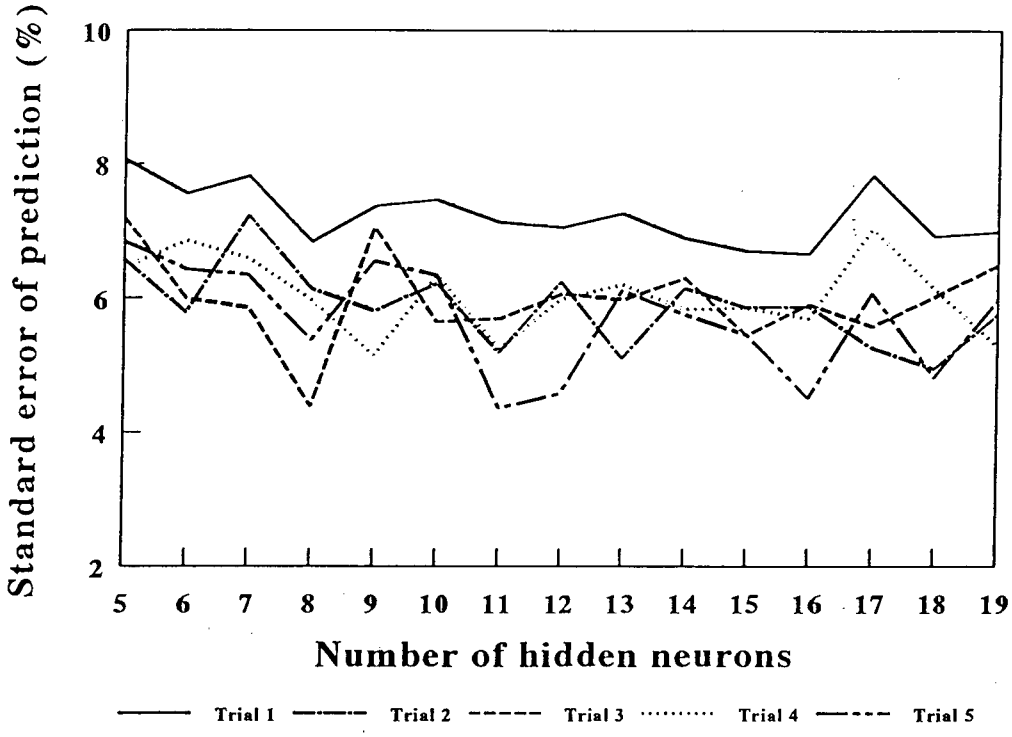

Figure 20. Standard error of prediction (%) by artificial neural networks (ANN) using different hidden neurons for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture: training data include testing data.

Figure 21 shows the performance of the cross validated ANN. Except in trial 2, there was a small difference for SEP with different numbers of hidden neurons. Compared with Figure 20, the SEP was much greater. These trends were similar to those for milk inoculated with *P. fragi* and *P. fluorescens*.

The ANN with the smallest SEP was used for the comparisons with PCR and PLS. The number of factors for PLS and PCR were 5 and 6, respectively. Table 10 shows the comparisons of the three methods. A model was trained with all data. The mean SEP were 5.2%, 21.1% and 25.1% for ANN, PLS and PCR, respectively. There were significant differences ($P < 0.01$) among the three methods. In the Turkey HSD test, the SEP of the ANN was significantly different from those of PLS and PCR ($P < 0.01$). The difference between PLS and PCR was significant ($P < 0.05$). Coefficients of variation for the SEP were 16.8%, 14.3% and 9.5% for the ANN, PLS and PCR, respectively. These values suggest that prediction ability of the ANN varied depending on testing data, even though the ANN consistently gave smaller SEP than PLS and PCR. PCR consistently had poorer prediction ability than the ANN and PLS. The mean r^2 were 0.98, 0.71 and 0.56 for the ANN, PLS and PCR, respectively. There were significant differences ($P < 0.01$) among the three methods. The r^2 of the ANN was significantly different from those of PLS and PCR ($P < 0.01$). The difference between PLS and PCR was also significant ($P < 0.05$).

Table 11 shows the cross validation results of ANN, PLS and PCR. The mean SEP were 18.8%, 26.9% and 26.2% for the ANN, PLS and PCR, respectively. They were significantly different ($P < 0.01$) from the one-way ANOVA. From a Turkey HSD test, the

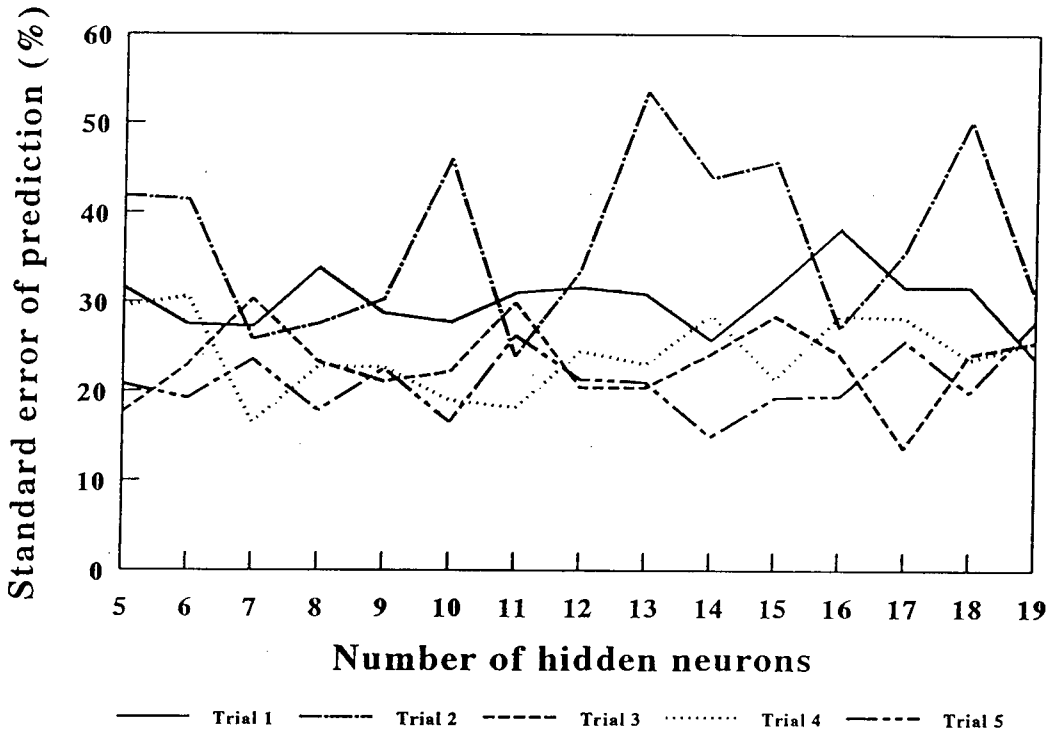

Figure 21. Standard error of prediction (%) by artificial neural networks (ANN) using different hidden neurons for *L. lactis*, *E. aerogenes*, *B. subtilis* and a mixed culture: training data do not include testing data.

Table 10. Comparison of prediction ability of artificial neural networks (ANN), partial least squares regression analysis (PLS) and principal component regression analysis (PCR) for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture: training data include testing data.

Trial	SEP (%) [a]			r^{2} [b]		
	ANN	PLS	PCR	ANN	PLS	PCR
1	6.7	22.8	27.6	0.97	0.73	0.45
2	5.1	24.5	26.9	0.98	0.63	0.60
3	4.9	22.3	24.0	0.98	0.63	0.60
4	5.1	17.5	25.4	0.98	0.74	0.45
5	4.4	18.4	21.6	0.98	0.82	0.70
Mean	5.2**[A1,A2]	21.1**[A1,B1]	25.1**[A2,B1]	0.98**[A3,A4]	0.71**[A3,B2]	0.56**[A4,B2]
SD [c]	0.9	3.0	2.4	0.004	0.08	0.11
CV (%) [d]	16.8	14.3	9.5	0.46	11.4	19.4

* Significant ($P < 0.05$), ** significant ($P < 0.01$) in one-way ANOVA test.
[A1, A2, A3, A4] Significant ($P < 0.01$), [B1,B2] significant ($P < 0.05$) in a Turkey HSD test.
[a] Standard error of prediction was divided by range of experimental values in testing.
[b] Coefficient of determination.
[c] Standard deviation.
[d] Coefficient of variation.

Table 11. Comparison of prediction ability of artificial neural networks (ANN), partial least squares regression analysis (PLS) and principal component regression analysis (PCR) for *L. lactis, E. aerogenes, B. subtilis* and a mixed culture: training data do not include testing data.

	SEP (%) [a]			r^2 [b]		
Trial	ANN	PLS	PCR	ANN	PLS	PCR
1	24.8	29.9	29.1	0.58	0.37	0.50
2	24.2	26.3	27.9	0.59	0.61	0.51
3	14.3	26.1	25.6	0.84	0.52	0.48
4	15.7	26.7	26.2	0.81	0.43	0.46
5	15.0	25.5	22.1	0.83	0.59	0.70
Mean	18.8**^{A,B1}	26.9**^{A}	26.2**^{B1}	0.73*^{B2,B3}	0.50*^{B2}	0.53*^{B3}
SD [c]	5.2	1.7	2.7	0.13	0.10	0.10
CV (%) [d]	27.8	6.3	10.2	18.2	20.4	18.3

* Significant ($P < 0.05$), ** significant ($P < 0.01$) in one-way ANOVA test.
[A] Significant ($P < 0.01$), [B1, B2, B3] significant ($P < 0.05$) in a Turkey HSD test.
[a] Standard error of prediction was divided by range of experimental values in testing.
[b] Coefficient of determination.
[c] Standard deviation.
[d] Coefficient of variation.

ANN had a significantly smaller SEP than PLS ($P < 0.01$) and PCR ($P < 0.05$). No significant difference was found between PLS and PCR. The mean r^2 were 0.73, 0.50 and 0.53 for the ANN, PLS and PCR, respectively. They were significantly different ($P < 0.05$). The differences between the ANN and PLS and the ANN and PCR were significant ($P < 0.05$) in the Turkey HSD test. The difference between PLS and PCR was not significant. ANN gave the best predictive ability. The values for the SEP and r^2 of PCR for the cross validated data were better than those for PLS. However, the differences were not significant ($P > 0.05$). Therefore, there was no difference of predictive ability between PLS and PCR ($P > 0.05$).

From the above two experiments, it is concluded that the ANN gave the best prediction ability among three supervised methods. The prediction ability of PLS was better than PCR. However, the performance of each method was dependent on the content of training and testing data; the more data, the better the prediction ability. Each method gave better predictive ability when trained with all data.

The ANN and PLS may be the most advanced methods. However, supervised classification requires reliable sample grouping in advance. If the sample grouping relies on subjective methods, it immediately affects the accuracy of classification. Since the ANN are supervised methods, a large data set is required for accurate, more reliable classification. Although a great deal of theoretical and experimental work remains to be done, it appears that the ANN technique can be employed as a simple, rapid computer technique for accurate classification of milk quality in the future.

C. Flavour Defects Due to Chemical Reactions

1. Multivariate Analysis of Gas Chromatographic Results

a) Principal Component Analysis (PCA)

PCA was performed on the eleven normalized gas chromatographic peak areas of 44 milk samples. The first four principal components (PC) had eigen values greater than one and accounted for 72.7% of the total variance (Table 12). This means that 11 peaks in the original gas chromatographic data were extracted into four PC with 27.3% loss of the information. The first and second PC were plotted in Figure 22. Light-induced, oxidized and heated milk samples were separated into their groups. The cooked milk samples were not clearly separated. Most of them were close to the negative control and heated milk samples. A few of them were misclassified as oxidized milk samples. Plots of heated milk were close to the normal milk samples (negative control). This suggests that the patterns of GC for cooked and heated milk samples were similar to that of milk without a treatment. Heated and light-induced samples were separated into their groups in a three dimensional plot (Figure 23). Cooked samples were closer to heated samples, while oxidized samples were closer to light induced samples. But no clear separation between cooked and oxidized milk samples was observed. The third PC scores of light-induced samples were generally smaller than those of other samples.

Factor loadings for the first four PC are shown in Table 13. The higher the absolute value of the loading the more the component contributed to the variance among the samples. Peaks 4, 8 and 11 had a high loading (≥ 0.7) in PC1. Peaks 3 and 6 had

Table 12. Results of cumulative eigen values for gas chromatographic data for pasteurized milk samples.

Factor	Eigen value	Variance	Cumulation (%)
1	2.536	23.052	23.052
2	2.451	22.280	45.332
3	1.890	17.182	62.514
4	1.121	10.190	72.704

Table 13. Factor loadings (pattern) from principal component analysis of gas chromatographic data for pasteurized milk samples.

	Principal Component (PC)			
Peak No.	1	2	3	4
1	0.212	0.450	-0.591	0.051
2	-0.020	0.219	-0.870	0.218
3	-0.159	0.818	0.029	0.029
4	0.760	0.382	0.132	0.242
5	0.081	0.265	0.718	0.545
6	0.197	-0.818	-0.134	0.074
7	0.068	-0.504	-0.192	0.771
8	0.781	0.131	-0.107	-0.184
9	-0.569	0.285	0.304	0.027
10	0.567	-0.445	0.278	-0.282
11	0.763	0.308	0.122	0.030

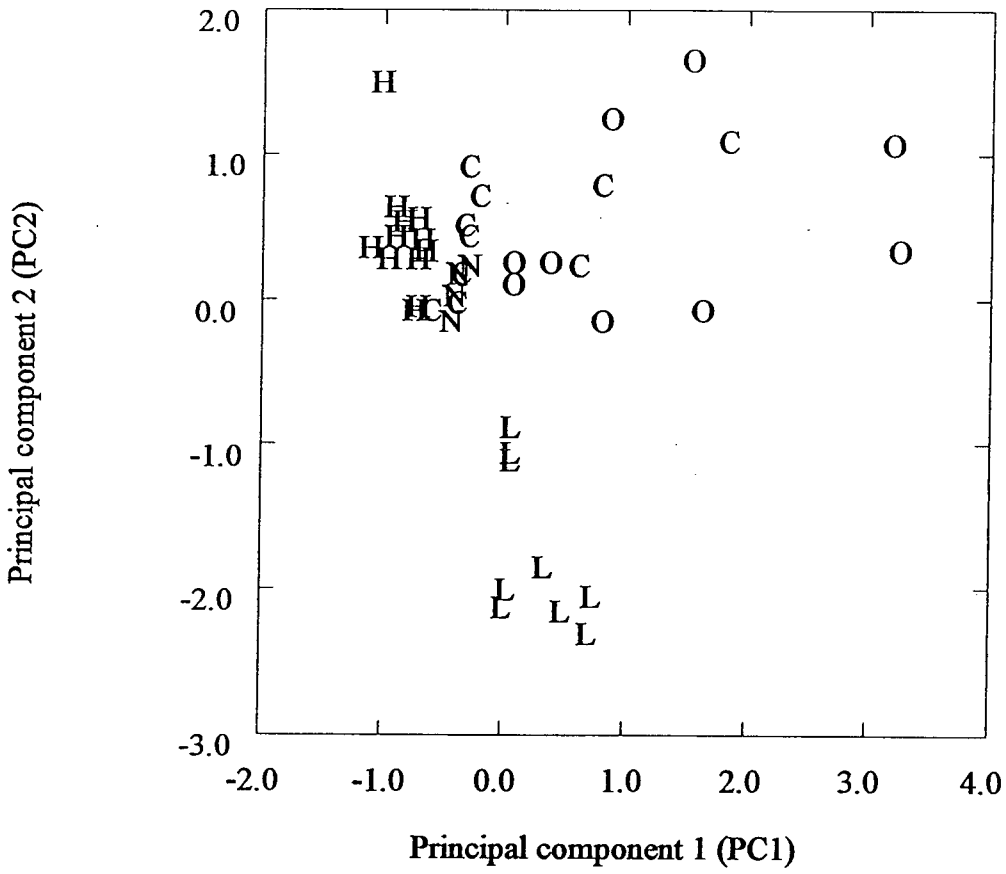

Figure 22. Principal component analysis plot (two dimensional) from gas chromatographic data for pasteurized milk samples with various treatments. N = Negative control. C = Cooked. H = Heated. L = Light-induced. O = Oxidized.

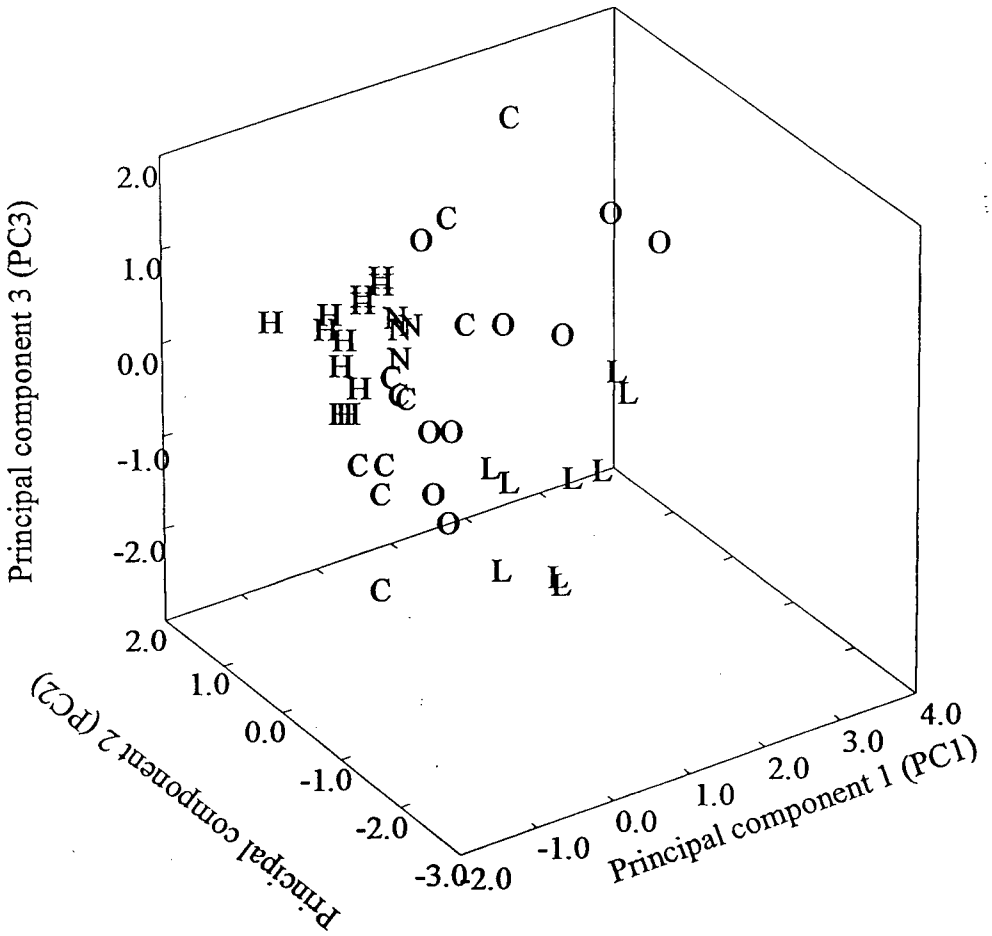

Figure 23. Principal component analysis plot (three dimensional) from gas chromatographic data for pasteurized milk samples with various treatments. N = Negative control. C = Cooked. H = Heated. L = Light-induced. O = Oxidized.

high loadings (≥ 0.8) in PC2. Peaks 2 and 5 had high loadings (≥ 0.7) for PC3.

b) Principal Component Similarity Analysis (PCS)

The first four PC from PCA and the variance they each explain were subjected to PCS analysis. PCS was carried out using the pasteurized milk without treatments (day 0 after pasteurization) as a reference. The coefficients of determination as well as the slopes between samples and the reference were calculated. Figure 24 shows a PCS plot of gas chromatographic peak areas. Most of the light-induced, oxidized and heated milk samples were separated into groups. The light-induced milk had, in general, smaller coefficients of determination than other milk. The oxidized milk had a larger slope. Cooked milk samples were not well differentiated and close to the point of reference (Coefficient of determination = 1, Slope = 1).

To account for the difference between the sample and the reference, the adjusted PCA scores of the samples were plotted against the adjusted PCA scores of the reference (Figure 25). The largest deviation of light-induced milk was accounted for by PC2 which was heavily loaded by peaks 3 and 6 (Table 13). Therefore, these peaks contribute to the substantial difference between the standard and the sample. In oxidized milk, PC1 was greatly differentiated. Little difference was observed between standard and cooked or heated milk. Figure 26 shows chromatograms of pasteurized milk samples with various treatments.

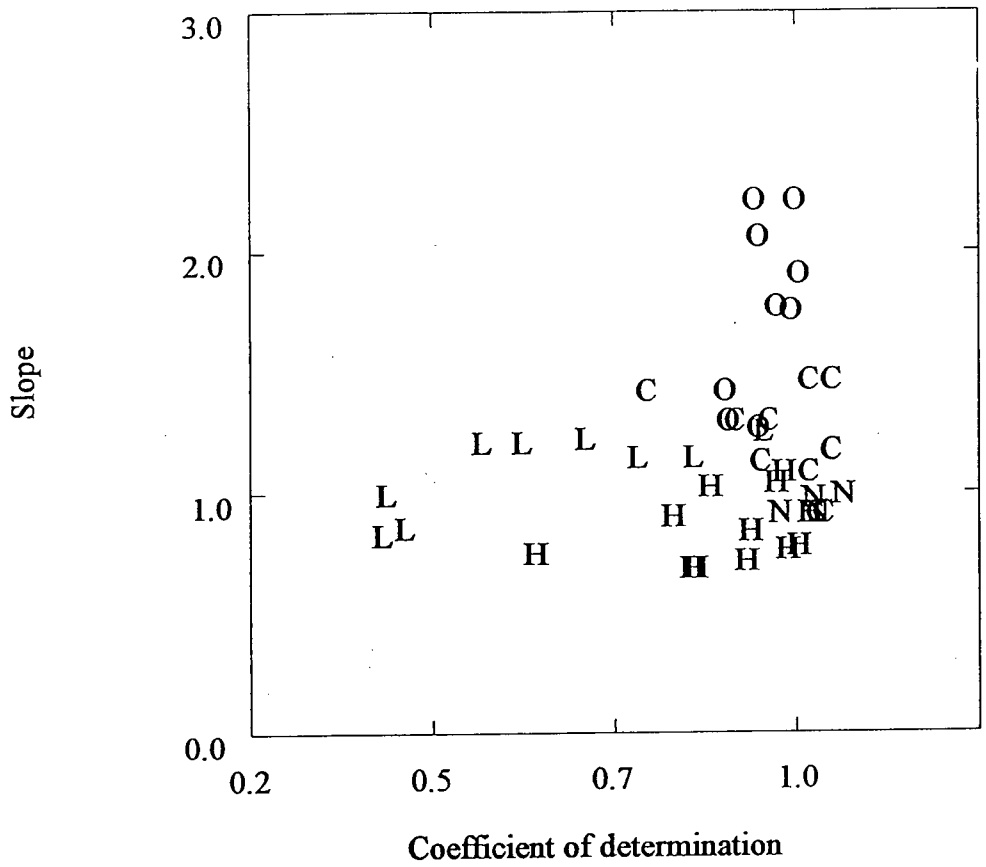

Figure 24. Principal component similarity analysis plot of gas chromatographic data for pasteurized milk samples with various treatments. Pasteurized milk without treatments was used as a reference. N = Negative control. C = Cooked. H = Heated. L = Light-induced. O = Oxidized. The coordinates for the reference are (1,1).

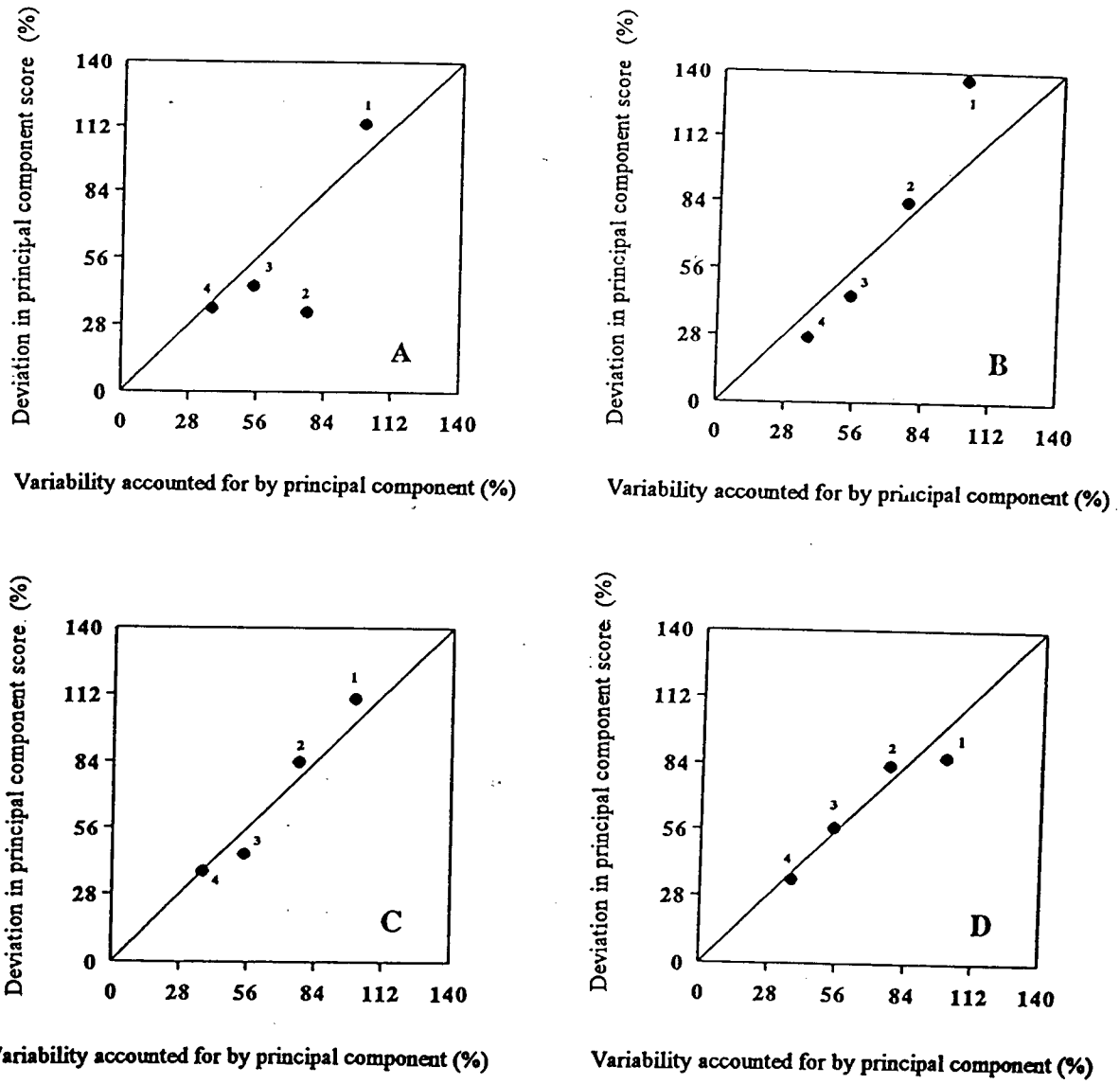

Figure 25. Adjusted principal component scores of pasteurized milk samples with various treatments. Adjusted principal component scores were computed according to steps 1-3 in Figure 1. The 45° line represents the match with principal component scores of the reference. A = Light-induced. B = Oxidized. C = Cooked. D = Heated.

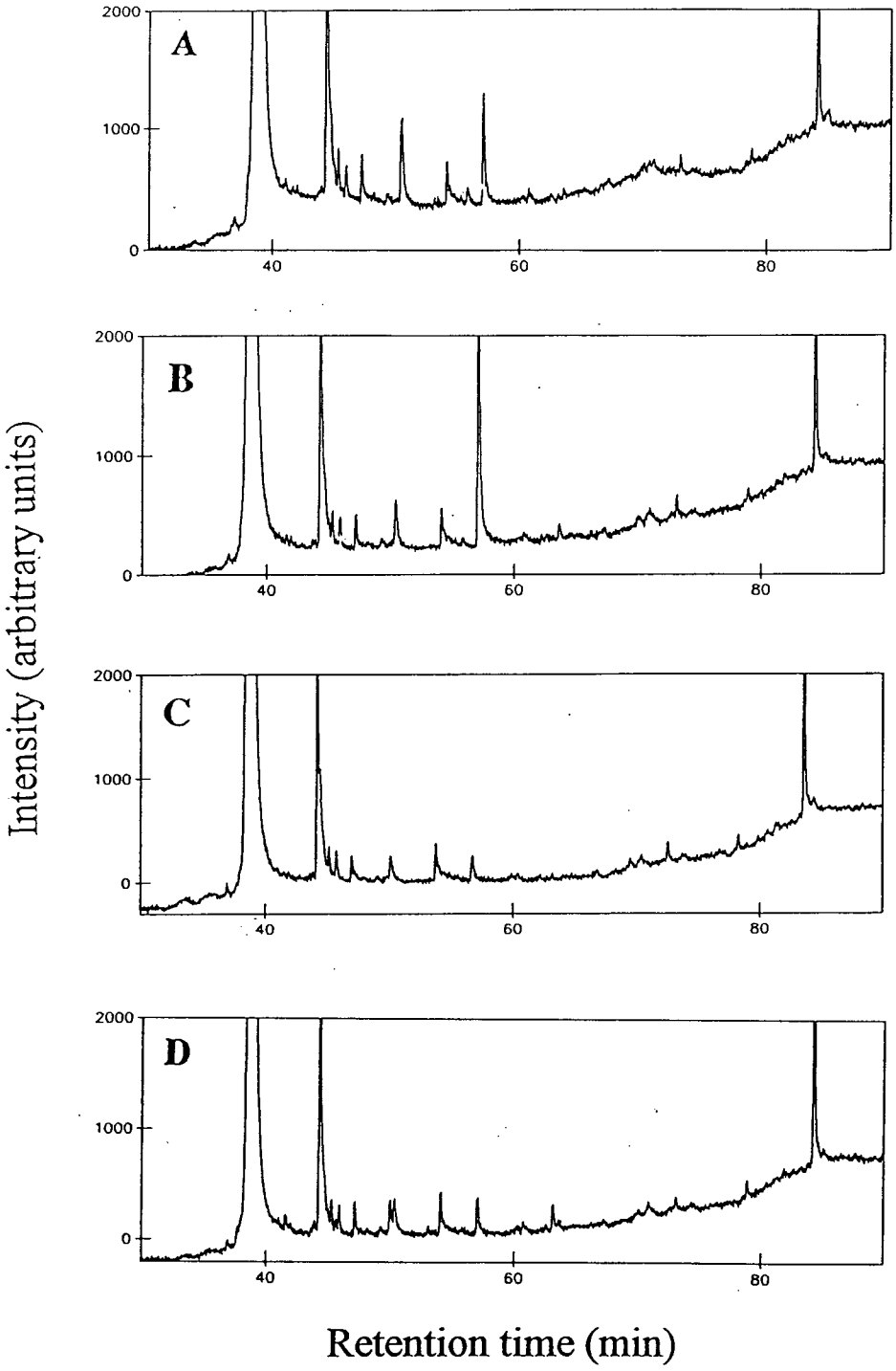

Figure 26. Gas chromatograms of pasteurized milk samples with various treatments. A = Light-induced. B = Oxidized. C = Cooked. D = Heated.

2. Sensory Analysis

a) Aroma

F-values of the two-way analysis of variance (ANOVA) test of unstandardized sensory scores for smelling are shown in Table 14. F-values for all attributes were significantly different among panellists. Among the treatments, cooked and heated flavour were significant ($P < 0.001$). Metal and rancid flavour were also significant ($P < 0.05$).

The sensory scores of each attribute from each panellist were standardized (Table 15). The procedure for standarization was described in the MATERIALS AND METHODS section (p. 49). There were no significant differences among the panellists. Cooked, heated, metal, rancid and acceptability were significantly different among the various treatments. There were no significant differences between the panellist and treatment interactions.

b) Taste

The two-way ANOVA test of unstandardized sensory scores for tasting showed significant differences in acid, astringent, bitter, light-induced and rancid flavour among the panellists (Table 16). Cooked, light induced, metal and rancid were significantly different among various treatments. Analysis of variance of standardized sensory flavour scores indicated significant differences between treatments: acid, cooked, heated, light induced, metal and rancid (Table 17). Many more F-values calculated for the given

Table 14. F-values for two-way analysis of variance of unstandardized sensory scores from smelling.

	Acid	Astringent	Bitter	Cooked	Heated	Light	Metal	Rancid	Accept
Panellist (P)	2.823**	8.290***	3.310***	2.584**	3.374**	3.258***	2.821**	3.449***	3.146***
Treat (T)	1.889	2.051	1.702	13.95***	20.491***	1.522	3.732*	3.727*	19.334***
P * T	1.793*	1.536	0.971	1.355	1.846*	0.517	0.765	0.892	0.703

Significant at * P < 0.05, ** P < 0.01, *** P < 0.001; rest not significant.

Table 15. F-values for two-way analysis of variance of standardized sensory scores[a] from smelling.

	Acid	Astringent	Bitter	Cooked	Heated	Light	Metal	Rancid	Accept
Panellist (P)	0.283	0.744	0.492	0.716	0.406	1.569	0.866	0.435	0.338
Treat (T)	2.194	1.803	2.169	14.259***	20.834***	2.787	5.170**	4.667**	23.728***
P * T	0.052	1.030	0.727	1.070	0.646	0.99	0.836	0.470	1.025

a Scores of each attribute were standardized within each judge.
Significant at * P < 0.05, ** P < 0.01, *** P < 0.001; rest not significant.

Table 16. F-values for two-way analysis of variance of unstandardized sensory scores from tasting.

	Acid	Astringent	Bitter	Cooked	Heated	Light	Metal	Rancid	Accept
Panellist (P)	3.811***	4.921***	2.440*	1.334	0.859	2.696***	1.779	2.317*	2.738**
Treat (T)	1.162	0.277	1.408	5.725***	2.026	4.187**	23.217***	4.412**	24.739***
P * T	0.386	1.854**	0.793	1.352	1.191	0.637	1.515	1.348	1.319

Significant at * P < 0.05, ** P < 0.01, *** P < 0.001; rest not significant.

Table 17. F-values for two-way analysis of variance of standardized sensory scores[a] from tasting.

	Acid	Astringent	Bitter	Cooked	Heated	Light	Metal	Rancid	Accept
Panellist (P)	1.251	1.013	1.097	0.707	0.412	1.768	0.679	1.756	2.499*
Treat (T)	2.781*	0.496	1.145	5.343***	10.121***	5.752***	22.218***	4.175**	28.079***
P * T	0.689	0.879	0.661	1.426	0.800	1.085	0.707	1.246	1.140

a Scores of each attribute were standardized within each judge.
Significant at * P < 0.05, ** P < 0.01, *** P < 0.001; rest not significant.

attributes were significantly different among treatments when analyzed by tasting than by smelling.

3. Multivariate Analysis of Sensory Analysis Results

a) Principal Component Analysis (PCA)

(1) Aroma

In order to determine which variables discriminate among samples, principal component analysis (PCA) was applied. PCA of milk samples yielded three PC with eigen values greater than one, which accounted for 72.1% of the total variance (Table 18). Figures 27 and 28 show two and three dimensional PCA plots of sensory scores for smelling. The heated milk samples were differentiated from other samples. Some light-induced and oxidized samples were also differentiated. Some panellists could differentiate milk samples such as light-induced, oxidized and heated flavour. However, most samples were misclassified by smelling. PCA was not considered very successful in differentiating milk samples. Table 19 shows the loadings of the first three PC. The first PC was composed of sensory parameters such as bitter and rancid. This indicated that bitter and rancid are particularly important when evaluating the samples. The second PC was a combination of cooked and heated attributes. The third PC was a combination of light-induced and astringent.

Table 18. Results of cumulative eigen values for sensory scores from smelling pasteurized milk samples with various treatments.

Factor	Eigen value	Variance	Cumulation (%)
1	3.072	38.41	38.05
2	1.538	19.23	57.63
3	1.159	14.48	72.11

Table 19. Factor loadings (pattern) from principal component analysis of sensory scores from smelling pasteurized milk samples with various treatments.

	Principal Component (PC)		
Attributes	1	2	3
Acid	0.670	0.075	0.182
Astringent	0.593	0.112	-0.611
Bitter	0.720	0.178	-0.344
Cooked	0.501	-0.781	-0.021
Heated	0.421	-0.831	0.069
Light	0.594	0.187	0.584
Metal	0.660	0.261	0.471
Rancid	0.732	0.290	-0.257

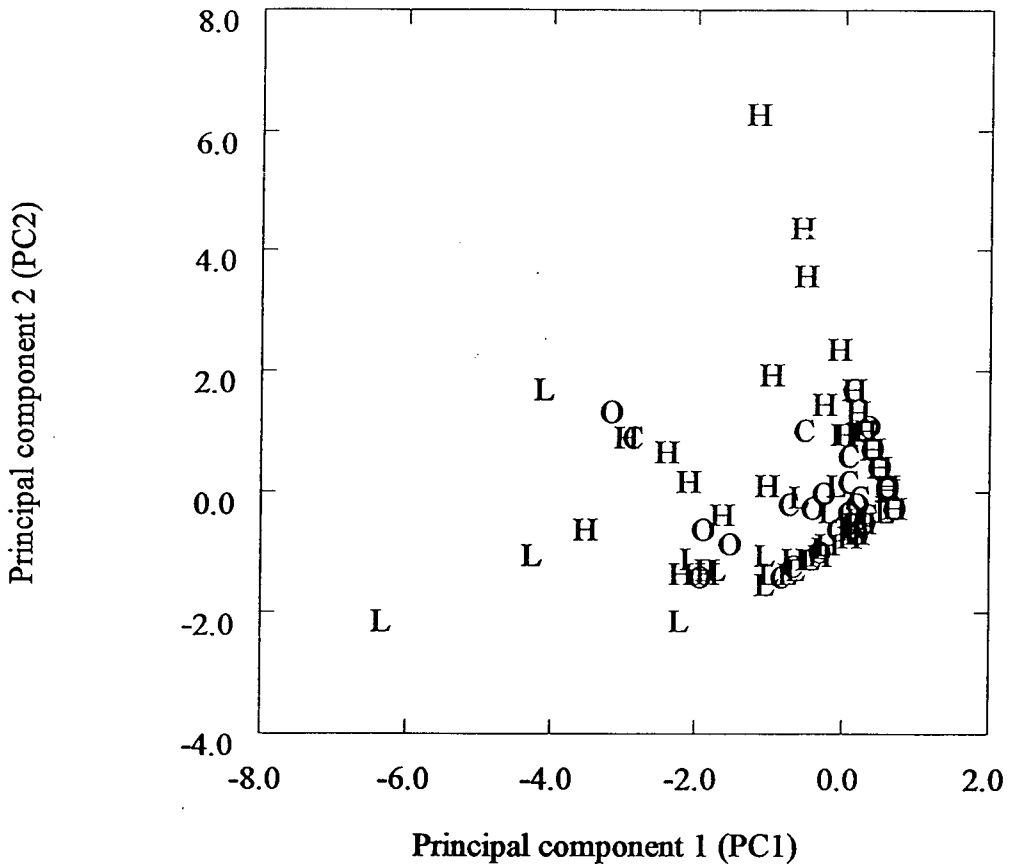

Figure 27. Principal component analysis plot (two dimensional) from sensory scores for smelling pasteurized milk samples with various treatments. C = Cooked. H = Heated. L = Light-induced. O = Oxidized.

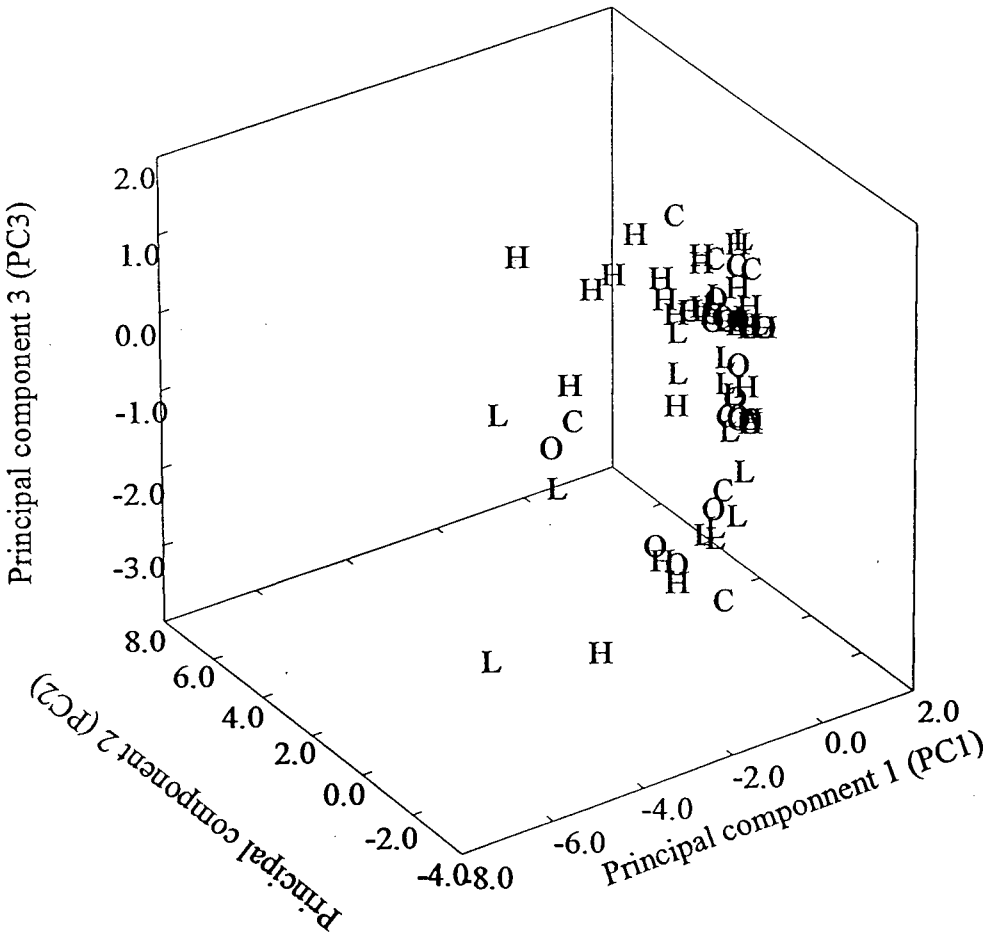

Figure 28. Principal component analysis plot (three dimensional) for sensory scores from smelling pasteurized milk samples with various treatments. C = Cooked. H = Heated. L = Light-induced. O = Oxidized.

(2) Taste

Three PC were extracted, explaining 59.3% of the total variance in the sensory scores from tasting. These are presented in Table 20. Figures 29 and 30 show two and three dimensional PCA plots. Milk samples were differentiated by taste more accurately than by smell. While light-induced and heated milk samples were relatively well separated, the oxidized and cooked samples were not clearly differentiated. However, none of them was separated into a single group. The component loadings of each variance for the first three PC are shown in Table 21. By definition, the first PC contains the most important information and includes the more important attribute (Manly, 1986). The first PC contrasted acid, astringent, bitter and rancid flavours. These attributes are important for differentiating milk samples. The second PC was differentiated by cooked and heated flavour. They were negatively related to the second PC. Bitterness was also negatively correlated to the second PC. The third PC is composed of light-induced flavour (positively), astringent (negatively) and bitterness (negatively).

b) Principal Component Similarity Analysis (PCS)

(1) Aroma

The first three PC, which accounted for 72% of the total variance were used for the PCS analysis. Figure 31 shows the PCS plot of sensory scores for smelling. The untreated milk sample was used as a reference. More points were scattered in

Table 20. Results of cumulative eigen values for sensory scores from tasting pasteurized milk samples with various treatments.

Factor	Eigen value	Variance	Cumulation (%)
1	2.477	30.958	30.958
2	1.232	15.403	46.361
3	1.054	13.172	59.533

Table 21. Factor loadings (pattern) from principal component analysis of sensory scores from tasting pasteurized milk samples with various treatments.

	Principal Component (PC)		
Attributes	1	2	3
Acid	0.613	0.072	0.272
Astringent	0.623	0.106	-0.440
Bitter	0.665	-0.236	-0.431
Cooked	0.410	-0.647	0.079
Heated	0.188	-0.737	0.290
Light	0.538	0.304	0.559
Metal	0.593	0.250	0.333
Rancid	0.652	0.211	-0.294

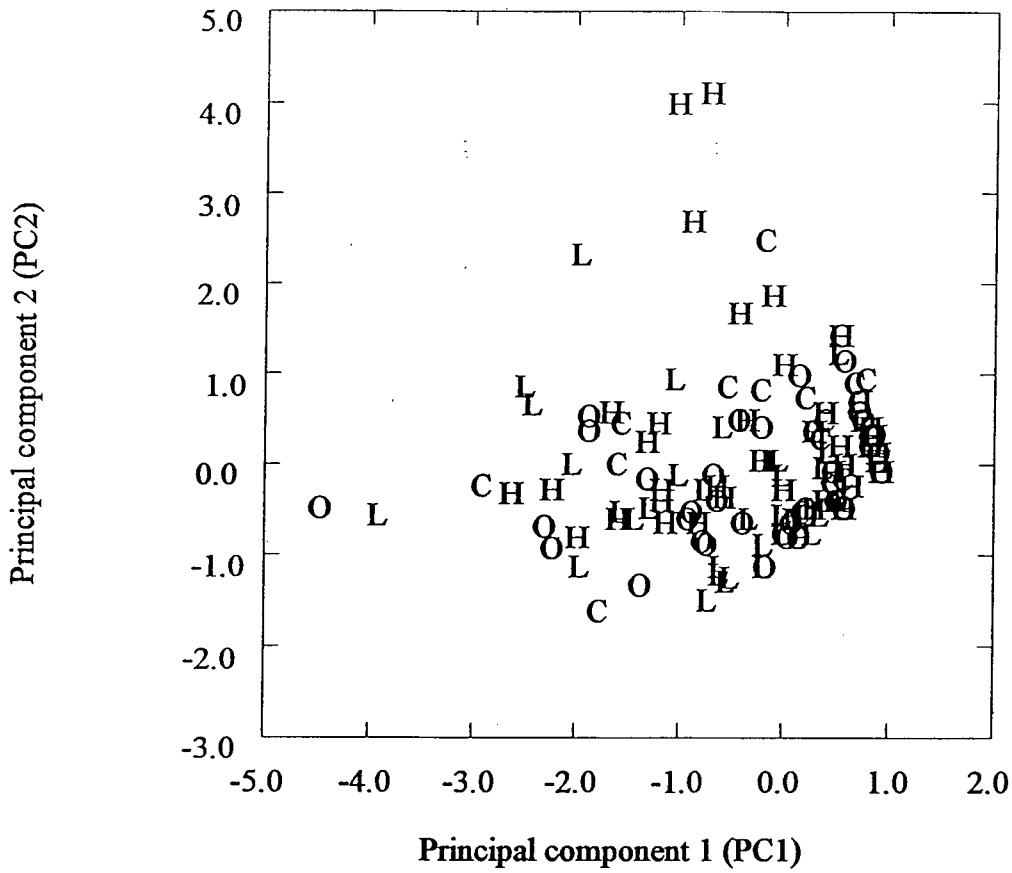

Figure 29. Principal component analysis plot (two dimensional) for sensory scores from tasting pasteurized milk samples with various treatments. C = Cooked. H = Heated. L = Light-induced. O = Oxidized.

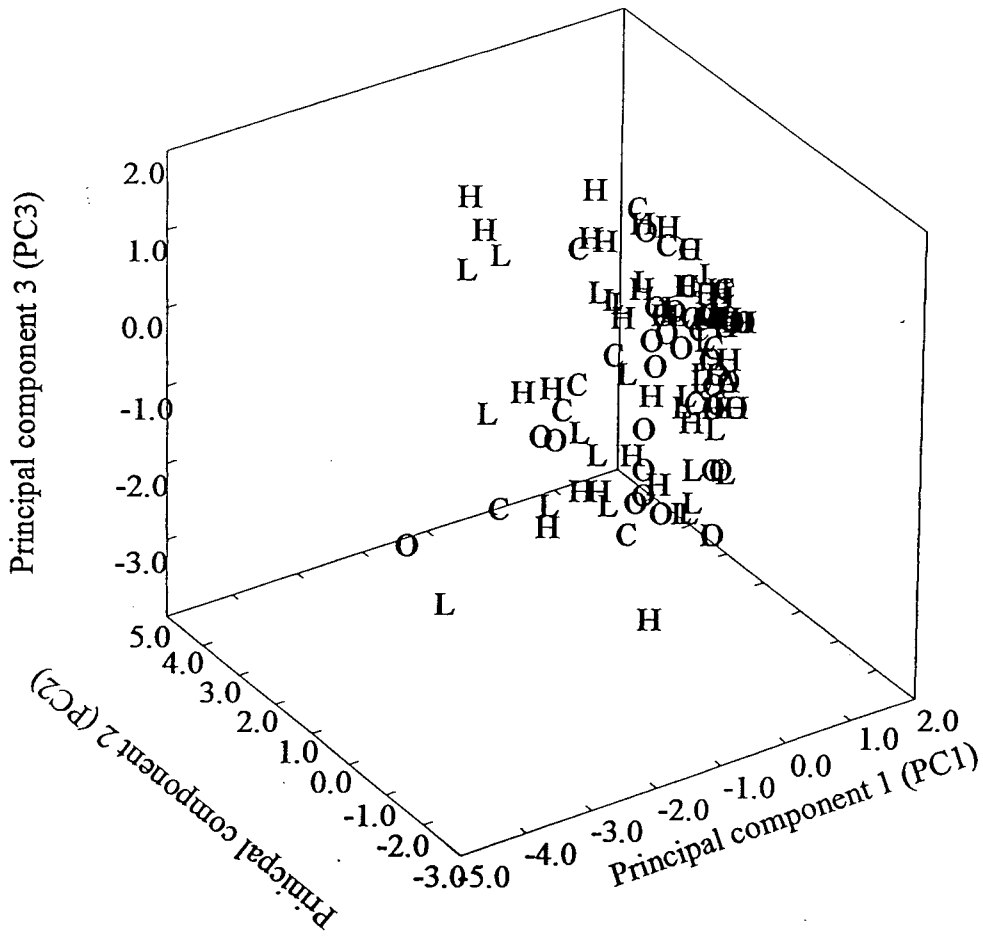

Figure 30. Principal component analysis plot (three dimensional) for sensory scores from tasting pasteurized milk samples with various treatments. C = Cooked. H = Heated. L = Light-induced. O = Oxidized.

comparison to the PCA plots (Figures 27 and 28). In the PCS plot, milk samples were differentiated more clearly than in the PCA plots. In particular, light-induced and heated milk samples were differentiated more distinctly than other samples. This is because more PC scores are used in the computation for PCS than PCA. However, no clear separation was observed in the PCS plot.

(2) Taste

Principal component analysis of sensory scores for tasting provided three PC, which accounted for 59.5% of total variation. These PC scores were used for the computation of PCS. Untreated pasteurized milk was used as a reference. Figure 32 shows the PCS plot. Compared with the PCS plot for smelling, these samples were more scattered and no clear separation into groups was observed. In general, most oxidized and cooked milk samples were widely scattered around the reference. The plots of light-induced and heated milk samples indicated differentiation from the reference by tasting. However, the panellists could not distinguish the difference between these samples. One reason for this result is that the sensitivity of the panellist is an important factor. In this study they were given one training session, because they were already experienced in sensory evaluation. This is further described in the MATERIALS AND METHODS section (p. 47). If more trained panellists or an expert in the dairy industry were used, they might have distinguished between light-induced and heated milk samples, because trained panellists typically detect smaller

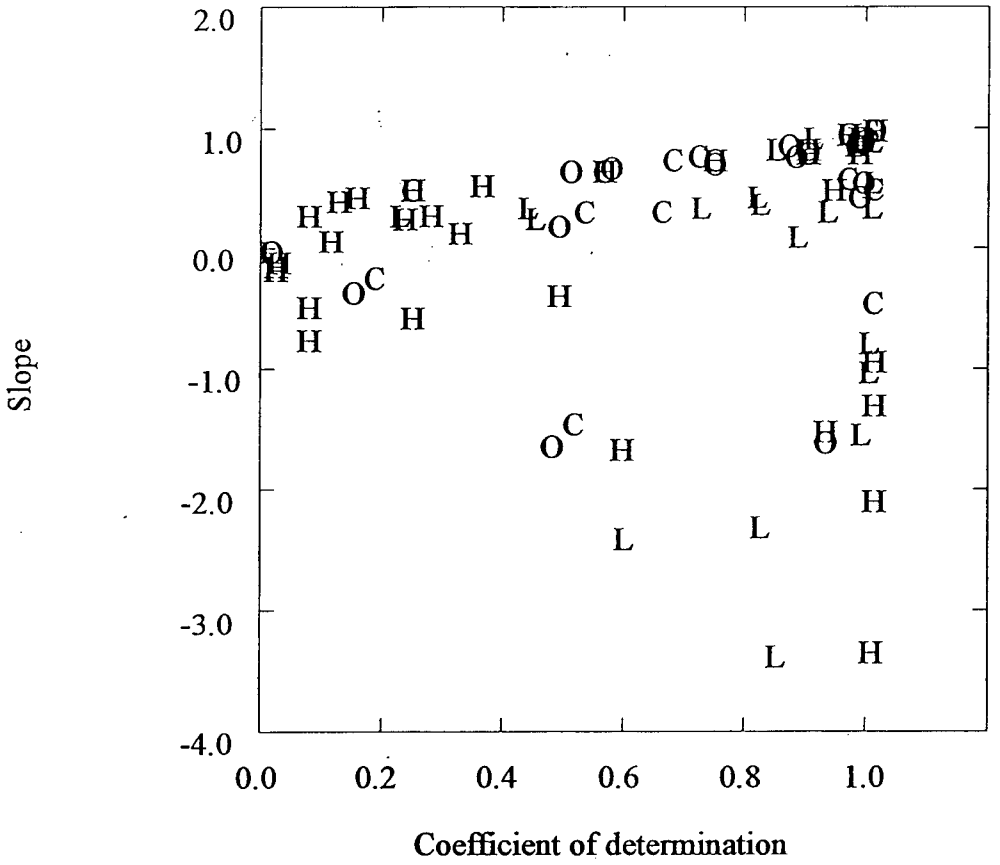

Figure 31. Principal component similarity analysis plot of sensory scores from smelling pasteurized milk samples with various treatments. Pasteurized milk without treatment was used as a reference. C = Cooked. H = Heated. L = Light-induced. O = Oxidized. The coordinates for the reference are (1,1).

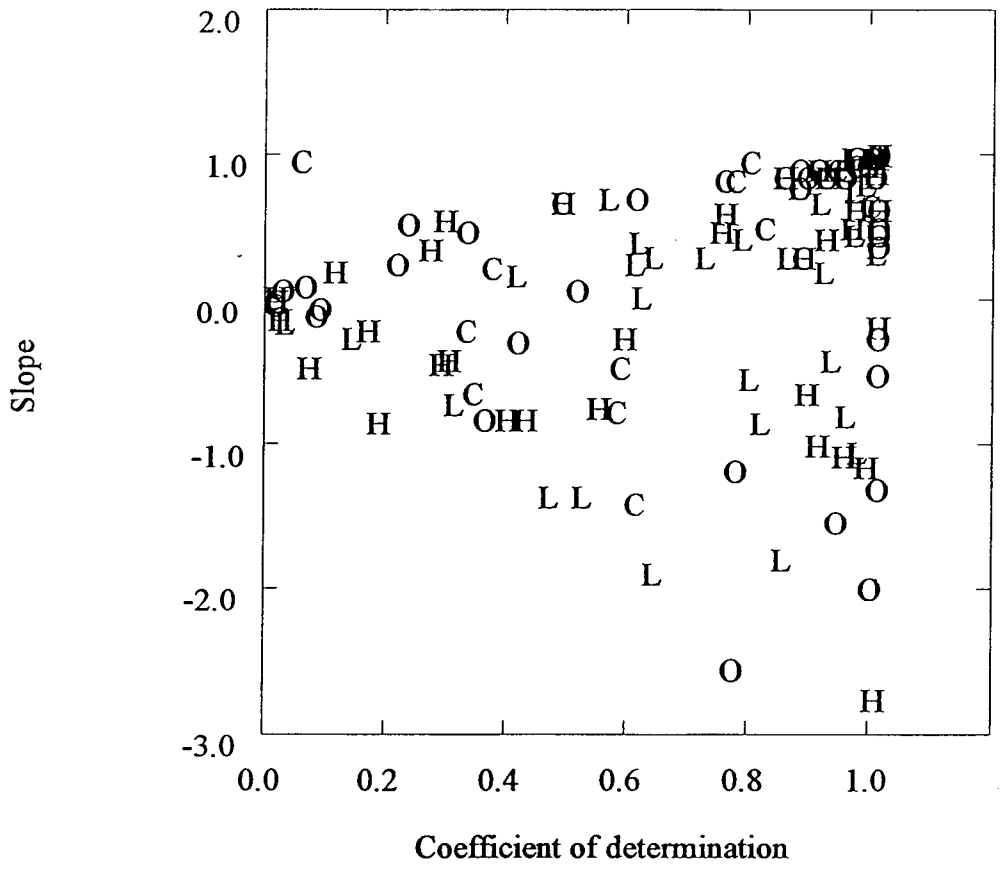

Figure 32. Principal component similarity analysis plot of sensory scores from tasting pasteurized milk samples with various treatments. Pasteurized milk without treatments was used as a reference. C = Cooked. H = Heated. L = Light-induced. O = Oxidized. The coordinates for the reference are (1,1).

differences among samples than do untrained panellist (Robert and Vickers, 1994).

On the other hand, there is considerable literature which compares trained panellists with untrained panellists. In the study by Shinesio et al. (1990), a trained panellist, who had extensive training as a descriptive sensory panel for 12 years, was unable to describe the intensities of the samples. Roberts and Vickers (1994) reported that the trained panels agreed with preference rating by the untrained panels. Powers (1984) also mentioned that even though panellists have been well trained, their judgements may sometimes be coloured to a certain extent by cultural differences.

It is apparent that consistent results in sensory evaluation requires panellists with long experience and well defined frames of reference as well as long exposure to the special product.

4. Principal Component Similarity Analysis with Sensory Scores

Flavour cannot be measured directly by instrument. It is an interaction between consumer and products (Piggot, 1990). A gas chromatogram can only provide information about volatiles in a food sample. Therefore, sensory scores were incorporated into principal component similarity analysis plot. Figure 33 shows the principal component similarity analysis (PCS) plot incorporated with unstandardized acceptability scores for smelling. The methods for contoured scatterplot were described in the MATERIALS AND METHODS section (p. 49).

In the ADSA scoring guide, the following values are assigned: "no criticism = 10", "accept moderately = 6", "accept slightly = 2" and "not accept = 0". Oxidized, cooked and light-induced milk samples had higher scores and were judged as acceptable when smelled. Heated milk samples had lower scores than the other milk samples. However, they were still moderately acceptable.

Figure 34 shows the PCS plot incorporated with standardized acceptability scores for smelling. Standardization was conducted within each panellist. It is difficult to judge the acceptance level by these values. Oxidized, cooked and light-induced milk samples had similar acceptance levels. Heated milk samples were separated into a group.

Figure 35 shows the PCS plot incorporated with unstandardized acceptability scores for tasting. Oxidized and cooked milk samples had higher acceptance scores. Most of the light-induced and heated milk samples had scores between "accept moderately" and "accept slightly".

Figure 36 shows the PCS plot incorporated with standardized acceptability scores for tasting. Milk samples were separated into two groups according to their acceptance levels. One was oxidized and cooked milk samples, the other was light-induced and heated milk samples.

These results suggest that oxidized, cooked and light-induced milk samples had similar acceptance levels for smelling. Tasting of light induced milk samples gave lower acceptance scores and had a similar acceptance level as for heated milk samples. Oxidized

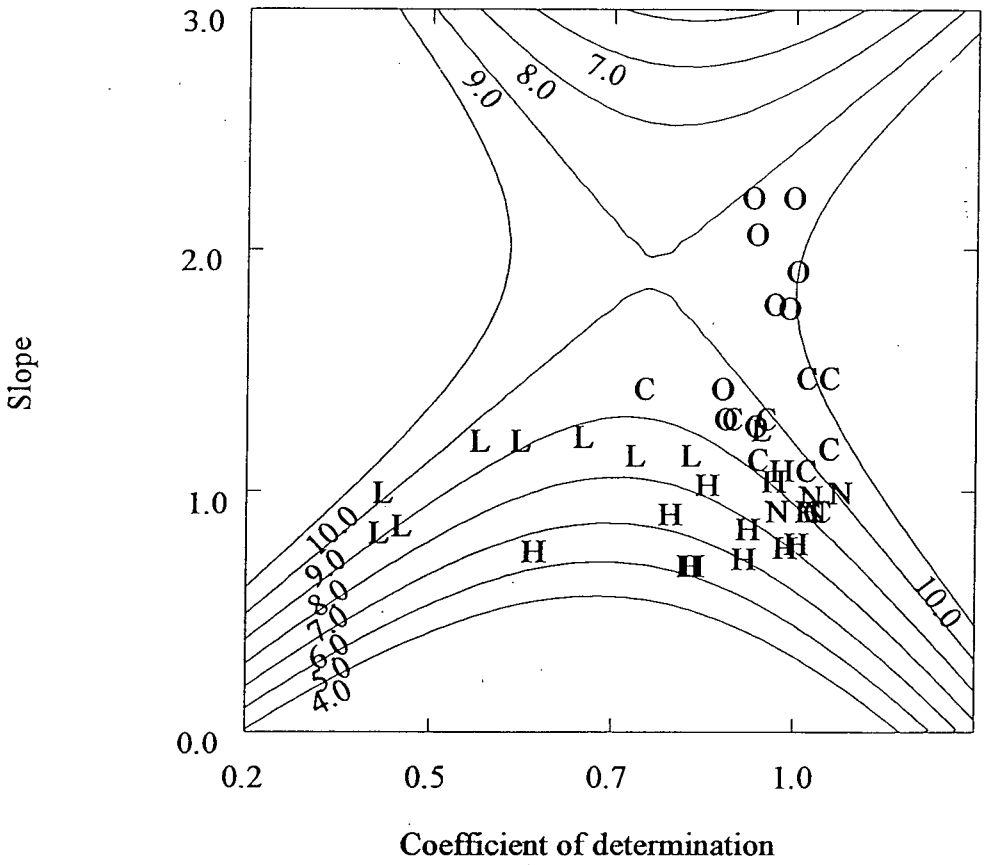

Figure 33. Principal component similarity analysis plot of gas chromatographic peak areas with unstandardized acceptability scores for smelling. Pasteurized milk without treatment was used as a reference. N = Negative control. C = Cooked. H = Heated. L = Light-induced. O = Oxidized. The coordinates for the reference are (1,1). The values for contouring are follows: "no criticism = 10", "accept moderately = 6", "accept slightly = 2" and "not accept = 0".

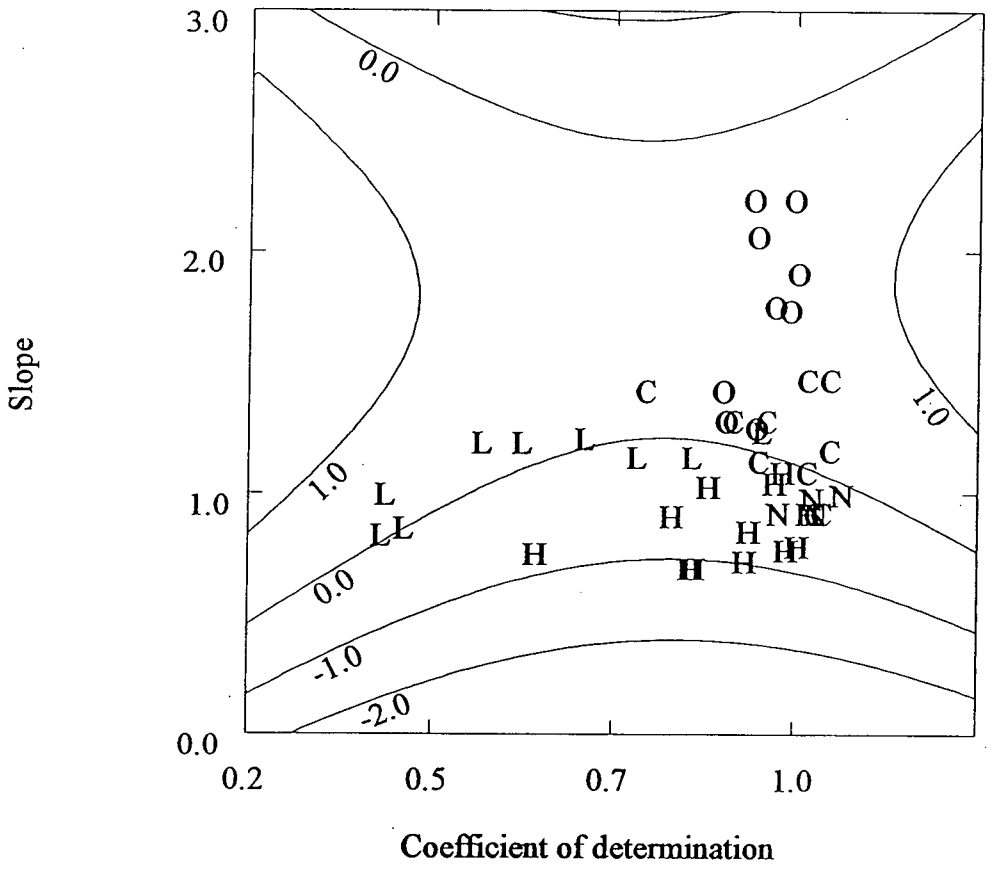

Figure 34. Principal component similarity analysis plot of gas chromatographic peak areas with standardized acceptability scores for smelling. Pasteurized milk without treatments was used as a reference. N = Negative control. C = Cooked. H = Heated. L = Light-induced. O = Oxidized. The coordinates for the reference are (1,1). The values -2.0 to 1.0 in the coutour represent the standardized acceptability score. Higher number scores are more acceptable. Lower number scores are less acceptable.

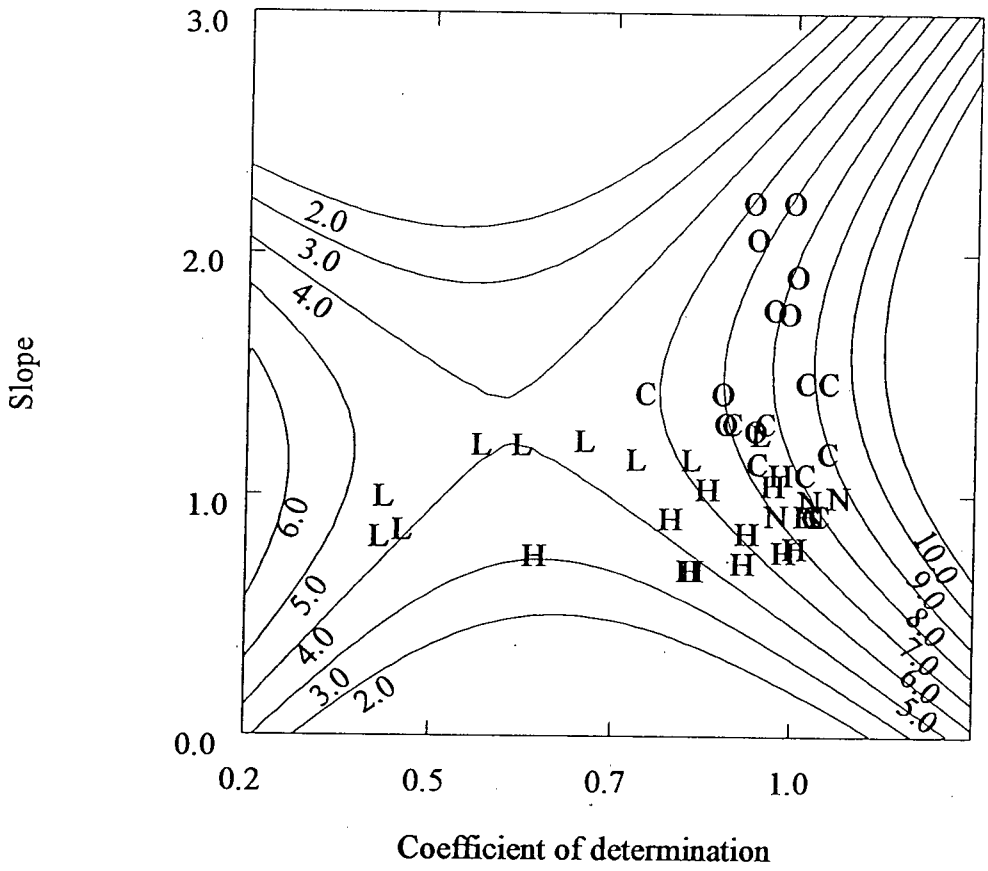

Figure 35. Principal component similarity analysis plot of gas chromatographic peak areas with unstandardized acceptability scores for tasting. Pasteurized milk without treatments was used as a reference. N = Negative control. C = Cooked. H = Heated. L = Light-induced. O = Oxidized. The coordinates for the reference are (1,1). The values for contouring are follows: "no criticism = 10", "accept moderately = 6", "accept slightly = 2" and "not accept = 0".

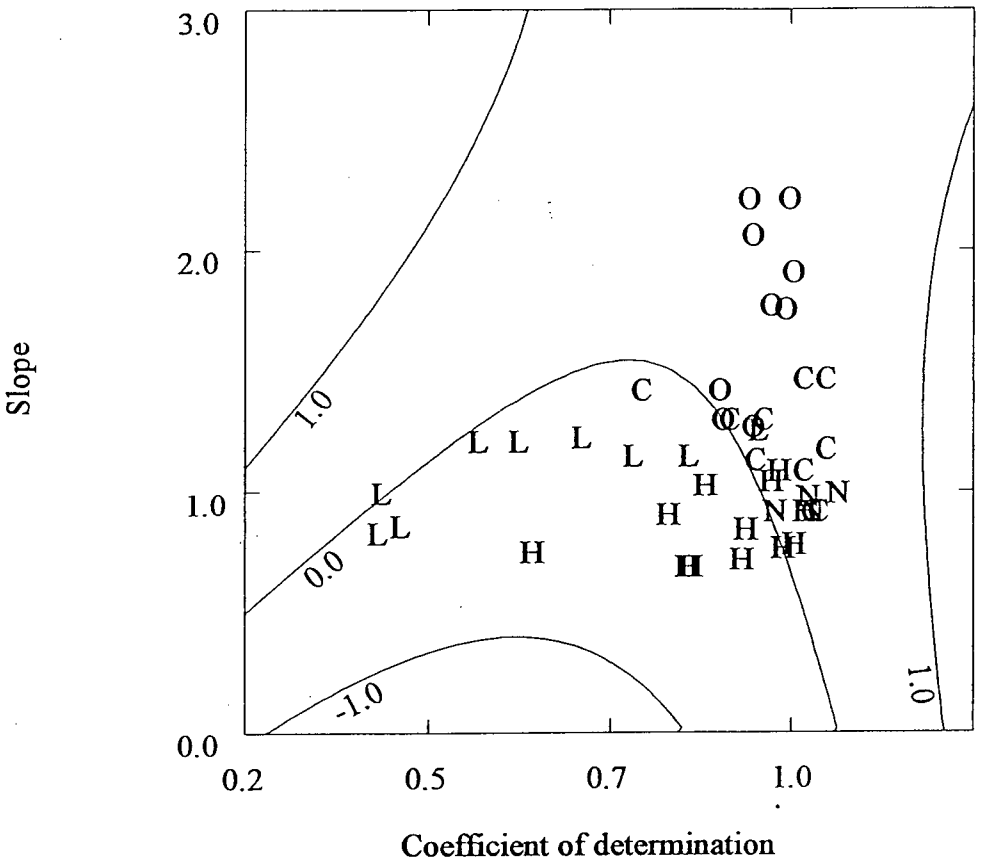

Figure 36. Principal component similarity analysis plot of gas chromatographic peak areas with standardized acceptability scores for tasting. Pasteurized milk without treatment was used as a reference. N = Negative control. C = Cooked. H = Heated. L = Light-induced. O = Oxidized. The coordinates for the reference are (1,1). The values -1.0 to 1.0 in the contour represent the standardized acceptability score. Higher number scores are more acceptable. Lower number scores are less acceptable.

and cooked milk samples tended to have a higher acceptance level for both smelling and tasting.

Sensory analysis cannot be replaced totally by instrumental analysis for quality control of milk, because acceptability is dependent upon consumer preference. Consumers, like semi-expert panellists, can be presumed to be unable to consistently differentiate off-flavours in milk. Sensory evaluation will continue to be important, particularly in product development. However, when considering the factors such as the difficulties of employing and training persons to be experts in sensory analysis, and sensory limitations of individuals, instrumental analysis appears more capable of objectively identifying presence and intensities of particular off-flavours in incoming raw milk samples.

CHAPTER V

CONCLUSIONS

This research was undertaken because of the need to develop a fast and objective method for detection of off-flavours in milk. Milk was treated to develop several off-flavours. Data from a low-cost gas chromatograph and from sensory evaluation were subjected to computer aided multivariate analyses.

The following conclusions can be drawn from this study:

1. A new portable gas chromatograph (SRI model 8610) was used for this research. It was found to give chromatograms adequate for classification of milk samples subjected to various treatments.

2. As a new multivariate analysis technique, principal component similarity (PCS) analysis was applied to peak areas in chromatograms and was capable of classifying UHT-sterilized milk samples with regards to bacterial species and storage time. The advantage of PCS is that classification can be made with incomplete information on sample groups and without the need for testing a large number of samples. It is applicable to continuing situations with the capacity of detecting abnormal samples. Causes of the abnormality are readily explained; therefore, this method can perhaps be used for quality assurance and food safety for other food. The GC-PCS system has potential for partially replacing slow and expensive microbial assays for classification of bacterial species.

For comparison with PCS, other existing multivariate analyses were also applied. There are two categories of classification: supervised and unsupervised, depending on whether the sample grouping is known. In this research one unsupervised and three supervised multivariate analyses were applied to GC data of UHT-sterilized milk for comparisons with PCS.

The unsupervised method, principal component analysis (PCA), was first examined. PCS classified samples more clearly than PCA. Three supervised multivariate analyses were then applied: artificial neural networks (ANN), partial least squares regression analysis (PLS) and principal component regression analysis (PCR). The statistical parameters of coefficient of determination (r^2) and standard error of prediction (SEP) were used to estimate predictive ability of each method. ANN gave the best mean r^2 and SEP among the supervised methods. The coefficient of variation of SEP of the ANN was much larger than that of PLS and PCR. Even though the ANN generally had a smaller SEP and larger r^2 than PLS and PCR, the content of training and testing data had a great influence on prediction ability of the ANN.

Generally, the ANN was able to make better predictions than PLS and PCR. This indicates that the relationship between dependent and independent variables may be non-linear. The ANN was also faster and easier to use than PLS and PCR. The results from using all data was better than cross-validated results. This suggests that a larger data set increases prediction ability for the ANN.

3. Instrumental gas chromatographic analysis was more effective than sensory evaluation as a means of distinguishing milk samples with various treatments. Pasteurized milk was treated to develop different intensities of off-flavours (light-induced, oxidized, cooked and

heated). The samples were analyzed by GC and sensory analysis. PCS and PCA were applied. GC analysis could classify samples into groups more clearly than sensory analysis. Tasting was more effective than smelling for differentiating among samples which had been given different treatments. Sensory analysis cannot be replaced totally by instrumental analysis for quality control of milk. However, when considering factors such as the difficulties of employing and training people to be experts in sensory analysis, as well as sensory limitations of individuals, instrumental analysis may be considered desirable for quality control of milk.

4. A combination of GC analysis and multivariate chemometric methods may have great potential in quality evaluation of milk. Once this system were established, it might provide valuable assistance in determining quality of not only milk and dairy products but also of other food products.

REFERENCES

Aishima, T. 1979. Classification of soy sauce on principal components in GC profiles. Agric. Biol. Chem. 43: 1905-1910.

Aishima, T. 1991. Discrimination of liquor aromas by pattern recognition analysis of responses from a gas sensory array. Anal. Chim. Acta. 243: 293-300.

Aishima, T. and Nakai, S. 1986. Centroid mapping optimization: a new efficient optimization for food research and processing. J. Food Sci. 51: 1297-1300, 1310.

Aishima, T. and Nakai, S. 1991. Chemometrics in flavor research. Food Rev. Int. 7: 33-101.

Aishima, T., Wilson, D. L. and Nakai, S. 1987. Application of simplex algorithm to flavor optimization based on pattern similarity of GC profiles. Ch. 4 in *Flavour Science and Technology*, M. Martens, G. A. Dalen and H. Russwurm (Eds.), p. 501-508. John Wiley & Sons, Ltd., Chichester, UK.

American Dairy Science Association, 1987. The ADSA scoring guide for off-flavors of milk and cream. Cited in F. W. Bodyfelt, J. Tobias and G. M. Trout (Eds.). 1988. *The Sensory Evaluation of Dairy Products*, p.140-141. Van Nostrand Reinhold, New York, NY.

Arteaga G. E. and Nakai, S. 1993. Predicting protein functionality with artificial neural networks: foaming and emulsifying properties. J. Food Sci. 58: 1152-1156.

Arteaga, G. E., Horimoto, Y., Li-Chan, E. and Nakai, S. 1994a. Partial least squares regression of fourth-derivative ultraviolet absorbance spectra predicts composition of protein mixtures: application to bovine caseins. J. Agric. Food Chem. 42: 1938-1942.

Arteaga, G. E., Li-Chan, E., Vazquez-Arteaga, M. C. and Nakai, S. 1994b. Systematic experimental designs for product formula optimization. Trends Food Sci. Technol. 5: 243-254.

Arteaga, G. E., Luebsen, B. E., Horimoto, Y. and Nakai, S. 1994c. Prediction of cheese variety by applying artificial neural networks to GC data. Presented at the Ann. Mtg., Inst. of Food Technologists, Atlanta, GA, June 25-29.

Auclair, J., Accolas, J-P., Vassal, L and Mocquot, G. 1981. Microbiological problems in cheese manufacture. Bull. IDF 136: 20-26.

Baardseth, P., Rosenfeld, H. J., Sundt, T. W., Skrede, G., Lea, P. and Slinde, E. 1995. Evaluation of carrot varieties for production of deep-fried carrot chips. I. Chemical aspects. Food Res. Int. 28: 195-200.

Badings, H. T. 1984. Flavors and off-flavors. Ch. 18 in *Dairy Chemistry and Physics*, P. Walstra and R. Jenness (Eds.), p. 336-357. John Wiley & Sons, Inc., New York, NY.

Badings, H. T. and de Jong, C and Dooper, R. P. M. 1985. Automatic system for rapid analysis of volatile compounds by purge-and-cold-trapping/capillary gas chromatography. J. High Resol. Chromatogr & Cromatogr. Commun. 8: 755-763.

Badings, H. T. and Neeter, R. 1980. Recent advances in the study of aroma compounds of milk and dairy products. Neth. Milk Dairy J. 34: 9-30.

Bailey, P. J. and Rohrback, B. G. 1994. Applications of chemometrics in the food and beverage industry. Food Technol. 48(4): 69-72.

Banks, J. M., Brechany, E. Y., Christie, W. W., Hunter, E. A. and Muir, D. D. 1992. Volatile components in steam distillates of cheddar cheeses as indicator indices of cheese maturity, flavour and odour. Food Res. Int. 25: 365-373.

Bassette, R. and Claydon, T. J. 1965. Characterization of some bacteria by gas chromatographic analysis of headspace vapors from milk cultures. J. Dairy Sci. 48: 775 (abstract).

Bassette, R., Bawdon, R. E. and Claydon, T. J. 1967. Production of volatile materials in milk by some species of bacteria. J. Dairy Sci. 50: 167-171.

Bassette, R., Fung, D. Y. C. and Mantha, V. R. 1986. Off-flavors in milk. CRC Crit. Rev. Food Sci. Nutr. 24: 1-52.

Bassette, R., Ozeris, S. and Whitnah, C. H. 1963. Direct gas chromatographic analysis of milk. J. Food Sci. 28: 84-90.

Bawdon, R. E. and Bassette, R. 1966. Differentiation of *Escherichia coli* and *Aerobacter aerogenes* by gas liquid chromatography. J. Dairy Sci. 49: 624-627.

Bekbölet, M. 1990. Light effects on food. J. Food Prot. 53: 430-440.

Bewig, K. M., Clarke, A. D., Roberts, C. and Unklesbay, N. 1994. Discriminant analysis of vegetable oils by near infrared reflectance spectroscopy. J. Am. Oil Chem. Soc. 71: 195-200.

Biggs, D. A. and McKenna, D. 1989. Alternative methods for infrared analysis of fat in milk: interlaboratory study. J. Assoc. Off. Anal. Chem. 72: 724-734.

Bishop, J. R. and White, C. H. 1986. Assessment of dairy production quality and potential shelf-life-a review. J. Food Prot. 49: 739-753.

Borggaard, C. and Thodberg, H. H. 1992. Optimal minimal neural interpretation of spectra. Anal. Chem. 64: 545-551.

Bradley, R. L. 1980. Effect of light on alteration of nutritional value and flavor of milk: a review. J. Food Prot. 43: 314-320.

Brainmaker software. 1992. 6th ed. California Scientific Software, Nevada, CA.

Burgess, K., Heggum, C., Walker, S. and van Schothorst, M. 1994. Spoilage and pathogenic bacteria in milk based products. Bull. IDF 292: 28-32.

Burton, H. 1983. Types of off-flavour. J. Soc. Dairy Technol. 36: 1-7.

Cadet, F., Bertrand, D., Robert, P., Maillot, J., Dieudonne, J. and Rouch, C. 1991. Quantitative determination of sugar cane sucrose by multidimensional statistical analysis of their mid-infrared attenuated total reflectance spectra. Anal. Spectrosc. 45: 166-172.

Capilla, C., Navarro, J. L., Sendra, J. M. and Izquierdo, L. 1988. Detection of orange juice dilution by canonical correlation analysis. Analy. Chim. Acta 212: 309-315.

Collins, S. J., Bester, B. H. and McGill, A. E. J. 1993. Influence of psychrotrophic bacterial growth in raw milk on the sensory acceptance of UHT skim milk. J. Food Prot. 56: 418-425.

Cousin, M. A. 1982. Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. J. Food Prot. 45: 172-207.

Cox, J. M. 1993. The significance of psychrotrophic pseudomonads in dairy products. Aust. J. Dairy Technol. 48: 108-113.

Datta, S. and Nakai, S. 1992. Computer-aided optimization of wine blending. J. Food Sci. 57: 178-182, 205.

Deeth, H. C. 1993. Lipase activity and its effect on milk quality. Aust. J. Dairy Technol. 48: 96-98.

Defernez, M. and Wilson, R. H. 1995. Mid-infrared spectroscopy and chemometrics for determining the type of fruit used in jam. J. Sci. Food Agric. 67: 461-467.

Dijksterhuis, G. 1995. Multivariate data analysis in sensory and consumer science: an overview of developments. Trends Food Sci. Technol. 6: 206-211.

Dillon, W. R. and Goldstein, M. (Eds.). 1984. *Multivariate Analysis*, John Wiley and Sons, New York, NY.

Dou, J., Toma, S. and Nakai, S. 1993. Random-centroid optimization for food formulation. Food Res. Int. 26: 27-37.

Downey, W. K. 1980. Review of the progress of dairy science: flavour impairment from pre- and post-manufacture lipolysis in milk and dairy products. J. Dairy Res. 47: 237-252.

Eerikäinen, T., Linko, P., Linko, S, Siimes, T. and Zhu, Y-H. 1993. Fuzzy logic and neural network applications in food science and technology. Trends Food Sci. Technol. 4: 237-242.

Ennis, D. M., Bowlines, H., Haring, H. and Cowman, P. 1982. Multivariate analysis in sensory evaluation. Food Technol. 36(11): 83-90.

Eyles, M. J. and Adams, R. F. 1986. Detection of microbial metabolites by gas chromatography in the examination of spoiled canned foods and related products. Int. J. Food Microbiol. 3: 321-330.

Fairbairn, D. J. and Law, B. A. 1986. Proteinases of psychrotrophic bacteria: their production, properties, effects and control. J. Dairy Res. 53: 139-177.

Forina, M., Lanteri, S. and Armanino, C. 1987. Chemometrics in food chemistry. Top. Curr. Chem. 141: 91-143.

Forss, D. A. 1971. The flavours of dairy fats: a review. J. Am. Oil Chem. Soc. 48: 702-710.

Forss, D. A. 1979. Review of the progress of dairy science: mechanisms of formation of aroma compounds in milk and milk products. J. Dairy Res. 46: 691-706.

Furtula, V., Nakai, S., Amantea, G. F. and Laleye, L. 1994a. Reverse-phase HPLC analysis of cheese samples aged by a fast-ripening process. J. Food Sci. 59: 528-532, 567.

Furtula, V., Nakai, S., Amantea, G. F. and Laleye, L. 1994b. Reverse-phase HPLC analysis of reference cheddar cheese samples for assessing accelerated cheese ripening. J. Food Sci. 59: 533-538.

Gianturco, M. A., Biggers, R. E. and Ridling, B. H. 1974. Seasonal variations in the composition of the volatile constituents of black tea. A numerical approach to the correlation between composition and quality of tea aroma. J. Agric. Food Chem. 22: 758-764.

Girard, B. and Nakai, S. 1991. Static headspace gas chromatographic method for volatiles in canned salmon. J. Food Sci. 56: 1271-1274.

Godwin, D. R., Bargmann, R. E. and Powers, J. J. 1978. Use of cluster analysis to evaluate sensory-objective relations of processed green beans. J. Food Sci. 43: 1229-1234.

Griffiths, M. W. and Phillips, J. D. 1986. The application of the pre-incubation test in commercial dairies. Aust. J. Dairy Technol. 41: 71-79.

Guarino, P. A. and Kramer, A. 1969. Gas chromatographic analysis of head-space vapors to identify micro-organisms in foods. J. Food Sci. 34: 31-37.

Hall, G. and Andersson, J. 1985. Flavour changes in whole milk powder during storage. III. Relationships between flavour properties and volatile compounds. J. Food Qual. 7: 237-253.

Hankin, L. and Anderson, E. O. 1968. Correlations between flavor scores, flavor criticism, standard plate count and oxidase count on pasteurized milks. J. Milk Food Technol. 32: 49-51.

Hansen, A. P., Turner, L. G. and Aurand, L. W. 1975. Fluorescent light-activated flavor in milk. J. Milk Food Technol. 38: 388-392.

Headley, L. M. and Hardy, J. K. 1989. Classification of whiskies by principal component analysis. J. Food Sci. 54: 1351-1358.

Heeschen, W. 1991. Methods for assessing the bacteriological quality of raw milk from the farm. Bull. IDF 256: 1-62.

Heymann, H. and Noble, A. C. 1987. Descriptive analysis of commercial cabernet sauvignon wines from California. Am. J. Enol. Vitic. 38: 41-44.

Hibbert, D. B. 1993. Genetic algorithms in chemistry. Chemom. Intell. Lab. Syst. 19: 277-293.

Horimoto, Y., Durance, T., Nakai, S. and Lukow, O. M. 1995. Neural networks vs principal component regression for prediction of wheat flour loaf volume in baking tests. J. Food Sci. 60: 429-433.

Hosono, A., Elliott, J. A. and McGugan, W. A. 1973. Production of ethylesters by some lactic acid and psychrotrophic bacteria. J. Dairy Sci. 57: 535-539.

Jaddou, H. A., Pavey, J. A. and Manning, D. J. 1978. Chemical analysis of flavour volatiles in heat-treated milks. J. Dairy Res. 45: 391-403.

Jansson, P. A. 1991. Neural networks: an overview. Anal. Chem. 63: 375A-362A.

Jennings, W. G., Viljhalmsson, S. and Dunkley, W. L. 1962. Direct gas chromatography of milk vapors. J. Food Sci. 27: 306-308.

Jeon, I. J. 1991. Pattern recognition techniques for food research and quality assurance. Ch. 12 in *Instrumental Methods for Quality Assurance in Foods*, D. C. Fung and R. F. Matthews (Eds.), p. 271-298. Marcel Dekker, Inc., New York, NY.

Jeon, I. J. 1993. Undesirable flavors in dairy products. Ch. 5 in *Food Taints and Off-flavours*, M. J. Saxby (Ed.), p. 122-149. Blackie Academic & Professional, Glasgow, UK.

Keller, W. J. and Kley, D. H. 1971. Headspace gas chromatography for objectively determining intensity of haylage flavor in raw milk. J. Dairy Sci. 55: 574-576.

Kwan, W-O. and Kowalski, B. R. 1980. Correlation of objective chemical measurements and subjective sensory evaluations. Wines of *Vitis Vineifera* variety 'Pinot Noir' from France and the United States. Anal. Chim. Acta. 122: 215-222.

Lamberto, M. and Saitta, M. 1995. Principal component analysis in fast atom bombardment-mass spectrometry of triacylglycerols in edible oils. J. Am. Oil Chem. Soc. 72: 867-871.

Law, B. A. 1979. Reviews of the progress of dairy science: enzymes of psychrotrophic bacteria and their effects on milk and milk products. J. Dairy Res. 46: 573-588.

Lawrence, J. 1991. *Introduction to Neural Networks*, 3rd ed. S. Luedeking (Ed.), California Scientific Software, Nevada, CA.

Lawrence, J. and Peterson, A. 1992. *Brainmaker: User's Guide and Reference Manual*, 6th ed. S. Luedeking and J. Fredrickson (Eds). California Scientific Software, Nevada, CA.

Lee, I., Fatemi, S. H., Hammond, E. G. and White, P. J. 1995. Quantitation of flavour volatiles in oxidized soybean oil by dynamic headspace analysis. J. Am. Oil Chem. Soc. 72: 539-546.

Lee, M. L., Smith, D. L. and Freeman, L. R. 1979. High-resolution gas chromatographic profiles of volatile organic compounds produced by microorganisms at refrigerated temperatures. Appl. Environ. Mirobiol. 37: 85-90.

Lee, Y-Z., Wong, R. M. and Nakai, S. 1990. Preparation of colorless globin from bovine hemoglobin using sodium alginate. J. Food Sci. 55: 577-578.

Lee-Yan, S., Ying-Hsiu, T. O. and Shun-Jen, T. 1991. Flavour characteristic compounds found in the essential oil of *Ocimum basilicum* L. with sensory evaluation and statistical analysis. J. Agric. Food Chem. 39: 939-943.

Leland, J. V., Reineccius, G. A. and Lahiff, M. 1987. Evaluation of copper-induced oxidized flavor in milk by discriminant analysis of capillary gas chromatographic profiles. J. Dairy Sci. 70: 524-533.

Li-Chan, E., Nakai, S. and Wood, D. F. 1987. Muscle protein structure-function relationships and discrimination of functionality by multivariate analysis. J. Food Sci. 52: 31-41.

Lin, J. C. C., Nagy, S. and Klim, M. 1993. Application of pattern recognition techniques to sensory and gas chromatographic flavor profiles of natural orange aroma. Food Chem. 47: 235-245.

Lopez-Fandino, R., Corzo, N., Villamiel, M., Delgado, T., Olano, A. and Ramos, M. 1993. Assessment of quality of commercial UHT milks by chromatographic and electrophoretic methods. J. Food Prot. 56: 263-264, 269.

Lotus 123 (Release 3.1). 1990. Lotus Development Corp., Cambridge, MA.

Lucasius, C. B. and Kateman, G. 1993. Understanding and using genetic algorithms. Part I. Concepts, properties and context. Chemom. Intell. Lab. Syst. 19: 1-33.

Manly, R. F. J. 1986. *Multivariate Statistical Methods: A Primer*, Chapman and Hall, London, UK.

Martens, M., Martens, H. and Wold, S. 1983. Preference of cauliflower related to sensory descriptive variables by partial least squares (PLS) regression. J. Sci. Food Agric. 34: 715-724.

Martens, H. and Naes, T. (Eds.). 1989. *Multivariate Calibration*, John Wiley & Sons, Ltd., Chichester, UK.

Mawatari, M., Nagashima, Y., Aoki, T., Hirota, T and Yamada, M. 1991. Sensory characterization of beer flavor using cluster analysis. J. Am. Soc. Brew. Chem. 49: 59-64.

McKellar, R. C. 1981. Development of off-flavors in ultra-high temperature and pasteurized milk as a function of proteolysis. J. Dairy Sci. 64: 2138-2145.

Nakai, S. 1990. Computer-aided optimization with potential application in biorheology. J. Jap. Soc. Biorheology 4: 143-150.

Nakai, S., Koide, K. and Eugster, K. 1984. A new mapping super-simplex optimization for food product and process development. J. Food Sci. 49: 1143-1148, 1170.

Nicholson, J. W. G. 1993. Spontaneous oxidized flavour in cow's milk. Bull. IDF 281: 2-12.

Norback, J. P. 1994. Natural language computer control of crucial steps in cheese making. Aust. J. Dairy Technol. 49: 119-122.

Page, S. W. 1986. Pattern recognition methods for the determination of food composition. Food Technol. 40 (11): 104-109.

Peak 2 (Version 2.0). 1991. SRI Instruments, Inc., Las Vegas, NV.

Pham, A-M. and Nakai, S. 1984. Application of stepwise discriminant analysis to high pressure liquid chromatography profiles of water extract for judging ripening of cheddar cheese. J. Dairy Sci. 67: 1390-1396.

Pierami, R. M. and Stevenson, K. E. 1976. Detection of metabolites produced by psychrotrophic bacteria growing in milk. J. Dairy Sci. 59: 1010-1015.

Pierce, M. M. and Wehling, R. L. 1994. Comparison of sample handling and data treatment methods for determining moisture and fat in cheddar cheese by near-infrared spectroscopy. J. Agri. Food Chem. 42: 2830-2835.

Piggot, J. R. 1990. Relating sensory and chemical data to understand flavor. J. Sensory Studies 4: 261-272.

Pino, J. A., Rosado, A., Goire, J. and Roncal, E. 1995. Evaluation of flavor characteristic compounds in dill herb essential oil by sensory analysis and gas chromatography. J. Agric. Food Chem. 43: 1307-1309.

PLS plus. 1992. Galactic Industries Corp., Salem, NH.

Poste, L. M., Mackie, D. A., Butler, G. and Larmond, E. 1991. *Laboratory Methods for Sensory Analysis of Food*, p. 2-15. J. Buckly (Ed.), Canada Communication Group-Publishing Center, Ottawa, ON.

Powers, J. J. 1984. Using general statitical programs to evaluate sensory data. Food Technol. 38(6): 74-82, 84.

Powers, J. J. and Keith, E. S. 1968. Stepwise discriminant analysis of gas chromatographic data as an aid in classifying the flavour quality of foods. J. Food Sci. 33: 207-213.

Powers, J. J. and Quinlan, M. C. 1974. Refining of methods for subjective-objective evaluation of flavour. J. Agric. Food Chem. 22: 744-749.

Quinlan, M. C., Bargmann, R. E., El-Galalli, Y. M. and Powers, J. J. 1974. Correlations between subjective and objective measurements applied to grape jelly. J. Food Sci. 39: 794-799.

Reddy, M. C., Bills, D. D., Lindsay, R. C., Libbey, L. M., Millar III, A. and Morgan, M. E. 1968. Ester production by *Pseudomonas fragi*. 1. Identification and quantification of some esters produced in milk cultures. J. Dairy Sci. 51: 656-659.

Resurreccion, A. V. A. 1988. Applications of multivariate methods in food quality evaluation. Food Technol. 42(11): 128-134.

Resurreccion, A. V. A. and Shewfelt, R. L. 1985. Relationships between sensory attributes and objective measurements of postharvest quality of tomatoes. J. Food Sci. 50: 1242-1245, 1256.

Resurreccion, A. V. A., Shewfelt, R. L., Prussia, S. E. and Hurst, W. C. 1987. Relationships between sensory and objective measures of postharvest quality of snap beans as determined by cluster analysis. J. Food Sci. 52: 113-123.

Rizzolo, A., Polesello, A. and Polesello, S. 1992. Use of headspace capillary GC to study the development of volatile compounds in fresh fruit. J. High Resol. Chromatogr & Chromatogr. Commun. 15: 472-477.

Roberts, A. K. and Vikers, Z. M. 1994. A comparison of trained and untrained judges' evaluation of sensory attribute intensities and liking of cheddar cheeses. J. Sensory Studies 9: 1-20.

Rubico, S. M., Resurreccion, A. V. A. and Beuchat, L. R. 1988. Comparison of sensory properties and headspace volatiles of a peanut beverage processed at different temperature and time conditions. J. Food Sci. 53: 176-180.

Sawyer, F. M., Cardello, A. V., Prell, P. A., Johnson, E. A., Segars, R. A., Maller, O. and Kapsalis, J. 1984. Sensory and instrumental evaluation of snapper and rockfish species. J. Food Sci. 49: 727-733.

Schafer, M. L., Peeler, J. T., Bradshaw, J. G., Hamilton, C. H. and Carver, R. B. 1982. A rapid gas chromatographic method for the identification of sporeformers and nonsporeformers in swollen cans of low-acid foods. J. Food Sci. 47: 2033-2037.

Schlegel, W. F., Jenette, E., Kayster, D. S., Block, B., Nussbaum, R., Hickes, W. F. and Liptik, B. G. 1969. Safety instrumentation. Ch. 10 in *Instrument Engineers' Handbook. Vol. 1. Process Measurement*, B. G. Liptik (Ed.), p. 1093. Chilton Book Co., Radnor, PA.

Servili, M., Conner, J. M., Piggott, J. R., Withers, S. J. and Paterson, A. 1995. Sensory characterisation of virgin olive oil and relationship with headspace compostion. J. Sci. Food Agri. 67: 61-70.

Shah, N. D. 1994. Psychrotrophs in milk: a review. Milchwissenschaft 49: 432-437.

Shinesio, F., Risvik, E. and Rødbotten, M. 1990. Evaluation of panelist performance in descriptive profiling of rancid sausage: a multivariate study. J. Sensory Studies 5: 33-52.

Shipe, W. F., Bassette, R., Deane, D. D., Dunkley, W. L., Hammond, E. G., Harper, W. J., Kleyn, D. H., Morgan, M. E., Nelson, J. H. and Scanlan, R. A. 1978. Off-flavours of milk: nomenclature, standards and bibliography. J. Dairy Sci. 61: 855-869.

Simon, P. W., Peterson, C. E. and Lindsay, R. C. 1980. Correlations between sensory and objective parameters of carrot flavour. J. Agric. Food Chem. 28: 559-562.

Skura, B. J., Craig, C. and McKellar, R. C. 1986. Effect of disruption of an N_2-overlay on growth and proteinase production in milk by *Pseudomonas fluorescens*. Can. Inst. Food Sci. Technol. J. 19: 104-106.

SlideWrite Plus™. 1995. Advanced Graphics Software, Inc., Sunnyvale, CA.

Smeyers-Verbeke, J., Massart, D. L. and Coomans, D. 1977. Application of linear discriminant analysis to the differentiation of pure milk from different species and mixtures. J. Assoc. Off. Anal. Chem. 60: 1382-1385.

Smithwell, N. and Kailasapathy, K. 1995. Psychrotrophic bacteria in pasteurized milk: problems with shelf life. Aust. J. Dairy Technol. 50: 28-31.

Speck, M. L. (Ed.). 1976. *Compendium of Methods for Microbiological Examination of Foods*, p. 30 & 50. American Public Health Association, Inc., Washington, DC.

Spencer, M. D., Pangborn, R. M. and Jennings, W. G. 1978. Gas chromatographic and sensory analysis of volatiles from cling peaches. J. Agric. Food Chem. 26: 725-732.

Stead, D. 1986. Microbial lipase: their characteristics, role in food spoilage and industrical uses. J. Dairy Res. 53: 481-505.

Sutherland, B. J. 1994. ANN application to cheese making response from a cheese making researcher. Aust. J. Dairy Technol. 49: 122-124.

Thomas, E. L. 1981. Trends in milk flavors. J. Dairy Sci. 64: 1023-1027.

Toan, T. T., Bassette, R. and Claydon, T. J. 1965. Methyl sulfide production by *Aerobacter aerogenes* in milk. J. Dairy Sci. 48: 1174-1178.

Ulberth, F. and Kneifel, W. 1992. Aroma profiles and sensory properties of yogurt and yogurt-related products. II. Classification of starter cultures by means of cluster analysis. Milchwissenschaft 47: 432-434.

Urbach, G. 1987. Dynamic headspace gas chromatography of volatile compounds in milk. J. Chromatogr. 404: 163-174.

Urbach, G. 1990. Headspace volatiles from cold-stored raw milk. Aust. J. Dairy Technol. 45: 80-85.

Vallejo-Cordoba, B. and Nakai, S. 1994a. Keeping-quality assessment of pasteurized milk by multivariate analysis of dynamic headspace gas chromatographic data. 1. Shelf life prediction by principal component regression. J. Agric. Food Chem. 42: 989-993.

Vallejo-Cordoba, B. and Nakai, S. 1994b. Keeping-quality assessment of pasteurized milk by multivariate analysis of dynamic headspace gas chromatographic data. 2. Flavour classification by linear discriminant analysis. J. Agric. Food Chem. 42: 994-999.

Vazquez-Arteaga, M. C. and Nakai, S. 1990. New method for meat formulation with emphasis in quality. Presented at Ann. Mtg., Inst. of Food Technologists, Anaheim, CA, June 16-20.

Vodovotz, Y., Arteaga, G. E. and Nakai, S. 1993. Principal component similarity analysis for classification and its application to GC data of mango. Food Res. Int. 26: 355-363.

Waltking, A. E. and Goetz, A. G. 1983. Instrumental determination of flavour stability of fatty foods and its correlation with sensory flavor responses. CRC Crit. Rev. Food Sci. Nutr. 19: 99-132.

Wellnitz-Ruen, W., Reineccius, G. A. and Thomas, E. L. 1982. Analysis of the fruity off-flavour in milk using headspace concentration capillary column gas chromatography. J. Agric. Food Chem. 30: 512-514.

Wilkinson, L. 1990. SYSTAT:The System for Statistics. SYSTAT, Inc., Evanston, IL.

Woo, A. H. and Lindsay, R. C. 1983. Statistical correlation of quantitative flavor intensity assessments and individual free fatty acid measurements for routine detection and prediction of hydrolytic rancidity off-flavors in butter. J. Food Sci. 48: 1761-1766, 1771.

Wu, L. S., Bargmann, R. E. and Powers, J. J. 1977. Factor analysis applied to wine descriptors. J. Food Sci. 42: 944-952.

Wythoff, B. J. 1993. Backpropagation neural networks: a tutorial. Chemom. Intell. Lab Sys. 18: 115-155.

Zadow, J. G. 1994. Application of artificial neural networks in the UHT operation - a response. Aust. J. Dairy Technol. 49: 118-119.

Zechman, J. M., Aldinger, S., Labows, J. N., Jr. 1986. Characterization of pathogenic bacteria by automated headspace concentration-gas chromatography. J. Chromatogr. 377: 49-57.

Zhou, W., Kailasapathy, D. G., Laing, D. G. and Ramachandran, M. 1994. Application of artificial neural networks (ANN) in UHT milk processing. Aust. J. Dairy Technol. 49: 117.

APPENDIXES

A. Computer Program for Peak Assignment

```
10    DIM RET(100), AREA(100), RRET(100), LL(200), UL(200), PEAK(200).
20    LL(1)=50 : UL(1)=52.97
30    LL(2)=53! : UL(2)=53.16
40    LL(3)=53.6 : UL(3)=53.9
50    LL(4)=58! : UL(4)=59.1
60    LL(5)=59.2 : UL(5)=59.99
70    LL(6)=60! : UL(6)=61.8
80    LL(7)=63! : UL(7)=63.5
90    LL(8)=63.6 : UL(8)=64
100   LL(9)=64.1 : UL(9)=65.6
110   LL(10)=66! : UL(10)=66.89
120   LL(11)=66.9 : UL(12)=67.4
130   LL(13)=67.5 : UL(13)=67.7
140   LL(14)=68.1 : UL(14)=68.9
150   LL(15)=71! : UL(15)=72
160   LL(16)=72.1 : UL(7)=72.5
170   LL(17)=74! : UL(17)=76.8
180   LL(18)=76.9 : UL(18)=77!
190   LL(19)=77.2 : UL(19)=77.7
200   LL(20)=78.2 : UL(20)=79.9
210   LL(21)=80! : UL(21)=81.9
220   LL(22)=82 : UL(22)=82.7
230   LL(23)=82.75 : UL(23)=83.4
240   LL(24)=83.5 : UL(24)=86
250   INPUT "ENTER NAME OF FILE TO ANALYZE"; NAMES$: OPEN NAMES$
      FOR INPUT AS #1: INPUT "ENTER NUMBER OF PEAKS"; NUMPEAK: FOR
      I=1TO NUMPEAK: INPUT #1, RET(I), AREA(I)
260   RRET (I)=(INT(RET()*100)/100
270   NEXT I: CLOSE
280   INPUT "DO YOU WANT TO PRINT FILE?; A$
290   IF A$="N" THEN 340
```

```
300     LPRINT TAB(20) "FILE NAME >>"; NAME$
310     FOR I=1 TO NUMPEAK
320     LPRINT I, RRET(I), AREA(I)
330     NEXT I
340     FOR I=1 TO NUMPEAK
350     FOR J=1 TO 24
360     IF RRET (I)>=LL(J) AND RRET(I) <=UL(J) THEN PEAK(J)=PEAK(J) +
        AREA(I)
370     NEXT J
380     NEXT I
390     PRINT "RESULTS"
400     LPRINT TAB(20) "RESULTS FOR FILE>>; NAME$
410     FOR I=1 TO 24
420     PRINT I, PEAK(I)
430     NEXT I
440     INPUT "ENTER NAME OF FILE FOR OUTPUT"; RESULT$
450     OPEN RESULTS$ FOR OUTPUT AS #1: FOR I=1 TO 24: PRINT #1, I,
        PEAK(I):   NEXT I
460     CLOSE
```

B. Computer Program for Principal Component Similarity Analysis

```
5     REM VERSION NOVEMBER 25 WITH DR NAKAI IDEA WITH PRINTS
      AND INPUT
7     REM OPTION OF FILE TO SAVE CORRECTED PCA IS NOT
      WORKING!!!!!!!!!!!!!
10    DIM M(30),L(112)
20    DIM N(112),CC(112)
30    DIM A(112,10),R(112)
40    DIM B(10,112)
50    DIM REF(10),S(50)
60    DIM VARB(30),K(30)
70    DIM Q(112,20),Y(112)
80    DIM X(112),POX(40),POY(40)
90    DIM X1(112),Y1(112)
92    CLS:SCALES$="CDEFGAB":PLAY "O4 XSCALES$;"
95    SCREEN 1:COLOR 12:KEY OFF
100   LOCATE 10,40:PRINT "PCA-SIMILARITY ANALYSIS":PRINT:PRINT
110   INPUT " PRESS ANY KEY TO CONTINUE",ZZ:CLS
115   SCREEN 2:SCREEN 0
130   INPUT "FILE NAME TO BE USED";FILE$
140   INPUT "HOW MANY CASES IN EACH FACTOR";C
150   INPUT "HOW MANY FACTORS WOULD YOU LIKE TO USE";F
160   INPUT "WHICH CASE (ROW NUMBER) DO YOU WANT TO BE YOUR
      STANDARD";S
165   INPUT "WOULD YOU LIKE TO USE ARBITRARY MAGNIFICATION
      ?";A$
166   IF A$="N" THEN GOTO 180
170   INPUT "WHAT WOULD YOU LIKE YOUR ARBITRARY
      MAGNIFICATION TO BE";M
180   FOR X=1 TO F
190   PRINT "WHAT IS THE % VARIANCE FOR FACTOR NUMBER";X
200   INPUT VARB(X)
210   NEXT
220   REM INPUTING FILES FROM PCA
230   OPEN FILE$ FOR INPUT AS #1
240   FOR I=1 TO C
250   FOR J=1 TO F
260   INPUT #1,A(I,J)
270   NEXT J
```

```
280     NEXT I
290     CLOSE #1
295     REM REFERENCE USED IN CALCULATION
300     FOR J=1 TO F
320     REF(J)=A(S,J)
330     NEXT J
331     REM conversion to 100 Dr. Nakai's idea
334     FOR I=1 TO F
338     RVARB(I)=VARB(I)
339     NEXT I
350     FOR I=1 TO C
360     FOR J=1 TO F
370     IF J=1 THEN S(J)=100
380     K(J)=S(J)-RVARB(J)
390     S(J+1)=K(J)
400     IF J=1 THEN Q(I,J)=((A(I,J)-REF(J))*RVARB(J))+100
410     IF J=1 THEN 430
420     Q(I,J)=((A(I,J)-REF(J))*RVARB(J))+S(J)
430     NEXT J
440     NEXT I
450     FOR I=1 TO F
460     FOR J=1 TO C
470     B(I,J)=Q(J,I)
480     NEXT J
490     NEXT I
500     REM SUBROUTINE:  REGRESSION
510     FOR P=1 TO C
520     SX=0:SY=0:SX2=0:SY2=0:SXY=0
530     FOR I=1 TO F
540     Y(I)=B(I,P)
550     X(I)=B(I,S)
560     NEXT I
570     FOR T=1 TO F
580     SX=SX+X(T)
590     SY=SY+Y(T)
600     SX2=SX2+X(T)^2
610     SY2=SY2+Y(T)^2
620     SXY=SXY+X(T)*Y(T)
630     NEXT T
640     REM SLOPE
650     L(P)=(F*SXY-SX*SY)/(F*SX2-SX^2)
660     REM COEFFICIENT OF CORRELATION
```

```
670     CC(P)=(SXY-SX*SY/F)/(SQR((SX2-(SX^2)/F)*(SY2-(SY^2)/F)))
680     R(P)=CC(P)^2
690     NEXT P
700     LPRINT "CASE","SLOPE","COEF. OF DETERM."
710     REM GRAPHICS
720     FOR P=1 TO C
730     X(P)=R(P)
740     Y(P)=L(P)
750     LPRINT  P,L(P),R(P)
760     PRINT  P,L(P),R(P):NEXT P
770     INPUT "NAME OF FILE FOR R^2 AND SLOPE";FILS$
780     OPEN FILS$ FOR OUTPUT AS #1
790     FOR I=1 TO C
800     PRINT #1,X(I),Y(I)
810     NEXT I
820     XMIN=0
830     YMIN=0
840     XMAX=1
850     YMAX=3
860     NPTS=C
870     XLAB$="COEFFICIENT OF DETERMINATION"
880     YLAB$="SLOPE"
890     SYM=1
900     SIZE=.5
910     XLEN=0
920     YLEN=0
930     XLIN=0
940     YLIN=0
950     XINC=0
960     MORE=0
970     GOSUB 1430
980     LOCATE 1,2:INPUT"PRESS ANY KEY TO CONTINUE (PRESS PRINT
        SCREEN TO PLOT GRAPH)";ZZ:CLS
990     INPUT "WOULD YOU LIKE TO CHANGE THE SCALE OF THE X AND Y
        COORDINATES (Y/N)";A$
1000    IF A$="N" THEN 1080
1010    IF A$="Y" THEN 1020
1020    PRINT "THESE ARE THE CURRENT X MIN. AND X MAX.";XMIN,XMAX
1030    INPUT "ENTER THE NEW X MIN, X MAX";XMIN,XMAX
1040    PRINT "THESE ARE THE CURRENT Y MIN. AND Y MAX.";YMIN,YMAX
1050    INPUT "ENTER THE NEW Y MIN, Y MAX";YMIN, YMAX
1060    GOSUB 1430
```

```
1070    LOCATE 1,2:INPUT"PRESS ANY KEY TO CONTINUE (PRESS PRINT
        SCREEN TO PLOT GRAPH)";ZZ:CLS
1080    INPUT"WOULD YOU LIKE TO SEE THE GRAPH OF ANY SPECIFIC
        CASE (Y/N)";Y$
1090    IF Y$="Y" THEN 1110
1100    IF Y$="N" THEN 1410
1110    INPUT "WHICH CASE # WOULD YOU LIKE TO SEE";CN
1120    XMIN=0:XMAX=0:YMIN=0:YMAX=O
1125    PRINT:PRINT TAB(15) "STANDARD=";S;:PRINT TAB(15) "VS.
        SAMPLE=";CN
1130    FOR  I=1 TO F
1140    X(I)=B(I,S):PRINT:PRINT "CORRECTED PCA(";I;")";:PRINT TAB(17)
        B(I,S);
1150    Y(I)=B(I,CN):PRINT TAB(30) B(I,CN)
1160    IF X(I)>AMAX THEN AMAX=X(I)
1170    IF X(I)<AMIN THEN AMIN=X(I)
1180    IF Y(I)>BMAX THEN BMAX=Y(I)
1190    IF Y(I)<BMIN THEN BMIN=Y(I)
1200    NEXT I
1205    PRINT:INPUT "DO YOU WANT TO SAVE THE CORRECTED PCA";MM$
1207    IF MM$="N" THEN 1210
1208    CLOSE #1:INPUT "FILE NAME TO SAVE RESULTS";NOM$:OPEN NOM$
        FOR OUTPUT AS #1:FOR I=1 TO F:PRINT #1,B(I,CN),B(I,S):NEXT
        I:CLOSE
1210    XMAX=AMAX
1220    XMIN=AMIN
1230    YMAX=BMAX
1240    YMIN=BMIN
1250    NPTS=F
1260    LAB1$="CASE #="
1270    CORNER=1
1280    XLAB$="STANDARD"
1290    YLAB$="CASE"
1300    LTYPE=0
1310    MORE=0
1320    GOSUB 1430
1330    MORE=1:X(1)=XMAX
1340    X(2)=XMIN
1350    Y(1)=X(1)
1360    Y(2)=X(2)
1370    LTYPE=1
1380    NPTS=2
```

```
1390    GOSUB 1430
1400    GOTO 1070
1410    PRINT:PRINT:PRINT TAB(30)"-------END OF PROGRAM--------":END
1420    '
1430    ' SUBGRAPH      May 10,1984          Bob Shannon
1440    '
1450    'SUBGRAPH is a BASIC subroutine that will plot any set of data.
1460    'It defines the coordinates of the screen, draws
1470    'the grid with tic marks, numbers, and labels on each axis.
1480    'It also prints a 3 line label in one corner. The user can specify
1490    'the size and proportion of the figure in inches. Either linear
1500    'or logarithmic axes are allowed on both ordinate and abcissa.
1510    '
1520    ' Alt-C controls the color of the plot, the entire figure is
1530    ' plotted in the chosen color. The color option does not
1540    ' affect the printer dump print density.
1550    '-------------------------------------------------------------
1560    'Calling sequence:
1570    '       1. MERGE "SUBGRAPH.bas" with your program
1580    '       2. Save a new copy of your program which includes subroutine
1590    '       3. Set up the parameters as defined below
1600    '       4. gosub 60000 at point where you want the plot
1610    '
1620    '-------------------------------------------------------------
1630    'Parameters:   Do not use these names elsewhere in your program
1640    '
1650    ' SYM          symbol type (0=none,1=open sq,2=fill sq,3=open tri
1660    '                4=fill tri,5=open cir,6=fill cir
1670    '                7=open diamond,8=filled diamond,9=X
1680    ' SIZE         Symbol size in % of axes length
1690    ' LTYPE         line type (0=none,1=solid,2=dashed,3=dotted,4=regression
1700    ' NPTS         is the number of data points
1710    ' X(I),Y(I)    arrays that contain the x and y data points
1720    ' XLEN,YLEN      x and y axis length in inches
1730    ' XMIN,YMIN      x any y minimum values
1740    ' XMAX,YMAX      x and y axis maximum values
1750    ' XLIN,YLIN      flag for linear(=0) or Log(=1) axis
1760    ' XINC,YINC      unit increment on each axis (valid only for linear)
1770    '              if these are <=0 they are calculated from data
1780    ' XLAB$,YLAB$    strings containing the axis labels
1790    ' LAB1$,LAB2$,LAB3$      three label lines
1800    ' CORNER        specifies the corner for the label
```

```
1810    '             (0=none,1=LL,2=LR,3=UL,4=UR)
1820    ' MORE          a flag that indicates whether this call is the
1830    '               first and so axes should be plotted, or if it
1840    '               is more data to go on the same axes (axes are
1850    '               not plotted if MORE=1), MORE=0 new figure.
1860    'MIRORSAVFIL'
1870    ' For more than one set of data on the same axes:
1880    '       1. Set up the first set of data and all other plot parameters
1890    '       2. GOSUB 60000 with MORE=0
1900    '       3. Set up second set of data (leave plot parameters unchanged)
1910    '            (you may change SYM, SIZE, LTYPE and NPTS for each set)
1920    '       4. GOSUB 60000 with MORE=1
1930    '       5. repeat steps 3 and 4 for each additional data set
1940    '
1950    '-------------------------------------------------------------
1960    '
1970    '
1980    ' Scale axes and plot them
1990    '
2000    IF YLEN<=0 THEN YLEN=5.5
2010    IF XLEN<=0 THEN XLEN=7.5
2020    IF MORE<>1 THEN SCREEN 2:CLS:KEY OFF
2030    KEY 20,CHR$(&H8)+CHR$(46):KEY (20) ON
2040    ON KEY (20) GOSUB 3460
2050    IF KLR.P=0 THEN KLR.P=15
2060    OUT 985,KLR.P
2070    XINC.P=XINC:YINC.P=YINC
2080    XRANGE.P=XMAX-XMIN:YRANGE.P=YMAX-YMIN
2090    IF XINC<=0 THEN XINC.P=10^(INT(LOG(XRANGE.P*.66)/LOG(10)))
2100    IF YINC<=0 THEN YINC.P=10^(INT(LOG(YRANGE.P*.66)/LOG(10)))
2110    XMIN.P=XINC.P*INT(XMIN/XINC.P):XMAX.P=XINC.P*(INT((XMAX/XI
        NC.  P)+1))XMIN.P=XINC.P*=YINC
2120    YMAX=YINC.P*INT((YMAX/YMINC.P)+1):UMIN.P=YINC.P
        *(INT(YMIN/YMINC.P))
2130    IFXLIN=1THENXMAX.P=LOG(XMAX)/LOG(10):XMIN.P=
        LOG(XMIN)/LOG(10)
2140    IFYLIN=1THENYMAX.P=LOG(YMAX)/LOG(10):YMIN.P=
        LOG(YMIN)/LOG(10)
2150    XRANGE.P=XMAX.P-XMIN.P:YRANGE.P=YMAX.P-YMIN.P
2160    DX=SIZE*XRANGE.P/100!:DY=SIZE*YRANGE.P/100!
2170    XT.P=XRANGE.P*(9!/XLEN):YT.P=YRANGE.P*(7!/YLEN)
2180    TICX=.03*XRANGE.P:TICY=.04*YRANGE.P
```

```
2190    XTRA=(XT.P-XRANGE.P)*9/XT.P:YTRA=(YT.P-YRANGE.P)*7/YT.P
2200    LBD.X=XMIN.P-(1!*XT.P/9)
2210    LBD.Y=YMIN.P-(1!*YT.P/7)
2220    UBD.X=XMAX.P+((XTRA-1!)*XT.P/9):UBD.Y=YMAX.P+((YTRA-
        1!)*YT.P/7)
2230    IF MORE<>1 THEN WINDOW (LBD.X,LBD.Y)-(UBD.X,UBD.Y)
2240    IF MORE<>1 THEN LINE (XMIN.P,YMIN.P)-(XMAX.P,YMAX.P),1,B
2250    XLOW.P=XMIN.P-LBD.X:YLOW.P=YMIN.P-LBD.Y
2260    XHI.P=XT.P-XRANGE.P-XLOW.P:YHI.P=YT.P-YRANGE.P-YLOW.P
2270    XP.P=.00159*(UBD.X-LBD.X)
2280    IFMORE<>1THENLINE(XMIN.P+XP.P,YMIN.P)-
        (XMAX.P+XP.P,YMAX.P),1,B
2290    STYLE=&HFFFF:IF LTYPE=0 THEN STYLE=&H0
2300    IF LTYPE=2 THEN STYLE=&HF0F0
2310    IF LTYPE=3 THEN STYLE=&HC0C0
2320    IF LTYPE=4 THEN STYLE=&H0
2330    IF MORE=1 THEN 3170
2340    '
2350    'label axes
2360    '
2370    XPOS.P=((XLOW.P+(XRANGE.P/2!))*80!/XT.P)-(LEN(XLAB$)/2)
2380    LOCATE 25,XPOS.P:PRINT XLAB$;
2390    YPOS.P=25!-(25!*((YLOW.P+(YRANGE.P/2!))/YT.P))-(LEN(YLAB$)/2!)
2400    FOR I=1 TO LEN(YLAB$):YT$=MID$(YLAB$,I,1):LOCATE
        I+YPOS.P,3:PRINT YT$;:NEXT I
2410    '
2420    ' Print label on figure in specified corner
2430    '
2440    '
2450    IF CORNER=0 THEN GOTO 2590
2460    MAXLEN=0:IF LEN(LAB3$)>MAXLEN THEN MAXLEN=LEN(LAB3$)
2470    IF LEN(LAB2$)>MAXLEN THEN MAXLEN=LEN(LAB2$)+1
2480    IF LEN(LAB1$)>MAXLEN THEN MAXLEN=LEN(LAB1$)+1
2490    IF CORNER=1 OR CORNER=2 THEN XPOS.P=((XLOW.P/XT.P)*80!)+3
2500    IF CORNER=3 OR CORNER=4 THEN
        XPOS.P=(((XLOW.P+XRANGE.P)/XT.P)*80!)-MAXLEN
2510    IF CORNER=2 OR CORNER=4 THEN YPOS.P=((YHI.P/YT.P)*26)+2
2520    IF CORNER=1 OR CORNER=3 THEN
        YPOS.P=(((YHI.P+YRANGE.P)/YT.P)*26!)-4!
2530    LOCATE YPOS.P,XPOS.P:PRINT LAB1$;CN
2540    LOCATE YPOS.P+1,XPOS.P:PRINT LAB2$;
2550    LOCATE YPOS.P+2,XPOS.P:PRINT LAB3$;
```

```
2560    '
2570    ' tic marks and numbers on linear x axis
2580    '
2590    IF XLIN=1 THEN 2710
2600    FOR XTIC=XMIN.P TO XMAX.P STEP XINC.P
2610     LINE (XTIC,YMIN.P)-(XTIC,YMIN.P+TICY),1
2620     LINE (XTIC+XP.P,YMIN.P)-(XTIC+XP.P,YMIN.P+TICY),1
2630     LINE (XTIC,YMAX.P-TICY)-(XTIC,YMAX.P),1
2640     LINE (XTIC+XP.P,YMAX.P-TICY)-(XTIC+XP.P,YMAX.P),1
2650     XPOS.P=(((XLOW.P+(XTIC-XMIN.P))/XT.P)*80!)-(LEN(STR$(XTIC))/2)
2660    LOCATE 23,XPOS.P:PRINT USING "#.##";XTIC;
2670    NEXT XTIC
2680    '
2690    '       tic marks and numbers on linear y axis
2700    '
2710    IF YLIN=1 THEN 2850
2720    FOR YTIC=YMIN.P TO YMAX.P STEP YINC.P
2730    LINE (XMIN.P,YTIC)-(XMIN.P+TICX,YTIC),1
2740    LINE (XMAX.P-TICX,YTIC)-(XMAX.P,YTIC),1
2750    YPOS.P=((YHI.P+(YMAX.P-YTIC))/YT.P)*26!
2760    XPOS.P=6-(LEN(STR$(YTIC))/2)
2770    IF YPOS.P>25 OR YPOS.P<1 THEN BEEP:GOTO 2800
2780    IF XPOS.P>80 OR XPOS.P<1 THEN BEEP:GOTO 2800
2790    LOCATE YPOS.P,XPOS.P:PRINT USING "##.#";YTIC
2800    NEXT YTIC
2810    '
2820    '       tic marks and numbers on log x axis
2830    '
2840    '
2850    IF XLIN=0 THEN 3010
2860    FOR CYC=-5 TO 5
2870    FOR LTIC=1 TO 10
2880    XTIC=LTIC*(10^CYC)
2890    LXTIC=LOG(XTIC)/LOG(10)
2900    IF LXTIC<=XMIN.P OR LXTIC>=XMAX.P THEN 2950
2910    LINE (LXTIC,YMIN.P)-(LXTIC,YMIN.P+TICY),1
2920    LINE (LXTIC+XP.P,YMIN.P)-(LXTIC+XP.P,YMIN.P+TICY),1
2930    LINE (LXTIC,YMAX.P-TICY)-(LXTIC,YMAX.P),1
2940    LINE (LXTIC+XP.P,YMAX.P-TICY)-(LXTIC+XP.P,YMAX.P),1
2950    NEXT LTIC
2960    IF LXTIC>=XMIN.P AND LXTIC<=XMAX.P THEN LOCATE
        23,(((XLOW.P+(LXTIC-XMIN.P))/XT.P)*80!)-1:PRINT XTIC;
```

```
2970    NEXT CYC
2980    '
2990    '       tic marks and numbers on log y axis
3000    '
3010    IF YLIN=0 THEN 3140
3020    FOR CYC=-5 TO 5
3030    FOR LTIC=1 TO 10
3040    YTIC=LTIC*(10^CYC)
3050    LYTIC=LOG(YTIC)/LOG(10)
3060    IF LYTIC<=YMIN.P OR LYTIC>=YMAX.P THEN 3090
3070    LINE (XMIN.P,LYTIC)-(XMIN.P+TICX,LYTIC),1
3080    LINE (XMAX.P-TICX,LYTIC)-(XMAX.P,LYTIC),1
3090    NEXT LTIC
3100    YPOS.P=((YHI.P+(YMAX.P-LYTIC))/YT.P)*26!
3110    XPOS.P=6-((LEN(STR$(YTIC))/2!))
3120    IF LYTIC>=YMIN.P AND LYTIC<=YMAX.P AND YPOS.P>=1 THEN
        LOCATE YPOS.P,XPOS.P:PRINT YTIC;
3130    NEXT CYC
3140    '
3150    ' now plot data on axes
3160    '
3170    SX=0:SY=0:SSX=0:SXY=0
3180    FOR I=1 TO NPTS
3190    X1(I)=X(I):IF XLIN=1 THEN X1(I)=LOG(X(I))/LOG(10)
3200    Y1(I)=Y(I):IF YLIN=1 THEN Y1(I)=LOG(Y(I))/LOG(10)
3210    IF I>1 THEN LINE(X1(I-1),Y1(I-1))-(X1(I),Y1(I)),1,,STYLE
3220    IF I>1 THEN LINE (X1(I-1)+XP.P,Y1(I-1))-(X1(I)+XP.P,Y1(I)),1,,STYLE
3230    IF SYM=1 THEN LINE (X1(I)-DX,Y1(I)-DY)-(X1(I)+DX,Y1(I)+DY),1,B
3240    IF SYM=1 OR SYM=2 THEN LINE(X1(I)-DX+XP.P,Y1(I)-DY)-
        (X1(I)+DX+XP.P,Y1(I)+DY),1,B
3250    IFSYM=2THENLINE(X1(I)-DX+XP.P,Y1(I)-DY)-
        (X1(I)+DX+XP.P,Y1(I)+DY),1,BF
3260    IF SYM=3 OR SYM=4 THEN LINE (X1(I)-DX,Y1(I)-DY)-(X1(I)+DX,Y1(I)-
        DY),1:LINE (X1(I),Y1(I)+DY)-(X1(I)-DX,Y1(I)-DY),1:LINE
        (X1(I),Y1(I)+DY)-(X1(I)+DX,Y1(I)-DY),1
3270    IF SYM=3 OR SYM=4 THEN LINE (X1(I)+XP.P,Y1(I)+DY)-
        (X1(I)+XP.P+DX,Y1(I)-DY),1:LINE (X1(I)+XP.P,Y1(I)+DY)-(X1(I)+XP.P-
        DX,Y1(I)-DY),1
3280    IF SYM=4 THEN PAINT (X1(I)+2*XP.P,Y1(I)),1
3290    IF SYM=5 OR SYM=6 THEN CIRCLE (X1(I),Y1(I)),DX:CIRCLE
        (X1(I)+XP.P,Y1(I)),DX
3300    IF SYM=6 THEN PAINT (X1(I)+2*XP.P,Y1(I)),1
```

```
3310    IF SYM=9 THEN LINE (X1(I)-DX,Y1(I)-DY)-(X1(I)+DX,Y1(I)+DY),1:LINE
        (X1(I)+DX,Y1(I)-DY)-(X1(I)-DX,Y1(I)+DY),1
3320    IF SYM=7 OR SYM=8 THEN LINE (X1(I),Y1(I)+DY)-
        (X1(I)+DX,Y1(I)),1:LINE -(X1(I),Y1(I)-DY),1:LINE -(X1(I)-
        DX,Y1(I)),1:LINE -(X1(I),Y1(I)+DY),1
3330    IF SYM=8 THEN PAINT (X1(I)+2*XP.P,Y1(I)),1
3340    SY=SY+Y1(I):SX=SX+X1(I):SSX=SSX+(X1(I)^2):SXY=SXY+(X1(I)*Y1(I))
3350    NEXT I
3360    IF LTYPE<>4 THEN RETURN
3370    '
3380    ' Regression line plotted
3390    :A=((NPTS*SXY)-(SX*SY))/((NPTS*SSX)-(SX*SX))
3400    B=(SY/NPTS)-(A*SX/NPTS)
3410    YMIN.P=(A*XMIN.P)+B:YMAX.P=(A*XMAX.P)+B
3420    LINE (XMIN.P,YMIN.P)-(XMAX.P,YMAX.P),1
3430    LINE (XMIN.P+XP.P,YMIN.P)-(XMAX.P+XP.P,YMAX.P),1
3440    '
3450    RETURN
3460    '
3470    ' key trap of Alt-C to change color
3480    '
3490    KLR.P=(KLR.P+1) MOD 128:IF KLR.P MOD 8=0 OR KLR.P MOD 16=0
        THEN KLR.P=KLR.P+1
3500    OUT 985,KLR.P
3510    RETURN
3520    OPEN "com1:9600,s,7,1,rs,cs65535,ds,CD" AS #1
3530    PRINT #1, "IN;SP1;IP1750,1100,8500,7550;"
3540    PRINT #1, "SCXMIN,XMAX,YMIN,YMAX;"
3550    PRINT #1 "PUXMIN,YMIN,
        PDXMAX,YMIN,XMAX,YMAX,XMIN,YMAX,XMIN,YMIN PU"
3560    PRINT #1,"SI.2,.3;TL1.5,0"
3570    I=0
3580    FOR XTIC=XMIN TO XMAX STEP XINC
3590    PRINT #1,"PA";X(I),",0":XT;"
3600    PRINT #1,"CP-2,-1;LB";X(I),+CHR$(3)
3610    I=I+1:NEXT XTIC
```

C. ADSA Scoring Guide for Sensory Evaluation

Flavour defects	Not detect	Slight	Definite	Pronounced	Unsellable
Acid	10	3	1	0	0
Astringent	10	8	7	6	0
Bitter	10	5	3	1	0
Cooked	10	9	8	6	0
Heated	10	9	8	6	0
Oxidized light induced	10	6	4	1	0
Oxidized metal induced	10	5	3	1	0
Rancid	10	4	1	0	0

	Accept	Accept moderately	Accept slightly	Not accept
Overall impression	10	6	2	0