INFLUENCE OF LACTOBACILLI AND STREPTOCOCCI ON THE VOLATILE COMPONENTS FOUND IN SOYMILK

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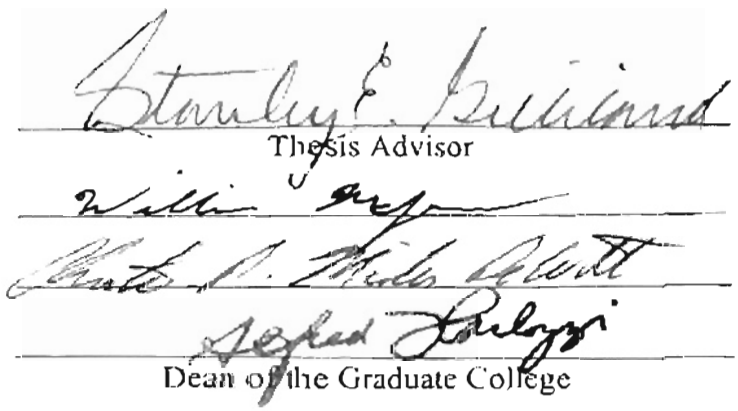

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Chapter I

Introduction

Americans as a society have embarked on a very rapid trend of moving toward more convenient, health conscious diets. With this in mind looking at the agricultural products available to the United States, soybeans are seen as having enormous potential for future product development. The National Agriculture Statistics Service and USDA reported that in 2001 there were ~ 74 million acres of soybeans planted resulting in a yield valued at 12 billion dollars. Thus soybeans are an economically important crop, which provides an excellent source of quality protein. Soybeans are comprised of 42-48% protein and 20-22% oil (Cheng and others 1990; Friedman and Brandon 2001). As much of the United States soybean production today is exported the window for soy based product development for domestic use is very open. As a result of soybeans being a very inexpensive source of many of the essential amino acids, they have sparked tremendous interest from the food industry. Currently foods such as cheese, drinks, miso, tempeh, tofu, and salami comprise the product list that are based from or contain soy (Friedman and Brandon 2001). Soy and soy products have increasingly made their mark as a popular meat substitute especially with the vegetarian consumers. Not only are soy and soy products readily available and inexpensive, but also a great amount of research has been published to support the many claims that consumption of these products offer numerous potential health and nutritional benefits. Considering the potential associated with consumption of soy and soy based products food science research has set many

goals for developing soy products that meet the needs and tastes of the general American consumer.

The objective of this study was to determine if supplementing soymilk with a selected species of a probiotic culture would be capable of reducing the volatile compounds that are responsible for the very undesirable "beany" flavor of soy products.

Chapter II

Review of Literature

Benefits of Foods Developed from Soy

Much of the interest in consumption of soy and soy derived products stems not only from the availability of soy and its inexpensiveness, but also from the extensive amount of published support that describes its potential beneficial effects on health and nutrition. Some of the potential benefits are the prevention of diseases such as cancer, diabetes, and obesity (Karleskind and others 1991; Friendman and Brandon 2001). Friedman and Brandon (2001) also have claimed that soy consumption may help in the protection against bowel and kidney diseases. Other reports have shown that the consumption of soy products can result in reduced levels of serum cholesterol, saturated fats, and lactose (Lee and others 1990; Karleskind and others 1991).

There has been much focus placed on examining the reduction in serum cholesterol through consuming a soybean diet. Sirtori and others (1995) reported, that a "soybean diet is currently the most potent dietary tool for treating hypercholesterolemia". The possible mechanism by which soy based diets effectively lower cholesterol levels is in the soy proteins' ability to regulate the low-density lipoprotein receptors in the liver (Manzoni and others 1998). The reduction of the cholesterol levels is reported to be as much as ~6-12% decrease in tested individuals (Friendman and Brandon 2001).

Undesirable Flavor in Soybean and Soy Products

According to the 2001 edition of the Annual Soybean Statistics Guide, which is sponsored by the United Soybean Board, there have been substantial increases in planting and production of soybeans. In addition the U.S exports of soybeans have increased ~200 million bushels in the last two years. The statistics from this source show that this level of exports totals almost half of the entire soybean production of the United States. This data supports the known fact that soybeans are far more important as a food crop in the Orient than they are here in America or in other Western Countries (Che Man and others 1989; Buono and others 1990; Moreira and others 1993; Wang and others 1998). Many authors have elucidated the major limiting factor with regards to consumer acceptability of soybeans and soy derived products in areas other than the Orient as being the development of an undesirable off-flavor that is described as "beany" (Wolf 1975; Rackis and others 1979; Che Man and others 1989; Cheng and others 1990; Srinivas and others 1992; Maheshwari and others 1995, 1997; Wang and others 1998). In realizing the nutritional and economical potential of soybeans, there has been an effort to increase the utilization of soybeans and soy products in the American diet. Much research has focused on determining the source of the beany flavor as well as possible methods for its elimination.

"Beany" Flavor Development

Several authors have shown evidence that the major component of soybean seeds responsible for the undesirable "beany" flavor is lipoxygenase isozymes (Arai and others 1970a; Rackis and others 1979; Moreira and others 1993). Wolf (1975) stated that

lipoxygenase was originally referred to as an enzyme in soybeans that was capable of oxidizing fat. It is known that soybean seeds not only contain significant amounts of lipoxygenase but also the substrates linoleic and linolenic acids (Moreira and others 1993). It is the physical impact of harvesting, transport, processing, and storage that allows interactions between lipoxygenase and these fatty acids that ultimately results in lower product quality (Davies and others 1987; Moreira and others 1993). These lipoxygenase-mediated conversions of the fatty acids result in the formation of volatile compounds that lead to the "beany" off flavor development (Sessa and Rackis 1977; Rackis and others 1979; Sessa 1979). These volatile compounds have been identified as aldehydes, ketones, and alcohols (Arai and others 1970a, 1970c; Sessa and Rackis 1977; Rackis and others 1979; Sessa 1979; Takahashi and others 1979b; Damodaran and Kinsella 1981). The implicated volatile compounds have been named as hexanal, hexenal, pentylfuran, heptanol, hexanol, pentanol, and ethyl vinyl ketone (Arai and others 1970a, 1970c; Sessa and Rackis 1977; Rackis and others 1979; Sessa 1979; Takahashi and others 1979b; Damodaran and Kinsella 1981; Maheshwari and others 1995, 1997; Wang and others 1998). The major class of compounds responsible for the "beany" flavor is the medium-chain aldehydes – pentanal, hexanal, and heptanal (Maheshwari and others 1995, 1997).

In addition to the formation of these undesirable carbonyl compounds, research has shown that these carbonyl flavor compounds bind very firmly to the proteins present in soy (Arai and others 1970c; Chiba and others 1979b; Damodaran and Kinsella 1981). Once these flavor compounds have bound to the protein compounds they become extremely resistant to removal by extraction, distillation, and most other conventional

methods of removal (Arai and others 1970c; Chiba and others 1979a; Damodaran and Kinsella 1981). Some evidence also suggests that through a gradual release of these carbonyls during storage and cooking the soy products' "beany" off flavor is enhanced (Chiba and others 1979b).

Attempts at Removal of "Beany" Flavor

As pointed out earlier the impact of the integration of more soy-derived products into the human diet offers abundant opportunity both economically and nutritionally. Because of the possible beneficial roles of soybeans in the diet, a substantial amount of research has been conducted on new methods that offer the answer to eliminating the "beany" flavor from these products.

Heat Treatments

In much of the research published referencing the action of lipoxygenase as the culprit for the development of the "beany" flavor the underlying theme to a majority of the proposed solutions was to use heat to eliminate the off flavor (Wolf 1975). The use of steam, controlled moist heat treatment known as toasting, and extremely high temperature treatments have all been examined as a means of producing "beany" free soy products. With regard to heat treatments two major results were seen: 1) the removal of the off flavor compounds was not complete and 2) qualities and functionalities of the soy proteins were severely effected (Chiba and others 1979a, 1979b; Che Man and others 1989). For example, the attempt at toasting the soy proteins did eliminate much of the flavor associated with the raw soy flour examined, however as is the case with most of

the heat treatments attempted a nutty or toasted flavor developed as well as a disagreeable darkened color change (Wolf 1975). Tuitemwong and others (1993) examined the use of rapid hydration hydrothermal cooking in order to produce high quality soymilk. Although methods such as these have an impact on the elimination of the off flavor compounds the processing cost that would be incurred as a result of the incorporation becomes very uneconomical for the soy product industry.

Acid Treatments

Much like the heat treatments, acid treatments resulted in significant inactivation of lipoxygenase; however, it resulted in the product having proteins with undesirable characteristics and functionality (Che Man and others 1989). The products were generally very bland. In addition, it seems very unfeasible to incorporate an acid treatment into the processing of soy products (Maheshwari and others 1995). The unfavorable economical feasibility and loss of protein functionality can also be observed with a method of extraction involving combinations of hexane and acetic acid (Srinivas and others 1992).

Supercritical CO_2 Technology

Looking at newer technological methods, Maheshwari and others (1995) examined the use of Supercritical Carbon Dioxide extraction on the removal of off flavors from soybean protein isolates. Although significant improved flavor characteristics resulted without impairing protein functionality they were unsuccessful at making the procedure economically feasible for soy product processing industry.

Enzymatic Treatments

The use of different protease treatments on soybeans has been examined extensively as a solution to the elimination of the undesirable "beany" flavor. These protease treatments have included the use aldehyde dehydrogenases such as bovine liver mitochondrial aldehyde dehydrogenase, a combination of aldehyde dehydrogenase and diaphorase, microbial proteases from *Aspergillus saitoi* such as molsin or aspergillopeptidase A and aspergillus acid carboxypeptidase, and aldehyde oxidases such as porcine liver aldehyde oxidase (Arai and others 1970a, 1970b; Chiba and others 1979a, 1979b; Takahashi and others 1979a, 1979b, 1980; Maheshwari and others 1997).

The use of aldehyde dehydrogenase was significantly successful at completely removing the "beany" flavor; however it is very impractical and uneconomical because of the requirement of NAD^+ as a cofactor (Chiba and others 1979a; Takahashi and others 1980; Maheshwari and others 1995). Even the combination of aldehyde dehydrogenase and diaphorase required a small amount of NAD+ in order to significantly reduce the "beany" flavor (Takahashi and others 1980).

The attempt at using microbial proteases was some what effective at reducing some of the "beany" flavor however it led to the development of other off flavors such as bitterness (Arai and others 1970b). This particular research did reveal that through combinations of different microbial proteases one could achieve a deodorized and debittered soy protein. However, there was no indication in the paper as to how this treatment affected the functionality of the soy proteins.

While several studies reported that the use of aldehyde oxidases was successful at reducing a great amount of the "beany" odor from soybean extracts, it was observed that the rate by which this was accomplished was significantly slower than other methods (Takahashi and others 1979b). The authors reported this observation was due to the fact that the flavor causing components were tightly bound to the proteins. The aldehyde oxidase was unable to interact as well with such tightly bound compounds compared to the ability of compounds such as aldehyde dehydrogenases. On the other hand, unlike the aldehyde dehydrogenases that require the NAD+ as a cofactor to exhibit this reduction, the aldehyde oxidases only require oxygen as the electron acceptor (Takahashi and others 1979b).

Addition of Flavor Compounds

American consumers are very familiar with yogurt products containing additional flavoring constituents such as fruits. However, the addition of such components to soy yogurt does not answer our dilemma of undesirable off flavors. As a matter of fact the addition of flavoring agents to soy yogurt often results in additional or certainly different off flavors (Gremli 1974). Actually, it is reported that the "beany" flavor itself will either suppress the added flavoring compound or combine with it to give an altered usually undesirable flavor (Gremli 1974). An attempt to mask the "beany" flavor rarely is successful. Another very critical point to make about the addition of flavoring agents is that many interact with and thereby affect the functionality of the soy proteins (Gremli 1974).

Genetically altering soybean seeds

Genetic modification was thought to be one possible solution to the off flavors associated with soybeans. Some research published by Davies and others (1987) examined soybeans that had the genes encoding for lipoxygenases genetically removed. Although the data from this research showed a significant reduction in the intensity of the "beany" flavor there was an increase in other undesirable flavors. In addition to these other flavors, the autoxidation of the soybean oil leading to the undesirable flavor compounds remained a problem for the genetically modified soybeans (Maheshwari and others 1995).

Potential Effect of Bacterial Reductase Activity

As many researchers have published work on the development of the "beany" flavor of soybean products, the industry has set forth many strides to eliminate the undesirable off flavors of these very valuable products. As described in the previous section the spectrum of attempts at this elimination is very broad. Considering the fact that volatile compounds that result from the action of lipoxygenase on fatty acids in soybeans cause the "beany" flavor (Sessa and Rackis 1977; Rackis and others 1979; Sessa 1979), a possible solution might be directed toward removal or reduction of those compounds. It is known that these volatile compounds are classified as aldehydes, ketones, and alcohols with the major contributor being the medium-chain aldehydes – pentanal, hexanal, and heptanal (Arai and others 1970a; Sessa and Rackis 1977; Rackis

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and others 1979; Sessa 1979; Takahashi and others 1979b; Damodaran and Kinsella 1981; Maheshwari and others 1995, 1997).

The concept of certain microorganisms having some form of reducing activity in food systems has recently established a good deal of interest. The mechanism, effect on flavor and odor, and the stability of this reducing action are not yet fully understood. Bhupathiraju and others (1999) examined the use of tetrazolium dye as an indicator of viability and reducing activity of certain anaerobic bacteria. These authors did observe reduction of the dye by numerous microorganisms that were tested. It was the hypothesis of these researchers that the reduction activity was due in part to the dehydrogenase system in the cell or certain electron transport system components. Additional research performed by Lin and Yen (1999) showed that certain organisms were very capable of producing catalases, peroxidases, and other compounds that possessed reducing activity. Saíde (2001) performed several studies on the antioxidative and reducing activity of lactobacilli and streptococci. By measuring this reducing activity using the TTC (2,3,5-Triphenyl Tetrazolium Chloride) method he reported that there were several species of lactobacilli that showed significant reducing activity while being grown in both modified MRS broth and nonfat cow's milk. He also reported that several species of streptococci were capable of significantly reducing the dye when grown in the milk. After examining the research in this area, the possibility of some of these lactic acid bacterial cultures to reduce the volatile compounds that are responsible for the expression of the "beany" flavor in soy products is very realistic. Lee (2001) examined the effect of lactic acid bacteria on n-hexanal, one of the maior contributors to the "beany" flavor. levels in

peanut milk. He reported that *S. thermophilus* was capable of significantly reducing the n-hexanal in 9 hours of incubation.

Probiotics in Soy Yogurt

The advantageous effects on human health received following the ingestion of foods containing probiotics has increasingly became a point of interest for health professionals as well as the health conscious community. Probiotics have been defined as selected viable microorganisms used as dietary supplements having potential for improving health of man or animal following ingestion (Gilliland 2001). It is the intent of this section to examine within a summarized outline the research that supports some of the potential health benefits associated with consumption of probiotics. The advantages described in the following section offer additional support to the incorporation of these cultures into soymilk products.

Currently the dairy industry gives a lot of attention to the availability of probiotics in fermented and nonfermented dairy foods. Consuming these live cultures offers several potential health benefits for the consumer. These potential benefits include control of intestinal infections, improved lactose utilization, control of some cancer, control of some serum cholesterol levels, and stimulation of the immune system.

Control of Intestinal Infections

Several species of lactobacilli and bifidobacteria have been implicated as having a significant role in maintaining the microflora of the intestinal tract (Gilliland and Speck

1977; Watkins and Miller 1983; Gilliland 1989; Perdigon and others 1995). Gibson and McCartney (1998) claimed that probiotics have an effect on the composition and/or the metabolic activity of the intestinal flora. The relationship and interaction between the microflora of the intestine and the host has been reported to result in the healthy survival of the host (Raibaud 1992). He continued to explain that most gastrointestinal disorders arise because these interactions and relationships become unbalanced. As a result of the interest in maintaining a balanced microflora in the gut, the antagonistic effect of probiotic cultures on other intestinal bacteria, specifically enteric pathogens, has been the purpose and goal of many research projects.

Gilliland and Speck (1977) performed a study that looked at the antagonistic action in vitro of several strains of *L. acidophilus* against a variety of pathogens including *S. aureus, C. perfringens, S. typhimurium,* and enteropathogenic *E. coli.* This research concluded that the antagonistic action of *L. acidophilus* that was observed against these pathogens was a result of a combination of factors including the acid production, hydrogen peroxide production, and other inhibitory substances.

Further studies performed using gnotobiotic chicks support the use of *L. acidophilus* as a means of treatment for controlling intestinal infections (Watkins and Miller 1983). The chicks were divided into two groups. The first group of chicks received a prophylactic treatment meaning they were treated with *L. acidophilus* followed by being challenged with a pathogen. The second group received a therapeutic treatment that is they were first challenged with the pathogen and then treated with the *L. acidophilus*. This research showed that both the prophylactic and therapeutic treatment significantly reduced the shedding of both pathogenic *S. typhimurium* and *S. aureus*. In

comparing the two methods of treatment with *L. acidophilus* the prophylactic administration was significantly more effective at reducing the pathogen than was the therapeutic administration.

Perdigon and others (1995) fed mice lactic acid bacteria for 7 days. At the end of this feeding the mice were challenged with *S. typhimurium*. The authors concluded that *L. casei* was significantly effective in the prevention of an infection against this pathogen.

More recently, research involving children was performed to evaluate the effect probiotic consumption had on rotavirus, the most common cause of acute childhood diarrhea (McNaught and MacFie 2001). The data was compiled from 204 undernourished Peruvian children between the ages of six months and twenty-four months. The project was carried out over a 15 month period. The summary of the data compiled was supportive of the beneficial use of probiotics in patients with infantile diarrhea.

Anticarcinogenic Properties

There have been several studies done in support of the ability of particular probiotics to influence the occurrence of certain cancers. While much research is still needed to fully understand the exact mechanisms, published work has shown that the use of probiotics can suppress some tumors in animal studies. Shahani and others (1983) reported that feeding bovine colostrum fermented with cultures of either *L. acidophilus* or *L. bulgaricus* for seven days to animals after receiving tumor implantation had a significant antitumor activity. In comparison to this animal study, Aso and others (1995) performed a project using 125 patients with superficial bladder cancer. These patients

were administered an oral preparation of *L. casei* and confirmed its efficacy for prevention of the reoccurrence of bladder cancers. Although there are numerous published papers examining the involvement of probiotics in suppression of tumor-growth the mechanism by which the probiotics exhibit this ability is thought to be closely linked to modulating the immune system (Perdigon and Alvarez 1992; Aso and others 1995).

Stimulation of the Immune System

With all of the interest in the potential benefits received by consuming probiotics much attention has been focused on the exact mechanisms by which these benefits are exhibited. There has been a great amount of data published over the past several years that suggest that one mechanism probiotics utilize in order to confer so many health benefits is through the stimulation of the immune system of the host (Perdigon and others 1995; Alvarez and others 1998; Matsuzaki 1998; Matsuzaki and Chin 2000). In addition to the specific potential health benefits previously mentioned, Isolauri and others (2000) reported that probiotic consumption by 27 infants having atopic eczema showed significant improvement at the end of the two month evaluation. It was suggested that the results were due to the effect the probiotic consumption had on the infants' immune response. Additional research has addressed the issue of poorly functioning immune systems in the elderly of New Zealand (Gill and others 2001). This project showed that the supplementation of the diets of the 13 elderly individuals with *L. rhamnosus* HN001 did enhance their cellular immunity thereby helping in warding off many infections and diseases.

Improved Lactose Utilization

Lactose maldigestion is a condition affecting a large number of people that has long been a hindrance for consumption of milk products. Some individuals suffering from this situation lack adequate production of the enzyme β-galactosidase in the intestine, which is responsible for the hydrolysis of lactose (Kim and Gilliland 1983; Potter and Hotchkiss 1995). As a goal of providing affected individuals with a suitable substitute, a great deal of research has pointed to consumption of milk products containing probiotics as one possible answer (Kim and Gilliland 1983; Gilliland and Lara 1988). Lactose malabsorbers can experience symptoms such as diarrhea, flatulence, and cramps following consumption of milk. Gilliland (1989) reported that this gastric distress is due from the formation of hydrogen gas by the action of microbes in the large intestine on undigested lactose. In an effort to eliminate the discomfort and inconvenience of lactose intolerance Kim and Gilliland (1983) examined the effect of milk supplemented with *L. acidophilus* on lactose digestion of twenty-nine different lactose malabsorbers. They reported that consumption of milk containing levels of *L. acidophilus* as low as 2.5×10^6 cells/ml did improve lactose utilization.

Influence of Serum Cholesterol Levels

According to the American Heart Association one of the leading causes of death in the United States for both men and women is coronary heart disease. The most important risk factors leading to coronary heart disease has been determined to be elevated serum cholesterol levels (Goldin and Gorbach 1992; Grundy 2000; Stamler and

others 2000). There has been much research done to point to the effect that the microflora of the gut has on the serum cholesterol levels of both the animal and human host. Much attention has been focused on the use of probiotics such as *L. acidophilus* to control levels of serum cholesterol. A group of 25 men were fed large quantities of milk fermented with a strain of lactobacillus for six days, and were then examined expecting to see increases in weight and serum cholesterol (Mann and Spoerry 1974). However, the large consumption of the milk fermented by the lactobacilli actually resulted in a decrease of the men's serum cholesterol. Shortly after this data appeared another study was done involving the examination of *L. acidophilus* on the serum cholesterol levels of infants (Harrison and Peat 1975). The *L. acidophilus* was administered through the incorporation of it into the infant formula. Following the feeding period the infants' serum cholesterol levels were measured, and the infants receiving the *L. acidophilus* had significantly lower levels of serum cholesterol than did the control group that did not receive any lactobacilli.

Further support of these findings was presented in a feeding trial using rat models that showed significant decrease in serum cholesterol levels of rats receiving milk fermented with *L. acidophilus* (Grunewald 1982).

Considering the findings just presented, much attention has turned to the mechanism by which these cultures were capable of imparting this effect on host. A study done using selected strains of *L. acidophilus* obtained from pig fecal samples revealed that although the strains grew well in the presence of bile and assimilated cholesterol from growth medium there was considerable variation among the isolates (Gilliland and others 1985; Buck and Gilliland 1994). The data from this study showed

that the cells of *L. acidophilus* when grown in the presence of cholesterol were actually removing the cholesterol from the medium. The feeding trial done in close relation to this study confirmed a significant reduction of serum cholesterol in the pig models, and also confirmed the variation seen through the use of the different isolates. A similar study using pigs as the model also examined the mechanism by which the strains of *L. acidophilus* lowered the serum cholesterol levels of the host. This study looked at the influence that lactobacilli had on total cholesterol, high density and low density cholesterol, and total bile acids. This study reported that the ability of *L. acidophilus* to lower serum cholesterol concentrations probably involved both assimilation of the cholesterol as well as the deconjugation of bile acids. Further support of this finding was reported by Brashears and others (1998). The bile salt deconjugation and cholesterol removal from media of *L. acidophilus* and *L. casei* were compared. The results were that the *L. acidophilus* assimilated the cholesterol where as the *L. casei* removed the cholesterol through bile salt deconjugation. This project showed the importance that in selecting a probiotic strain to administer this potential effect after consumption both maximum bile salt deconjugation and cholesterol assimilation must be taken into account.

A feeding trial involving hypercholesterolemic humans placed a significant value on the importance of *L. acidophilus* L1 and its effect on serum cholesterol level. Anderson and Gilliland (1999) reported that the above experiment resulted in a three to four percent reduction of serum cholesterol in hypercholesterolemic individuals. The authors concluded that a 6 to 10 % reduction in the risk for experiencing coronary heart disease was possible through the regular consumption of a "cholesterol-reducing *L. acidophilus*".

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CHAPTER III

REDUCTION OF LEVELS OF VOLATILE COMPONENTS ASSOCIATED WITH THE "BEANY" FLAVOR IN SOYMILK BY LACTOBACILLI AND STREPTOCOCCI

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ABSTRACT

Soymilk samples were analyzed for volatile compounds documented as being responsible for the "beany" off flavor. Methanol, acetaldehyde, ethanol, and hexanal were the four major volatiles detected. The concentrations of these compounds were measured in soymilk samples before and after being fermented with eight different cultures of lactic acid bacteria. While all eight of the cultures completely eliminated the hexanal in the cultured soymilk, there were considerable variations in the effects of the cultures on the other three compounds. All eight cultures caused significant reduction in levels of methanol. *Streptococcus thermophilus* OSU-2 was the only culture that significantly lowered the concentration of ethanol in the soymilk. All cultures except *Lactobacillus acidophilus* C19 and *Lactobacillus casei* E5 significantly lowered the level of acetaldehyde. Comparison of all of the cultures tested indicates that *Lactobacillus acidophilus* L1 offers the best potential for producing fermented soymilk with an improved volatile profile. It completely eliminated the acetaldehyde and hexanal peaks plus caused a significant reduction in the methanol. Although not significant, it also caused reduction in the concentration of ethanol.

INTRODUCTION

Soybeans have long been used in Asian countries for production of many traditional foods such as tofu and miso. Soybeans have recently become one of the most economical sources of food protein. In addition, their consumption offers potential health and nutritional benefits such as reduction in levels of serum cholesterol and saturated fats (Lee and others 1990; Manzoni and others 1998; Friendman and Brandon 2001).

A segment of the food industry has looked to soymilk as a possible substitute for the traditional dairy products. The hindrance however, is that the soymilk has a "beany" off-flavor that is very objectionable to the American consumer. The presence in soymilk of compounds such as aldehydes, ketones, and alcohols has been implicated as the source for the "beany" flavor (Rackis and others 1979; Takahashi and others 1979b; Damodaran and Kinsella 1981). Methods proposed for the removal or elimination of the off-flavors include heat treatments, acid treatments, enzymatic treatments, supercritical carbon dioxide extraction, genetic alteration of soybeans, and addition of flavor compounds (Che Man and others 1989; Srinivas and others 1992; Maheshwari and others 1997). While each of these processes has some potential for removal or masking of the beany flavor, they each have their own negative aspects ranging from interfering with the protein functionality to additional negative sensory attributes.

One possible solution in overcoming the off flavor problem of soy products, such as soy yogurt, is to use probiotic lactic acid bacteria exhibiting reductase activity during the fermentation. Although the mechanism is not fully understood, research supports the possibility that certain bacterial strains could reduce the volatile compounds that are

responsible for the "beany" flavor (Lin and Yen 1999; Saíde 2001; Lee 2001). In addition to the flavor improvement of the soy products, the consumer would receive potentially advantageous effects on human health as a result of ingestion of foods containing probiotics.

The objective of this study was to determine if fermenting soymilk with lactic acid cultures would reduce or eliminate the concentrations of volatile components associated with the "beany" flavor thereby resulting in a "yogurt-like" soy product that might be more acceptable to the consumer.

MATERIALS AND METHODS

Production of Soymilk

Choska soybeans were provided by Mr. Kent Keim, a research scientist in the Department of Plant and Soil Sciences at Oklahoma State University, for the production of fresh soymilk. Fifty grams of soybeans were soaked in 150 mL of deionized water at room temperature for ten hours. Following the soaking, the beans were drained through two layers of cheesecloth. The soybeans were then washed with ~300 mL volumes of deionized water three consecutive times. After each washing the soybeans were drained through two layers of cheesecloth. The soaked and washed soybeans were transferred into a blender cup containing 300 mL of cold deionized water, and blended for four minutes on the high speed using a laboratory blender (Waring Commercial, Model 31BL91, New Hartford Connecticut). The blender cup was then emptied into a porcelain Büchner funnel (12.2 cm diameter) with a fixed perforated plate (Fisher Scientific, Pittsburgh Pennsylvania) lined with three layers of cheesecloth, and connected to a one-liter vacuum flask. The flask was connected to the building vacuum system to aid in filtration. The filtered soymilk was adjusted to a final volume of 300 mL using deionized water. The soymilk was homogenized by using a laboratory homogenizer (Niro Soavi S.p.A., Model = PANDA; Parma, Italy) with the pressure settings at 75 bars for the second stage and 200 bars for the first stage. After homogenization, the soymilk was dispensed in 20 mL aliquots into screw cap test tubes, and autoclaved at 121°C for 15 minutes.

Maintenance of Cultures

All cultures used in this experiment were obtained from the stock culture collection of the Food Microbiology Laboratory at Oklahoma State University. The identity of the each culture was confirmed by testing fermentation patterns using API 50 CH kits (Bio Mérieux, Bruxelles Belgium) and by checking their catalase and Gram stain reaction. Cultures studied included two strains of *Lactobacillus acidophilus* (L1 and C19), two of *Lactobacillus casei* (E5 and E10), three of *Streptococcus thermophilus* (143, OSU-1, and OSU-2), and one of *Lactobacillus delbrueckii* ssp. *lactis* (RM2-5).

All of the cultures studied were maintained by weekly subculturing using 1% inoculum into lactobacilli MRS broth (Difco Laboratories, Detroit Michigan) followed by incubation for 18 hours at 37°C. Between subcultures, they were stored at 5°C. In addition, stock cultures of each were maintained by monthly subculturing in MRS agar stabs.

Immediately prior to the beginning of each experiment each culture was subcultured three times in the soymilk using 5% inocula and 12-hour incubation at 37°C.

Production of Fermented Soymilk

The tubes (20 mL) of soymilk were inoculated using 5% inocula with one of each of the strains of *Lactobacillus acidophilus* (L1 and C19), of *Lactobacillus casei* (E5 and E10), of *Streptococcus thermophilus* (143, OSU-1, and OSU-2), or *Lactobacillus delbrueckii* ssp. *lactis* (RM2-5). An uninoculated tube of soymilk was included as a control sample. The soymilk samples were placed in the 37°C incubator for 12 hours to allow for fermentation into a yogurt-like product.

Growth in the soymilk of each culture was measured in preliminary experiments to determine the hour of maximum growth. Samples were plated for bacterial counts every two hours for an 18-hour period. This data was used to select the 12-hour incubation period mentioned above that was used throughout this study.

Preparation of Samples for Head Space Analysis

The samples of fermented soymilk were removed from the 37°C incubator and placed in an ice-water bath for thirty minutes prior to analysis in order to stop growth. During this time, the internal standard (IS) solution was prepared by mixing 2 µL of 3-heptanone (Sigma, St. Louis Missouri) with 10 mL of n-hexadecane (Sigma, St. Louis Missouri) in a sterile screw cap tube. This internal standard solution was prepared fresh daily. For each sample that was to be analyzed, a 20 mL headspace vial (20mm Aluminum-Seal headspace vial, Kimble Glass Inc, Vineland New Jersey) was prepared containing one gram of sodium sulfate, 5 µL of the internal standard solution, and 2 grams of the sample. This was done without any mixing of the samples and as quickly as possible to reduce the loss of volatile compounds. As soon as the transfer of the 2 grams of sample was completed the lids (20mm Aluminum seal PTFE/Butyl pressure release, Kimble Glass Inc, Vineland New Jersey) were crimped onto the vials using manual a crimper (Fisher Scientific, Pittsburgh Pennsylvania), and the samples were mixed using a vortex mixer (Fisher Scientific, Pittsburgh Pennsylvania). The test tubes containing the samples were placed back in ice water for additional analyses.

Instrumentation and Operating Conditions

Headspace Analysis

A Hewlett-Packard (Palo Alto, California) model HP 7694 headspace autosampler was used to quantitate the headspace volatiles of soymilk. Samples were equilibrated for 60 minutes at 80°C. The temperature of the transfer line was 95°C and the sample injection needle temperature was 105°C. Additional conditions for the headspace autosampler included 4.8 seconds for pressurization, equilibration, and filling; and a 2-minute sample injection time. Conditions for headspace analysis were as described by Alonso (1999). See appendix D for a more detailed outline of all headspace parameters.

Gas Chromatographic Analysis

A Hewlett Packard (Palo Alto, California) model HP 6890 gas chromatograph equipped with a flame ionization detector (FID) was used to analyze the headspace samples of the soymilk. The column was a 30-meter long CP-Wax fused silica capillary column with 0.25 millimeter inside diameter and 0.25-micron film (Varian Inc., Palo Alto California). The column injector temperature and FID temperature were set at 210°C. Column type, injector temperature, and FID temperature were as described by Maheshwari and others (1995). The column temperature was maintained at 40°C for 5 minutes then increased as follows: 1°C per minute until reaching 42°C at which it was held for 1 minute, increased 7°C per minute until reaching 70°C at which it was held for 5 minutes, increased 10°C per minute until reaching 200°C at which it was held for 5 minutes. The temperature program was as reported by Alonso (1999). However, adjustments to some of the temperatures as well as the ramps and holding times were necessary due to the temperature of the room in which our GC was located. The flow

rate of the carrier gas (He) was 47 mL / min. Nitrogen, used as a make-up gas, was delivered at a rate of 30 mL / min. The air and hydrogen flow rates in the FID were set at 300 and 30 mL / min, respectively. The flow rates were as described by Maheshwari and others (1995). See appendix E for a more detailed outline of all GC parameters.

Concentration of Volatiles

Quantification of the concentration of the volatile components found in the soymilk samples was calculated and adjusted to a per 2 gram sample weight basis for comparison.

$$\text{Concentration of volatile} = \left[\frac{\text{Concentration of internal standard}}{\text{Peak area of internal standard}} \right] \times \text{Peak area of volatile}$$

Identification of the Volatile Components found in Soymilk

Soymilk samples were analyzed using a Hewlett Packard (Palo Alto, California) model HP 6890 gas chromatograph coupled with an Agilent (Palo Alto, California) Mass Selective (MS) Detector 5973. The conditions for the headspace analysis were performed using the exact same conditions as described in a previous section. In switching from the FID to the MS a majority of the GC parameters remained the same as well. However, due to the vacuum required to properly operate the MS, the GC flow rates had to be increased to maintain a positive pressure on the MS. The MS was operated in the scan mode with the m/z set at 10-400, a threshold of 150, and a sampling rate of 3.71 scans per second. Ultrapure helium, passed through a moisture, oxygen, and hydrocarbon trap, was used as the carrier gas.

Changing the flow rate to compensate for the vacuum used with the MS detector altered the retention times of the peaks recovered from the soymilk samples. In order to confirm that the peaks recovered with the MS detector were the same peaks that were detected with the FID, additional soymilk samples were assayed by the headspace and GC analysis using the FID with the higher flow rate settings required for the parameters of the MS detector. All other conditions remained the same. Ratios of the peak areas of the four major peaks detected using both flow rates were used to confirm peak identity. See appendix F for a more detailed outline of all parameters used.

Enumeration of Bacteria

The total numbers of *L. acidophilus*, *L. casei*, *S. thermophilus*, and *L. delbrueckii* ssp. *lactis* was determined by preparing dilutions according to the methods described in the Compendium of Methods for the Microbiological Examination of Foods (Vanderzant and Splittsloesser 1990). Appropriate dilutions were plated by the pour plate method with overlay using lactobacilli MRS agar. The plates were placed in plastic bags, taped closed, and incubated at 37°C for 48 hours. All colonies visible with a Quebec colony counter (Darkfield-Model 3325, Buffalo New York) were counted. Results were expressed as the number of colony forming units (cfu) per gram of sample.

Following the plating of each sample, the pH was measured by using an Accumet dual channel pH/ion meter (Fisher Scientific AR25, Pittsburg Pennsylvania) and recorded.

Statistical Analysis

Statistical analysis on this data was conducted such that the design was a split plot in a randomized complete block. Each of the replications was a block, the bacterial cultures were the main unit treatment, and each of the four major volatile compounds studied were considered as the subunit treatments. Statements of the SAS PROC MIXED procedure with the LSMEANS and Dunnett's test were used to compare the means of all culture treatments to that of the control for significant differences at the 5% level of confidence (SAS, 1985). The comparisons were made within each data set corresponding to the four individual volatile compounds that were examined.

RESULTS

Identification of Volatile Components found in Soymilk

Through a series of comparisons of headspace chromatograms from control samples of soymilk and chromatograms from the gas chromatograph connected to the mass spectrometer, four major peaks were identified. This procedure led to identification of the peak having a retention time of 4.61 minutes as methanol (Figure 1). Of the four peaks of interest this particular peak carried the least amount of confidence. The main reason for the uncertainty with this compound was due to the fact that the peak when detected using the mass spectrometer was contained within a very large peak for air. The second peak of interest found in the soymilk control samples detected at a retention time of 5.29 minutes was identified as acetaldehyde (Figure 1). Next, ethanol was identified with a high degree of confidence as the compound responsible for the peak that was detected at 9.51 minutes (Figure 1). The peak that was recovered from the soymilk controls at a retention time of 15.01 minutes was hexanal (Figure 1).

Influence of Fermentation of Soymilk on Concentration of Volatile Components

The influence of fermenting soymilk made from raw Choska soybeans with different bacterial strains on the concentrations of volatile components was evaluated. The chromatograms for all samples were assessed for the overall volatile component profiles. The concentration of each component found in the cultured soymilk samples was compared to the concentration of that particular component in the control soymilk

sample (Table 1). In addition to the component concentrations, Table 1 also shows pH values and plate counts of all samples.

Soymilk samples that were fermented with *S. thermophilus* 143, *S. thermophilus* OSU-1, *S. thermophilus* OSU-2, *L. acidophilus* L1, *L. acidophilus* C19, *L. casei* E5, *L. casei* E10, or *L. delbrueckii* ssp. *lactis* RM2-5 all had significantly ($P<0.05$) less methanol than did the control sample of soymilk. Most cultures had similar effects on acetaldehyde as they did on methanol. However, *L. acidophilus* C19 and *L. casei* E5 had no significant ($P<0.05$) effect on the level of acetaldehyde. Assessment of the ethanol concentration levels revealed very different results. The two samples prepared with *L. casei* E5 and *L. delbrueckii* ssp. *lactis* RM2-5 had, although not significant, higher concentrations of ethanol than found in the control soymilk samples. In contrast, the fermented soymilk prepared with *S. thermophilus* OSU-2 had significantly ($P<0.05$) less ethanol than did the soymilk control samples. All of the other cultured samples had concentration levels less but not significantly different than that of the soymilk control samples. All cultures completely eliminated hexanal from the soymilk during fermentation.

As observed in Table 1, there was substantial variation in the culture's ability to lower the pH of the soymilk during fermentation. Although all samples were below 6.38, which was the pH of the control soymilk, the two strains of *L. casei* had higher pH values than the other fermented samples with *L. casei* E10 having the highest at a pH of 5.02. On the other hand, *L. delbrueckii* ssp *lactis* RM2-5 created the lowest pH (3.91) of the fermented soymilks during growth.

The plate counts are also presented in Table 1 for all samples tested. All cultures grew well in the soymilk; however there still exists some variation in the bacterial counts. *L. acidophilus* L1 had the highest plate counts after the 12-hour incubation with 8.85 $\log_{10}$ CFU/mL. *S. thermophilus* OSU-2 was the sample that grew at the lowest levels in the soymilk with a plate count of 7.91 $\log_{10}$ CFU/mL.

DISCUSSION

There are several potential beneficial effects on health and nutrition possible through the consumption of soy and soy derived products that have been reported (Lee and others 1990; Manzoni and others 1998; Friendman and Brandon 2001). However, a majority of these products are not consumed in the United States due to undesirable "beany" flavor associated with them (Wolf 1975; Cheng and others 1990; Wang and others 1998).

The present study investigated the effects fermenting soymilk made from Choska soybeans on the concentrations of volatile components thought to be responsible for the off-flavor (beany) associated with soy products. Lactic acid bacteria exhibit variable reducing activity during growth (Lin and Yen 1999; and Saíde 2001). One possible end effect using selected cultures to ferment soymilk would be the reduction of the volatile components leading to a more acceptable flavor.

Initial samples of uninoculated soymilk were assayed as controls to determine the presence of volatile components. The chromatograms revealed four major peaks, one for each separate volatile component. The volatile compounds responsible for the off-flavor in soymilk have been classified as aldehydes, ketones, and alcohols with the major contributor being the medium-chain aldehydes – pentanal, hexanal, and heptanal (Rackis and others 1979; Takahashi and others 1979b; Damodaran and Kinsella 1981). Thus, the four major peaks found in the soymilk samples of the present study identified as methanol, acetaldehyde, ethanol, and hexanal are all of importance with regard to the flavor profile of soymilk.

Experiments were done to determine the influence of fermenting the soymilk with lactic acid bacteria on the concentration of each of these four volatile components. The concentration of each component varied among batches of soymilk as well among the samples of fermented soymilk. The greatest variation in the observations was seen in the concentrations of ethanol. Possible explanations for these variations include the volatility of the components of interest. Since the soymilk was autoclaved, there is a likelihood that some of the volatiles were lost during heating. Such changes in volatile concentration during autoclaving may not have been consistent for every batch of soymilk made. Variation among the influence of individual cultures was not surprising since for this group of bacteria variation among strains of a specific species for relative levels of metabolic activity is common. There is also the possibility that the reductase activity from a specified culture may vary depending on the type of compound present. For instance, the alcohol compounds may be more resistant to reduction than the aldehyde compounds. Thus selection of a specific strain for this reductase activity targeting specific compounds may be extremely important.

When looking at all bacterial strains studied *L. acidophilus* L1 appears to have had the greatest overall effect. *L. acidophilus* L1 completely eliminated the hexanal and the acetaldehyde, and significantly reduced the level of methanol. There was no significant effect on the concentration of ethanol by fermenting with *L. acidophilus* L1, however the average concentration was still lower than the level recorded for the control. Since aldehydes are a major contributing factor in off-flavors and alcohols comprise a much lower flavor threshold, the effect of *L. acidophilus* L1 seems to hold a substantial

possibility in producing a yogurt-like product from soymilk containing significantly less "beany" flavor.

More research is needed to investigate the effect that selected cultures have on the levels of the volatile compounds in relation to the perceived flavor of a yogurt-like product made from soymilk. As an example, samples of this yogurt-like product made from soymilk should be prepared using cultures or combinations of cultures shown to have greatest impact on the volatiles to determine through sensory analysis if flavor of the products is improved.

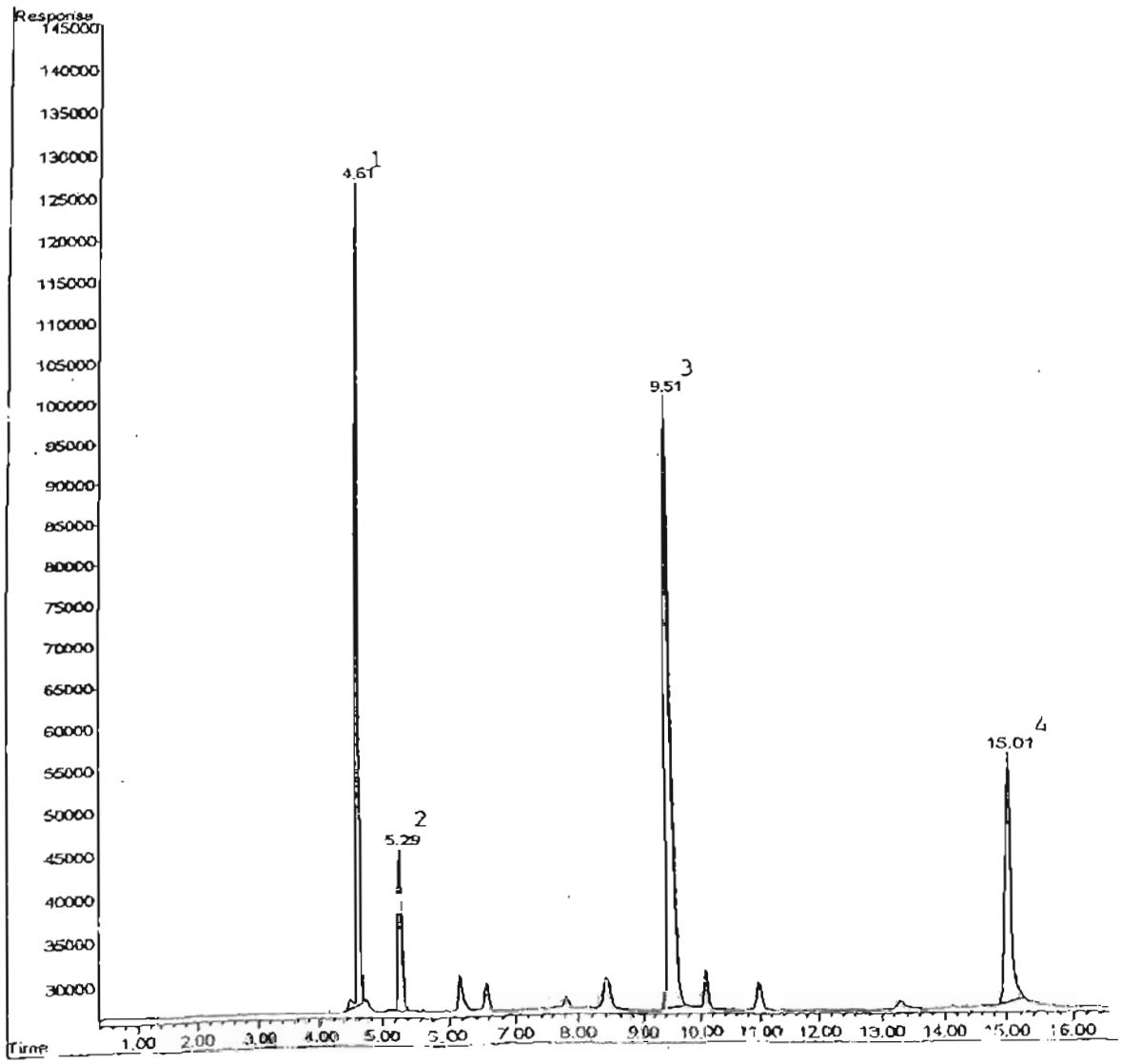

Figure 1. Capillary GC chromatogram of the headspace volatiles of a soymilk control sample. Peaks: 1, methanol; 2, acetaldehyde; 3, ethanol; 4, hexanal. Internal standard peak was detected at a retention time of 30.67 minutes (not shown).

Table 1. The growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	log_{10} CFU/g[2]
Control	6.38	21.0 (8.2)[3A]	12.9 (6.0) [A]	57.0 (36.5) [A]	17.9 (11.3) [A]	<0.0
S. thermophilus 143	4.52	2.0 (2.9) [B]	5.7 (5.2) [B]	39.8 (23.2) [A]	0.0 (0.0) [B]	8.48
S. thermophilus OSU-1	4.35	3.5 (4.0) [B]	6.4 (5.6) [B]	46.4 (28.0) [A]	0.0 (0.0) [B]	8.18
S. thermophilus OSU-2	4.43	1.4 (2.6) [B]	0.9 (2.4) [B]	31.1 (19.4) [B]	0.0 (0.0) [B]	7.91
L. acidophilus L1	4.13	1.8 (2.6) [B]	0.0 (0.0) [B]	41.8 (26.2) [A]	0.0 (0.0) [B]	8.85
L. acidophilus C19	4.31	4.2 (10.2) [B]	9.4 (11.1) [A]	54.2 (42.3) [A]	0.0 (0.0) [B]	8.61
L. casei E5	4.70	3.7 (5.2) [B]	7.3 (5.6) [A]	59.2 (23.7) [A]	0.0 (0.0) [B]	8.67
L. casei E10	5.02	3.0 (4.4) [B]	5.0 (5.5) [B]	51.5 (28.5) [A]	0.0 (0.0) [B]	8.57
L. del. ssp *lactis* RM2-5[4]	3.91	3.7 (4.4) [B]	1.2 (2.8) [B]	63.7 (40.7) [A]	0.0 (0.0) [B]	8.18

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.
[2]All measurements reported in the table are a mean from 8 replicate experiments; CFU = colony forming units.
[3]Values found in the parentheses represent the standard deviation associated with that value over the 8 replications.
[4]*Lactobacillus delbrueckii* ssp *lactis* RM2-5.
[AB]Values with different superscript letters within a column differ significantly ($P<0.05$).

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APPENDIX A

PROCEDURES FOR THE PRODUCTION OF HOMOGENIZED SOY MILK

Homogenization of Soymilk

The soymilk was homogenized by using a laboratory homogenizer (Niro Soavi S.p.A., Model = PANDA; Parma, Italy). The feedbox was filled with 500 mL of 60°C water in order to begin homogenization. The homogenizing pressure on the pressure gauge was zero and the hand wheels were completely loose before the power was turned on. Once running, the water was observed flowing constantly out of the outlet pipe. The second stagehand wheel was then turned clockwise until the pressure gauge read approximately 75 bars. The first stagehand wheel was then rotated clockwise until the pressure gauge read approximately 300 bars. The water was followed by 500 mL of 1% sodium hydroxide tempered to 60°C. Next, was 500 mL of 0.1% nitric acid tempered to 60°C, and last was 500 mL of 60°C water. Following the last water wash, the soymilk was run through, and collected into a separate container. As the soymilk neared the bottom of the feedbox the washing steps were repeated. Once the final water wash neared the end of the bottom of the feedbox the pressure was released first by completely loosening the first stagehand wheel, and then the second stagehand wheel. Once the pressure gauge read zero the power was shut off.

APPENDIX B

HEADSPACE PARAMETERS

MATRIX	
Matrix:	Water
Matrix boiling point:	100°C
TEMPERATURE	
Sample Oven:	80°C
Sample Valve:	95°C
Transfer Line:	105°C
TIME	
GC Cycle:	60.2 minutes
Sample Equilibration:	60.0 minutes
Vial Pressurization:	0.08 minutes
Loop Fill:	0.08 minutes
Loop Equilibration:	0.08 minutes
Sample Injection:	2.00 minutes
Oven Stabilization:	1.00 minute
SHAKING	
Agitation:	None
MODE	
Extractions:	1
Puncture Mode:	Single

APPENDIX C

GAS CHROMATOGRAPHY WITH FID PARAMETERS

OVEN

Initial Temperature:	40°C
Initial Time:	5.00 minutes

Ramps:	#	Rate	Final Temperature	Final Time
	1	1.00 minute	42°C	1.00 minute
	2	7.00 minutes	70°C	5.00 minutes
	3	10.00 minutes	200°C	5.00 minutes
	4	0.0 (Off)		

Post Temperature:	0°C
Post Time:	0.00 minute
Run Time:	35.00 minutes

FRONT INLET (SPLIT / SPLITLESS)

Mode:	Split
Initial Temperature:	210°C (ON)
Pressure:	5.90 psi (ON)
Split Ratio:	100:1
Split Flow:	43.9 mL/minute
Total Flow:	47.0 mL/minute
Gas Saver:	Off
Gas Type:	Helium

COLUMN 1

Capillary Column

Model Number:	Varian CP 8713 CP Wax 52CB
Maxium Temperature:	250°C
Nominal Length:	30.0 meters
Nominal Diameter:	250.0 micrometers
Nominal Film Thickness:	0.25 micrometers
Mode:	constant pressure
Pressure:	5.90 psi
Nominal Initial Flow:	0.4 mL/minute
Average Velocity:	13 cm/second
Inlet:	Front Inlet
Outlet:	Front Detector
Outlet Pressure:	ambient

AUX PRESSURE 3

Gas Type:	Helium
Initial Pressure:	14.00 psi
Initial Time:	0.00 minute

FRONT DETECTOR (FID)

Temperature:	210°C (ON)
Hydrogen Flow:	30.0 mL/minute (ON)

Air Flow:	300.0 mL/minute (ON)
Mode:	Constant makeup flow
Makeup Flow:	30.0 mL/minute (ON)
Makeup Gas Type:	Nitrogen
Flame:	On
Electrometer:	On
Lit Offset:	2.0

SIGNAL 1	
Data Rate:	20 Hz
Type:	front detector
Save Data:	On
Zero:	0.0 (Off)
Range:	0
Fast Peaks:	Off
Attentuation:	0

APPENDIX D

GAS CHROMATOGRAPHY WITH MS DETECTOR PARAMETERS

OVEN

Initial Temperature:	40°C
Initial Time:	5.00 minutes

Ramps:	#	Rate	Final Temperature	Final Time
	1	2.00 °C/minute	42°C	1.00 minute
	2	4.00 °C/minute	70°C	5.00 minutes
	3	13.00 °C/minute	200°C	5.00 minutes
	4	0.0 (Off)		

Post Temperature:	0°C
Post Time:	0.00 minute
Run Time:	34.00 minutes
Maximum Temperature:	250°C
Equilibration Time:	0.50 minute

FRONT INLET (SPLIT / SPLITLESS)

Mode:	Split
Initial Temperature:	210°C (ON)
Pressure:	5.89 psi (ON)
Split Ratio:	100:1
Split Flow:	89.5 mL/minute
Total Flow:	93.3 mL/minute
Gas Saver:	Off
Gas Type:	Helium

BACK INLET (SPLIT / SPLITLESS)

Mode:	Split
Initial Temperature:	50°C (Off)
Pressure:	0.00 psi (Off)
Total Flow:	45.0 mL/minute
Gas Saver:	Off
Gas Type:	Helium

COLUMN 1

Capillary Column

Model Number:	Varian CP 8713 CP Wax 52CB
Maxium Temperature:	250°C
Nominal Length:	30.0 meters
Nominal Diameter:	250.0 micrometers
Nominal Film Thickness:	0.25 micrometers
Mode:	constant flow
Pressure:	5.89 psi
Nominal Initial Flow:	0.9 mL/minute
Average Velocity:	34 cm/second
Inlet:	Front Inlet

Outlet:	MSD
Outlet Pressure:	vacuum

FRONT DETECTOR (FID)

Temperature:	250°C (Off)
Hydrogen Flow:	40.0 mL/minute (Off)
Air Flow:	450.0 mL/minute (Off)
Mode:	Constant makeup flow
Makeup Flow:	45.0 mL/minute (Off)
Makeup Gas Type:	Nitrogen
Flame:	Off
Electrometer:	Off
Lit Offset:	2.0

BACK DETECTOR (uECD)

Temperature:	250°C (Off)
Mode:	Constant makeup flow
Makeup Flow:	60.0 mL/minute (Off)
Makeup Gas Type:	Nitrogen
Electrometer:	Off

SIGNAL 1

Data Rate:	20 Hz
Type:	test plot
Save Data:	On
Zero:	0.0 (Off)
Range:	0
Fast Peaks:	Off
Attentuation:	0

SIGNAL 2

Data Rate:	20 Hz
Type:	test plot
Save Data:	Off
Zero:	0.0 (Off)
Range:	0
Fast Peaks:	Off
Attentuation:	0

THERMAL AUX 1

Use:	MSD Transfer Line Heater
Initial Temperature:	280°C (On)
Initial Time:	0.00 minute

AUX PRESSURE 3

Gas Type:	Helium
Initial Pressure:	0.00 psi (Off)

AUX PRESSURE 4

Gas Type:	Helium
Initial Pressure:	0.00 psi (Off)

AUX PRESSURE 5

Gas Type:	Helium
Initial Pressure:	0.00 psi (Off)

MS ACQUISTION PARAMETERS

GENERAL INFORMATION

Tune File:	ATUNE.U
Acquistion Mode:	Scan

MS INFORMATION

Solvent Delay:	0.00 minute
EM Absolute:	False
EM Offset:	0
Resulting EM Voltage:	1388.2

SCAN PARAMETERS

Low Mass:	10.0
High Mass:	400.0
Threshold	150
Sample #:	2 A/D samples 4
Plot 2 Low Mass:	30.0
Plot 2 High Mass:	200.0

MS ZONES

MS Quad:	150°C maximum 200°C
MS Source:	230°C maximum 250°C

APPENDIX E

IDENTITY OF CULTURES EXAMINED IN THE STUDY

API 50 CH Kit

CHL Medium

• Polypetone	10.00 g
• Yeast extract	5.00 g
• Tween 80	1.00 mL
• Dipotassium phosphate	2.00 g
• Sodium acetate $3H_2O$	5.00 g
• Diammonium citrate	2.00 g
• Magnesium sulfate $7H_2O$	0.20 g (200mg)
• Manganese sulfate $4H_2O$	0.05 g (50mg)
• Bromcresol purple	0.17 g
• Distilled water	1000 mL

Procedures

1. Culture the selected strains three times in MRS broth (medium). In order to obtain a stabilization of the biochemical traits.
2. Incubate at 37°C for 24 hours.
3. Transfer the culture into a sterilized centrifuge tube.
4. Centrifuge the culture for 10 minutes at 15,191 x g. (4° C- 9°C)
5. Remove the supernatant.
6. Wash the cells (pellet) with 10mL of CHL broth (the amount of wash solution depends on the size of the pellet).
7. Repeat steps 5 and 6 twice more.
8. Remove the supernatant and add 10 mL of CHL broth into the pellet and vortex.
9. Using a sterilized pipette, distribute the bacterial suspension into each of the compartments of the API 50CH strips.
10. Incubate the strips at 37°C for 24 hours under anaerobic conditions in Gas Pak chamber.
11. Read the reactions as a positive or negative result, and incubate for another 24 hours under the same condition to confirm the 24 hour readings.

Table 2. Confirmation of identity of cultures of *Lactobacillus acidophilus*

Test[1]	La[2]	La-L1	La-C19
Amygdalin	+	+	+
Arabinose	-	-	-
Cellobiose	+	+	+
Esculin	+	+	+
Fructose	+	+	+
Galactose	+	+	+
Gluconate	-	-	-
Glucose	+	+	+
Lactose	+	+	+
Maltose	+	+	+
Mannitol	-	-	-
Mannose	+	+	+
Melezitose	-	-	-
Melibiose	+/-	+	+
Raffinose	+/-	+	+
Rhamnose	-	-	-
Ribose	-	-	-
Salicin	+	+	+
Sorbitol	-	-	-
Sucrose	+	+	+
Trehalose	+/-	+	+
Xylose	-	-	-

[1] All cultures were Gram + rods; catalase negative; and did not grow at 15°C
[2] La=*Lactobacillus acidophilus*; reactions as listed in the 9th Edition of Bergey's Manual of Systematic Bacteriology.

Table 3. Confirmation of identity of cultures of *Lactobacillus casei*

Test[1]	Lc[2]	Lc-E5	Lc-E10
Amygdalin	+	+	+
Arabinose	-	-	-
Cellobiose	+	+	+
Esculin	+	+	+
Fructose	+	+	+
Galactose	+	+	+
Gluconate	+	-	-
Glucose	+	+	+
Lactose	+/-	-	-
Maltose	+	+	+
Mannitol	+	+	+
Mannose	+	+	+
Melezitose	+	+	+
Melibiose	-	-	-
Raffinose	-	-	-
Rhamnose	-	-	-
Ribose	+	+	+
Salicin	+	+	+
Sorbitol	+	+	+
Sucrose	+	+	+
Trehalose	+	+	+
Xylose	-	-	-

[1] All cultures were Gram + rods; catalase negative; and did not grow at 15°C
[2] Lc=*Lactobacillus casei*; reactions as listed in the 9th Edition of Bergey's Manual of Systematic Bacteriology.

Table 4. Confirmation of identity of cultures of *Lactobacillus delbrueckii* subsp. *lactis*

Test[1]	Ld[2]	Ld-RM2-5
Amygdalin	+	-
Arabinose	-	-
Cellobiose	+/-	-
Esculin	+	+
Fructose	+	+
Galactose	+/-	+
Gluconate	-	-
Glucose	+	+
Lactose	+	+
Maltose	+	+
Mannitol	-	-
Mannose	+	+
Melezitose	-	-
Melibiose	-	+
Raffinose	-	+
Rhamnose	-	-
Ribose	-	-
Salicin	+	-
Sorbitol	-	-
Sucrose	+	+
Trehalose	+	+
Xylose	-	-

[1] All cultures were Gram + rods; catalase negative; and did not grow at 15°C
[2] Ld=*Lactobacillus delbrueckii* subsp. *lactis*; reactions as listed in the 9th Edition of Bergey's Manual of Systematic Bacteriology.

Table 5. Confirmation of identity of cultures of *Streptococcus thermophilus*

Test[1]	St[2]	St-1	St-2	St-143
Arabinose	-	-	-	-
Fructose	+	-	-	+
Galactose	+/-	-	-	-
Glucose	+/-	+	+	+
Glycerol	-	-	-	-
Inuline	-	-	-	-
Lactose	+	+	+	+
Maltose	+/-	-	-	-
Mannitol	-	-	-	-
Mannose	+	-	-	-
Rhamnose	-	-	-	-
Salicin	-	-	-	-
Sorbitol	-	-	-	-
Sucrose	+	+	+	+
Xylose	-	-	-	-

[1] All cultures were Gram + cocci in pairs and chains; catalase negative
[2] St=*Streptococcus thermophilus*; reactions as listed in the 9th Edition of Bergey's Manual of Systematic Bacteriology.

APPENDIX F

COLLECTION OF RAW DATA FROM THE REDUCTION OF THE BEANY FLAVOR OF SOY YOGURT EXPERIMENT

Table 6. Collection of raw data from replication #1 showing the growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	$\log_{10}$ CFU/g[2]
Control	6.29	7.6	5.9	20.8	10.0	<0.0
S. thermophilus 143	4.44	6.3	7.9	34.7	0.0	8.48
S. thermophilus OSU-1	4.27	6.4	10.3	52.8	0.0	8.18
S. thermophilus OSU-2	4.35	6.1	6.9	40.3	0.0	7.91
L. acidophilus L1	4.08	3.3	0.0	29.9	0.0	8.85
L. acidophilus C19	4.20	0.0	0.0	0.0	0.0	8.61
L. casei E5	4.69	7.8	11.6	65.6	0.0	8.67
L. casei E10	4.73	0.0	0.0	15.5	0.0	8.57
L. del. ssp *lactis* RM2-5[3]	3.88	5.6	0.0	33.2	0.0	8.18

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.
[2]CFU = colony forming units.
[3]*Lactobacillus delbrueckii* ssp *lactis* RM2-5

Table 7. Collection of raw data from replication #2 showing the growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	log_{10} CFU/ g[2]
Control	6.38	20.6	8.1	22.5	13.8	<0.0
S. thermophilus 143	4.50	5.9	5.1	22.3	0.0	8.58
S. thermophilus OSU-1	4.29	6.0	8.9	42.9	0.0	8.46
S. thermophilus OSU-2	4.40	0.0	0.0	16.3	0.0	8.15
L. acidophilus L1	4.08	4.3	0.0	17.9	0.0	8.77
L. acidophilus C19	4.28	4.3	6.6	24.4	0.0	8.65
L. casei E5	4.77	5.5	9.7	57.3	0.0	8.56
L. casei E10	4.84	1.6	1.4	20.3	0.0	8.68
L. lactis RM2-5[3]	3.94	0.0	0.0	6.2	0.0	8.49

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.
[2]CFU = colony forming units.
[3]*Lactobacillus delbrueckii* ssp *lactis* RM2-5

Table 8. Collection of raw data from replication #3 showing the growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	log_{10} CFU/g[2]
Control	6.42	10.0	5.2	19.1	10.2	<0.0
S. thermophilus 143	4.54	3.8	0.0	14.6	0.0	8.40
S. thermophilus OSU-1	4.32	4.9	6.7	5.2	0.0	8.49
S. thermophilus OSU-2	4.40	5.1	0.0	19.5	0.0	8.11
L. acidophilus L1	4.16	6.4	0.0	31.0	0.0	8.71
L. acidophilus C19	4.32	29.1	0.0	29.1	0.0	8.57
L. casei E5	4.78	0.0	6.2	37.7	0.0	8.69
L. casei E10	5.57	5.5	5.9	33.7	0.0	8.26
L. lactis RM2-5[3]	3.88	11.9	0.0	39.8	0.0	8.49

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.
[2]CFU = colony forming units.
[3]*Lactobacillus delbrueckii* ssp *lactis* RM2-5

Table 9. Collection of raw data from replication #4 showing the growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	log_{10} CFU/g[2]
Control	6.35	26.8	18.3	67.3	28.9	<0.0
S. thermophilus 143	4.59	0.0	13.0	75.9	0.0	8.46
S. thermophilus OSU-1	4.45	0.0	0.0	0.0	0.0	8.26
S. thermophilus OSU-2	4.56	0.0	0.0	17.5	0.0	7.70
L. acidophilus 11	4.15	0.0	0.0	53.1	0.0	8.48
L. acidophilus C19	4.44	0.0	15.7	86.0	0.0	8.41
L. casei E5	4.71	0.0	0.0	57.6	0.0	8.18
L. casei E10	5.29	0.0	0.0	36.8	0.0	8.08
L. lactis RM2-5[3]	3.94	0.0	0.0	52.4	0.0	8.20

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.
[2]CFU = colony forming units.
[3]*Lactobacillus delbrueckii* ssp *lactis* RM2-5

Table 10. Collection of raw data from replication #5 showing the growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	log_{10} CFU/g[2]
Control	6.42	24.8	16.0	74.1	20.9	<0.0
S. thermophilus 143	4.57	0.0	9.8	61.7	0.0	8.46
S. thermophilus OSU-1	4.42	10.3	13.5	93.1	0.0	8.15
S. thermophilus OSU-2	4.51	0.0	0.0	37.6	0.0	8.11
L. acidophilus L1	4.17	0.0	0.0	76.6	0.0	8.73
L. acidophilus C19	4.34	0.0	0.0	32.0	0.0	8.48
L. casei E5	4.58	0.0	0.0	25.6	0.0	8.48
L. casei E10	4.79	0.0	11.4	60.4	0.0	8.57
L. lactis RM2-5[3]	3.94	6.0	1.3	108.2	0.0	8.93

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.
[2]CFU = colony forming units.
[3]*Lactobacillus delbrueckii* ssp *lactis* RM2-5

Table 11. Collection of raw data from replication #6 showing the growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	$\log_{10}$ CFU/g[2]
Control	6.47	29.1	11.3	41.2	2.8	<0.0
S. thermophilus 143	4.57	0.0	0.0	11.4	0.0	8.41
S. thermophilus OSU-1	4.43	0.0	0.0	28.7	0.0	8.52
S. thermophilus OSU-2	4.45	0.0	0.0	4.8	0.0	8.15
L. acidophilus L1	4.15	0.0	0.0	0.0	0.0	8.65
L. acidophilus C19	4.34	0.0	14.7	62.4	0.0	8.52
L. casei E5	4.58	14.3	16.0	105.5	0.0	8.32
L. casei E10	4.65	12.4	13.7	86.9	0.0	8.38
L. lactis RM2-5[3]	3.92	6.3	8.1	106.2	0.0	8.15

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.
[2]CFU = colony forming units.
[3]*Lactobacillus delbrueckii* ssp *lactis* RM2-5

Table 12. Collection of raw data from replication #7 showing the growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	$\log_{10}$ CFU/g[2]
Control	6.23	28.6	18.5	100.2	38.0	<0.0
S. thermophilus 143	4.44	0.0	9.4	43.2	0.0	8.30
S thermophilus OSU-1	4.24	0.0	11.5	72.4	0.0	8.46
S. thermophilus OSU-2	4.31	0.0	0.0	57.3	0.0	7.97
L. acidophilus L1	4.02	0.0	0.0	64.9	0.0	8.59
L. acidophilus C19	4.32	0.0	32.0	133.9	0.0	8.53
L. casei E5	4.67	2.0	4.9	53.7	0.0	8.74
L. casei E10	4.76	0.0	0.0	82.9	0.0	8.53
L. lactis RM2-5[3]	3.84	0.0	0.0	50.8	0.0	8.87

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.

[2]CFU = colony forming units.

[3]*Lactobacillus delbrueckii* ssp *lactis* RM2-5

Table 13. Collection of raw data from replication #8 showing the growth, acid production, and influence on volatile compound concentrations that various cultures have in soymilk.

Sample[1]	pH	Methanol ppm	Acetaldhyde ppm	Ethanol ppm	Hexanal ppm	log_{10} CFU/g[2]
Control	6.41	20.7	19.8	110.6	18.8	<0.0
S. thermophilus 143	4.53	0.0	0.0	54.2	0.0	8.34
S. thermophilus OSU-1	4.34	0.0	0.0	46.2	0.0	8.23
S. thermophilus OSU-2	4.37	0.0	0.0	55.5	0.0	8.08
L. acidophilus L1	4.11	1.6	0.0	60.6	0.0	8.62
L. acidophilus C19	4.27	0.0	6.1	66.1	0.0	8.52
L. casei E5	4.54	0.0	9.9	70.6	0.0	8.52
L. casei E10	4.81	4.8	7.5	75.2	0.0	8.11
L. lactis RM2-5[3]	3.91	0.0	0.0	114.6	0.0	8.95

[1]The control and samples containing a 5% inoculum of specified culture were incubated at 37°C for 12 hours.
[2]CFU colony forming units.
[3]*Lactobacillus delbrueckii* ssp *lactis* RM2-5

APPENDIX G

REPRESENTATIVE CHROMATOGRAMS OF EACH FERMENTED SOYMILK SAMPLE EXAMINED

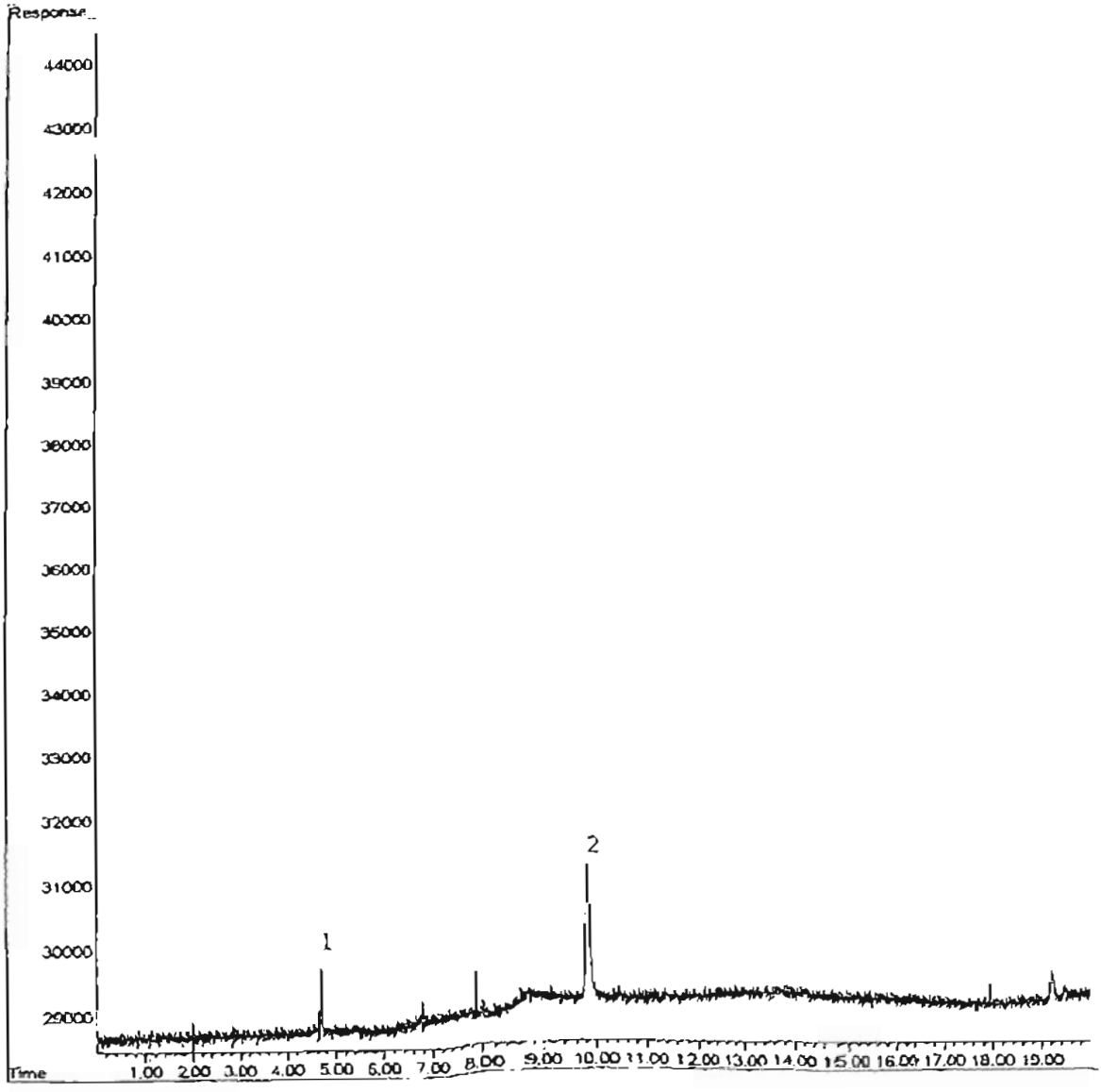

Figure 2. Capillary GC chromatogram of the headspace volatiles of a soymilk sample fermented with *L. acidophilus* L1. Peaks. 1, methanol; 2, ethanol.

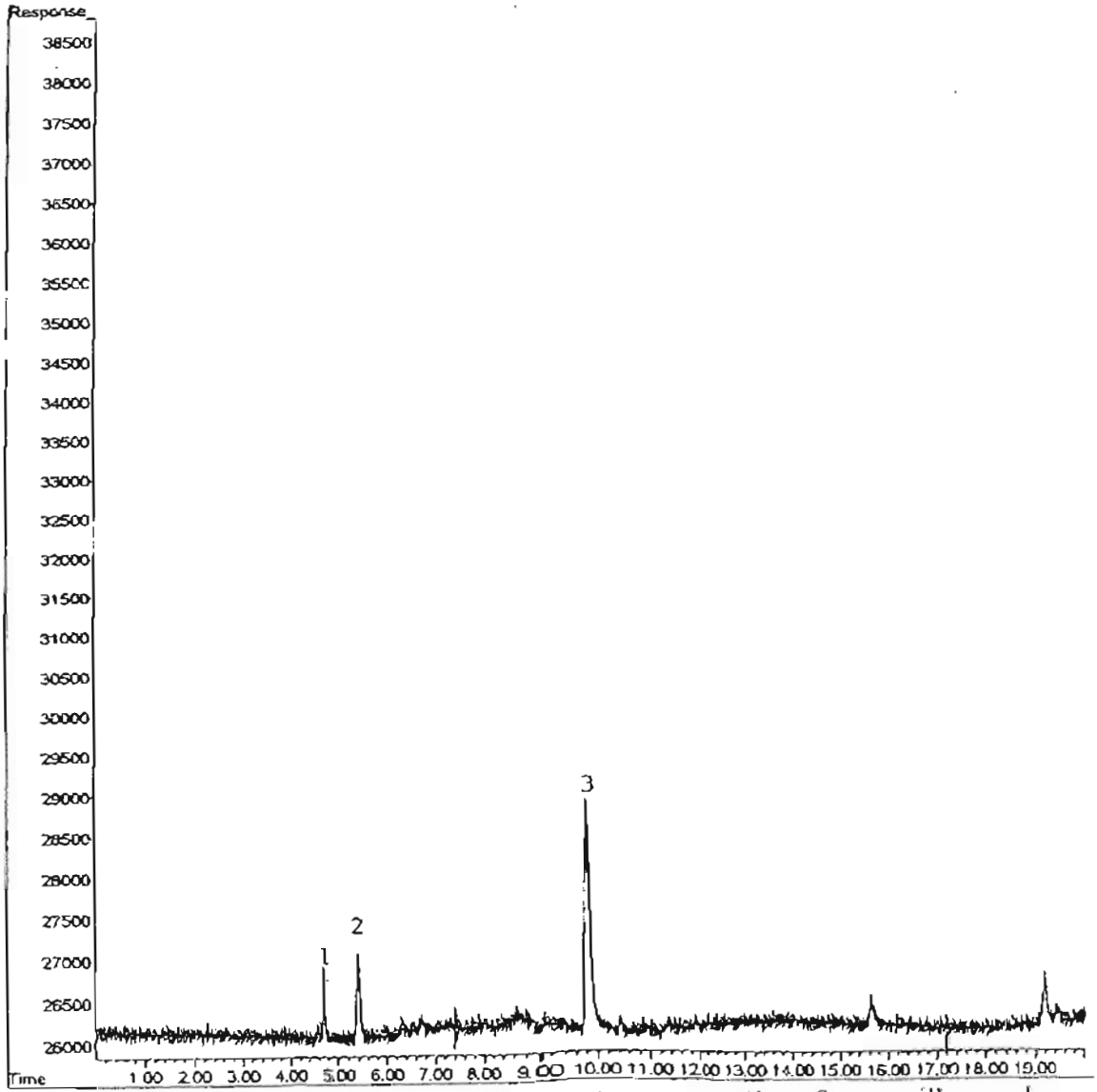

Figure 3 Capillary GC chromatogram of the headspace volatiles of a soymilk sample fermented with *L. acidophilus* C19 Peaks: 1, methanol, 2, acetaldehyde; 3, ethanol.

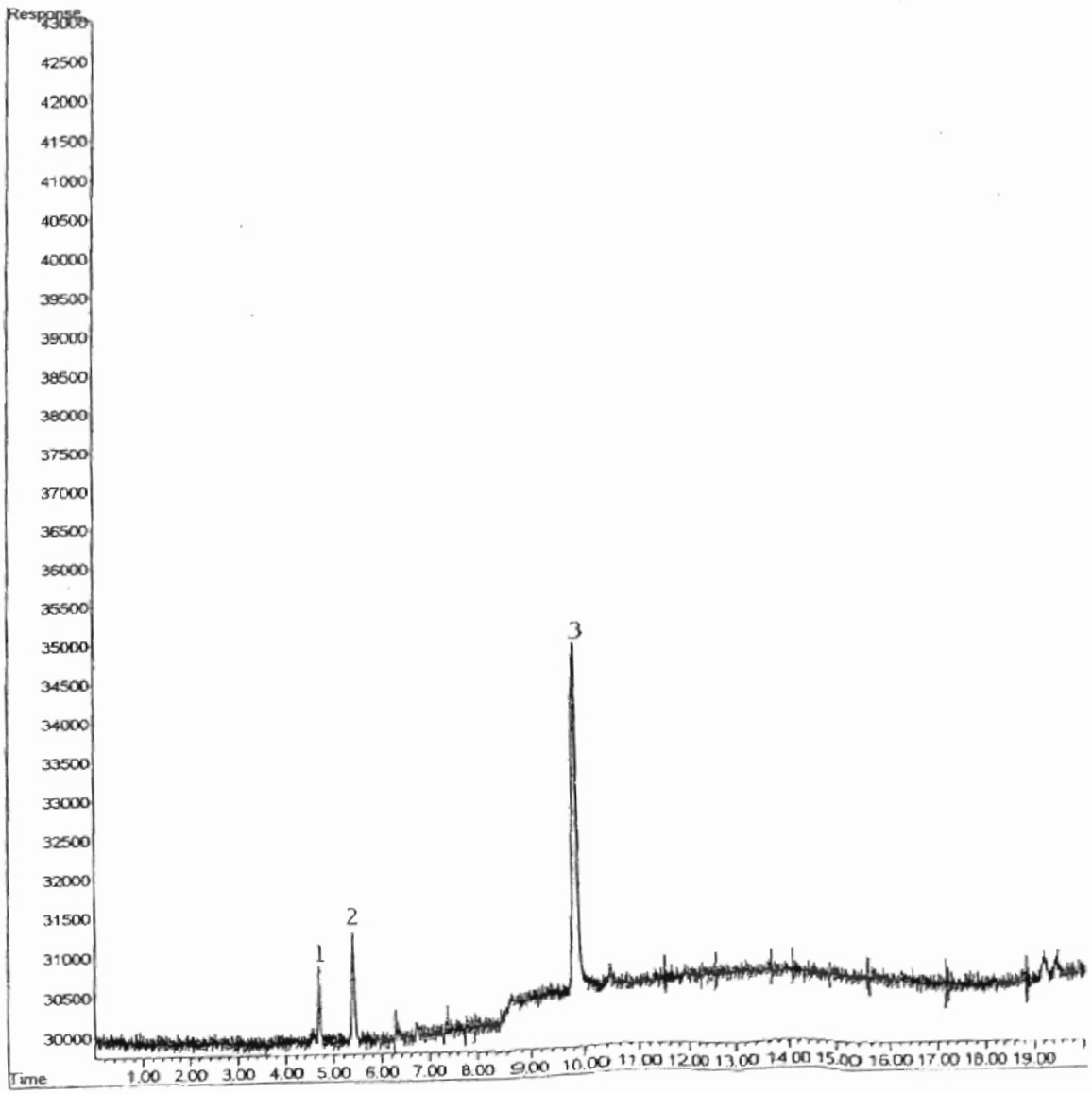

Figure 4 Capillary GC chromatogram of the headspace volatiles of a soymilk sample fermented with *L. casei* E5. Peaks: 1, methanol; 2, acetaldehyde; 3, ethanol.

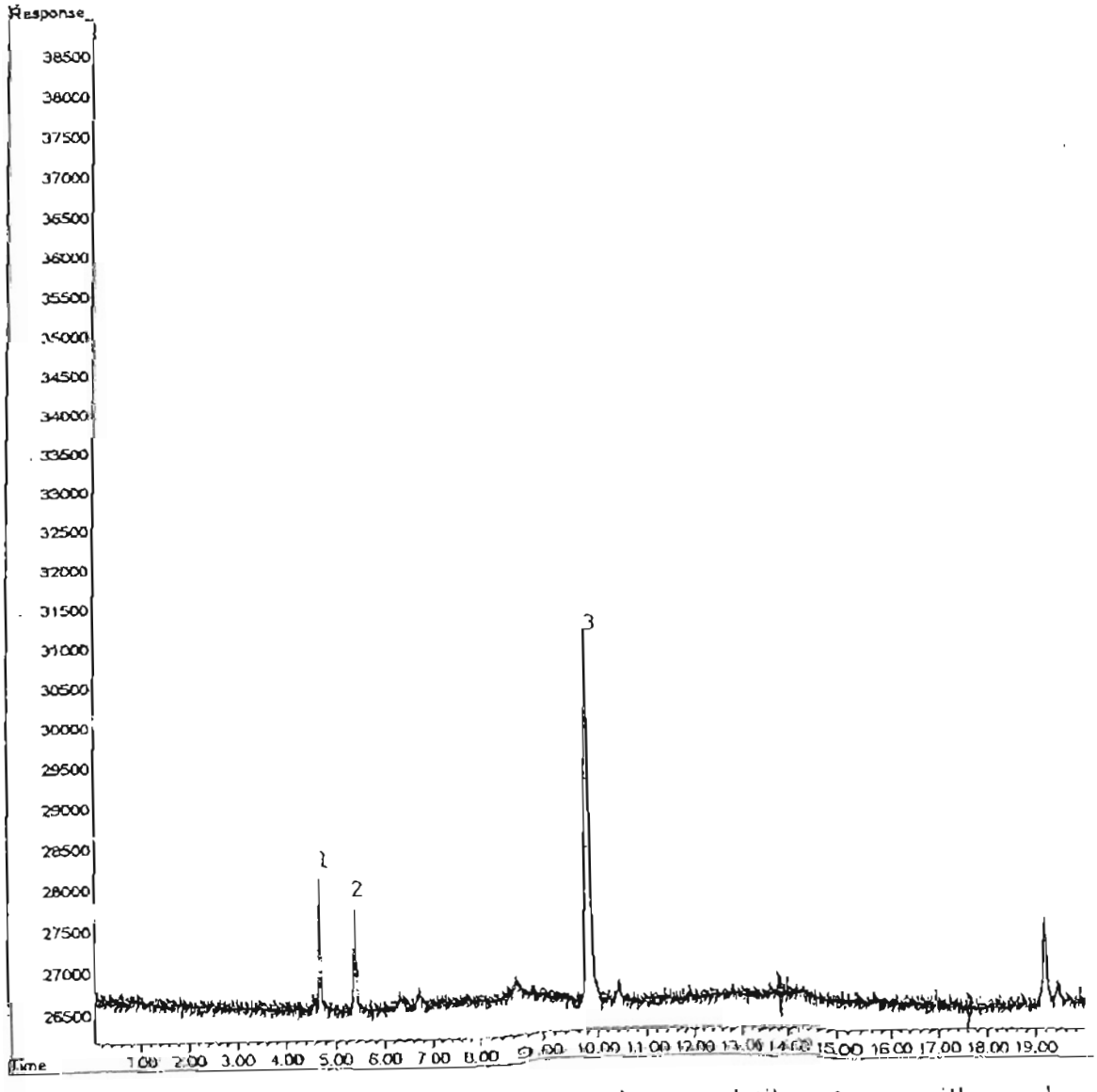

Figure 5. Capillary GC chromatogram of the headspace volatiles of a soymilk sample fermented with *L. casei* E10. Peaks: 1, methanol, 2, acetaldehyde; 3, ethanol.

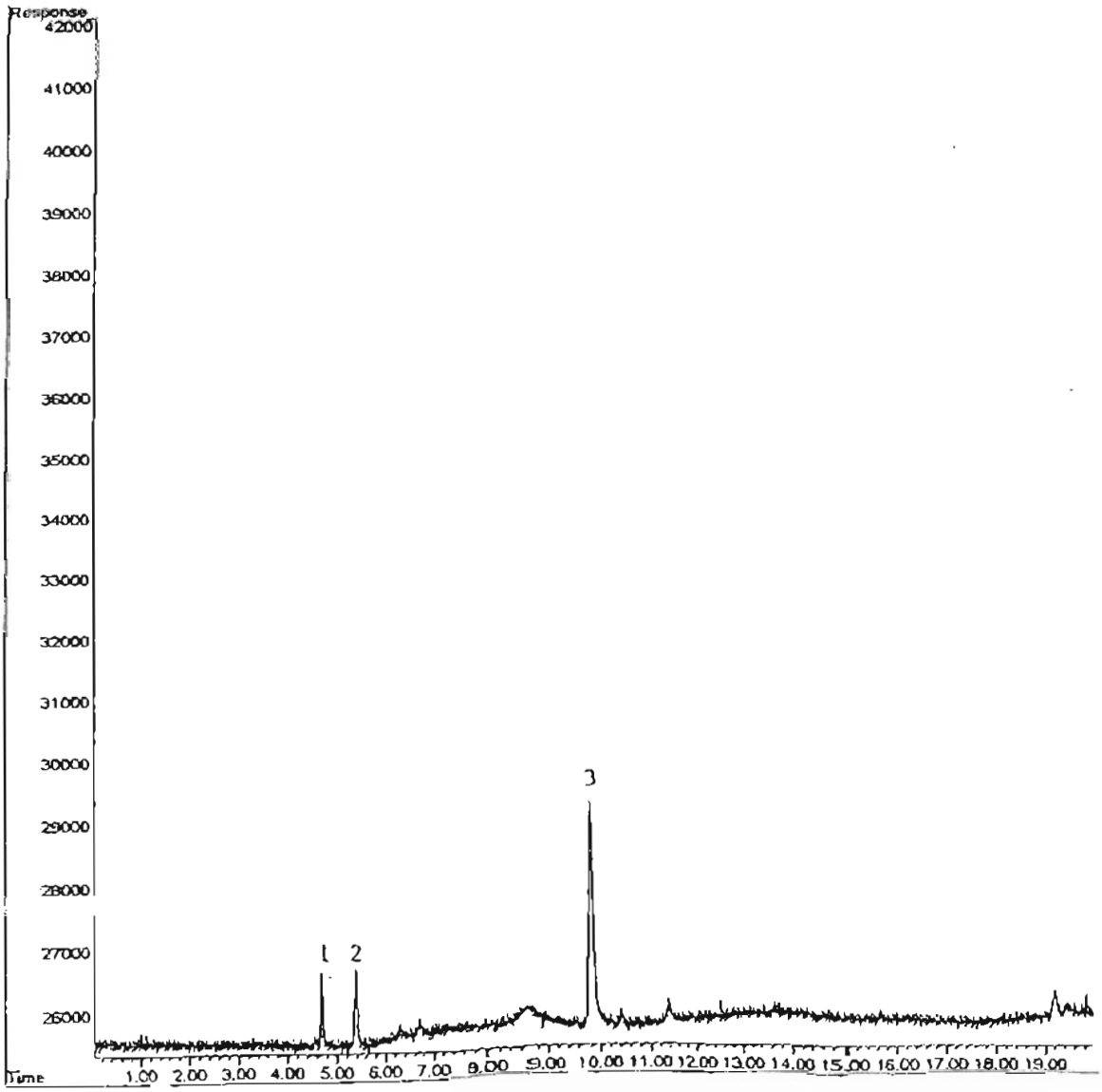

Figure 6 Capillary GC chromatogram of the headspace volatiles of a soymilk sample fermented with *S. thermophilus* OSU-1 Peaks 1, methanol, 2, acetaldehyde; 3, ethanol.

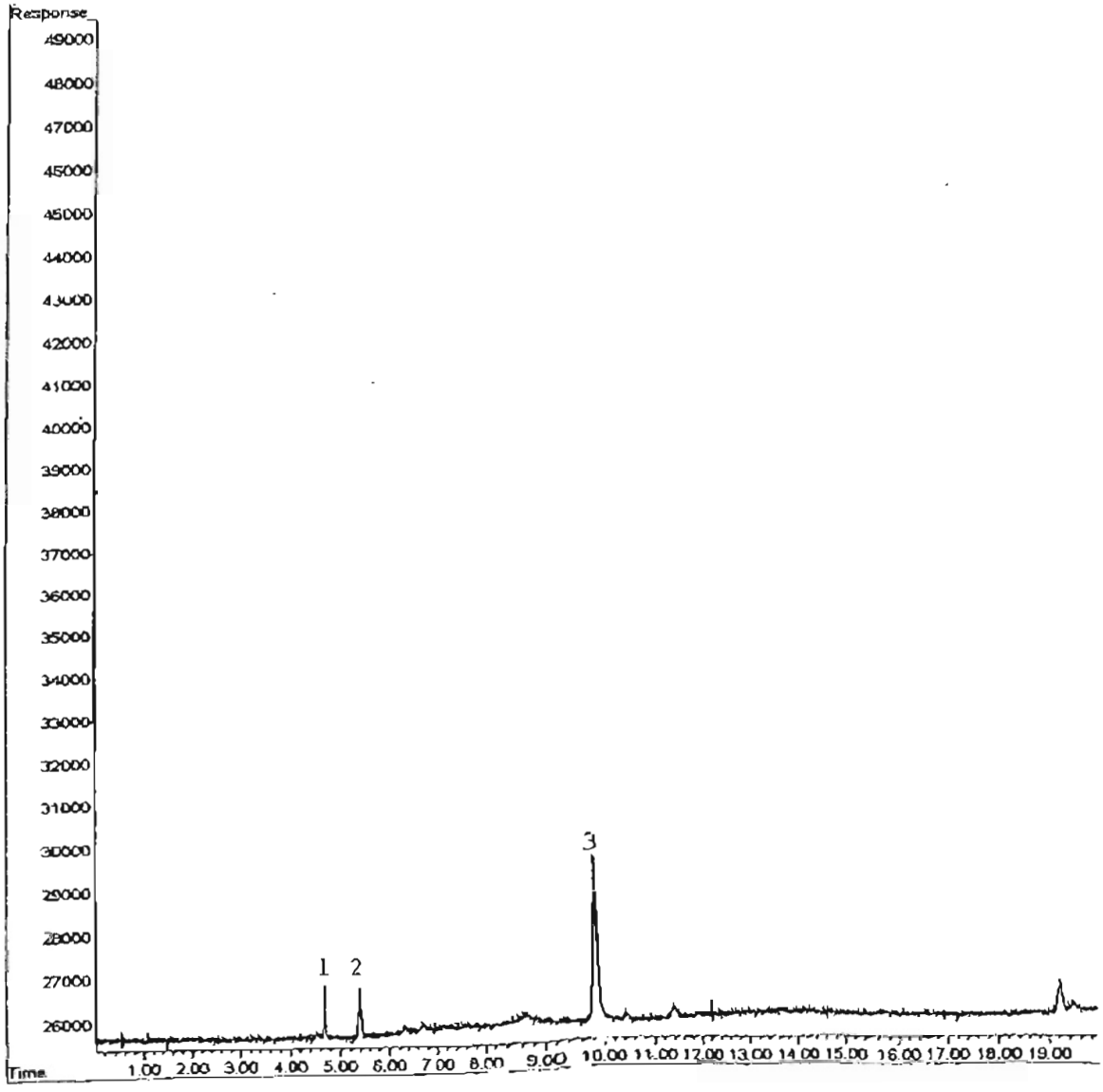

Figure 7 Capillary GC chromatogram of the headspace volatiles of a soymilk sample fermented with *S. thermophilus* OSU-2. Peaks: 1, methanol, 2, acetaldehyde; 3, ethanol

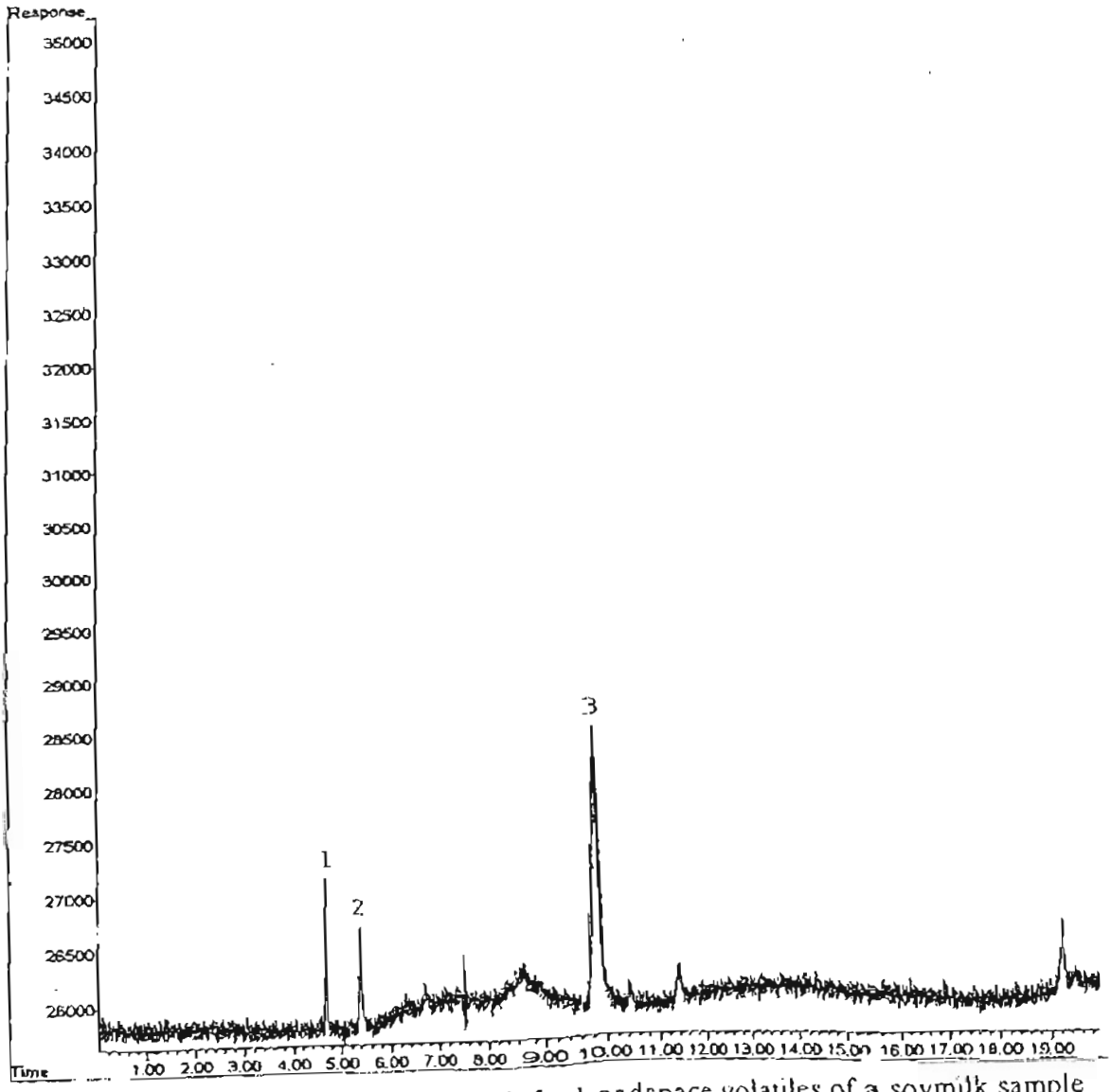

Figure 8. Capillary GC chromatogram of the headspace volatiles of a soymilk sample fermented with *S. thermophilus* 143. Peaks: 1, methanol; 2, acetaldehyde; 3, ethanol.

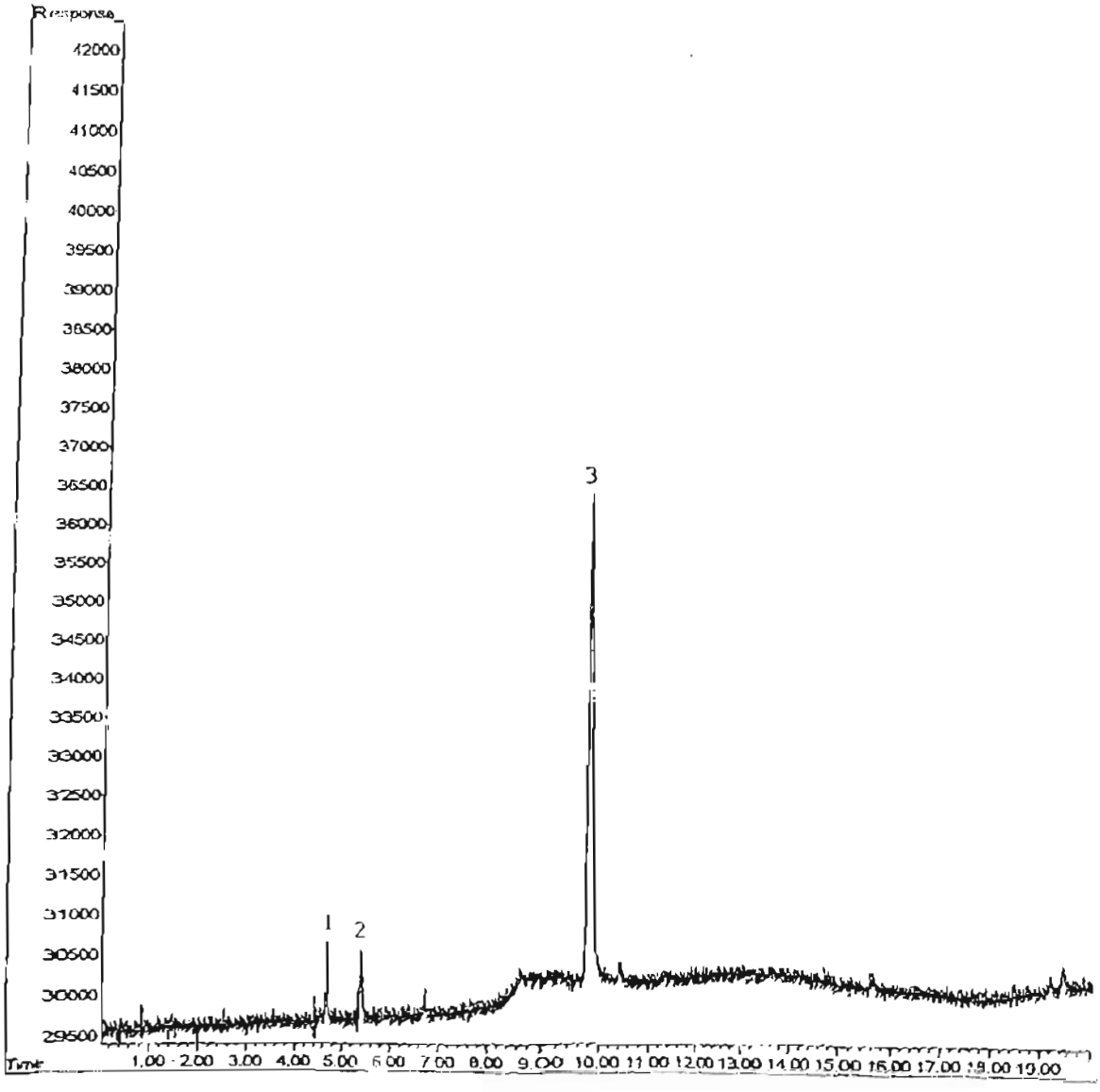

Figure 9. Capillary GC chromatogram of the headspace volatiles of a soymilk sample fermented with *L. delbrueckii* ssp *lactis* RM2-5. Peaks: 1, methanol; 2, acetaldehyde; 3, ethanol

APPENDIX H

IDENTIFICATION OF VOLATILE COMPOUNDS DETECTED IN THE SOYMILK CONTROL SAMPLES THROUGH USE OF THE MASS SPECTROMETER

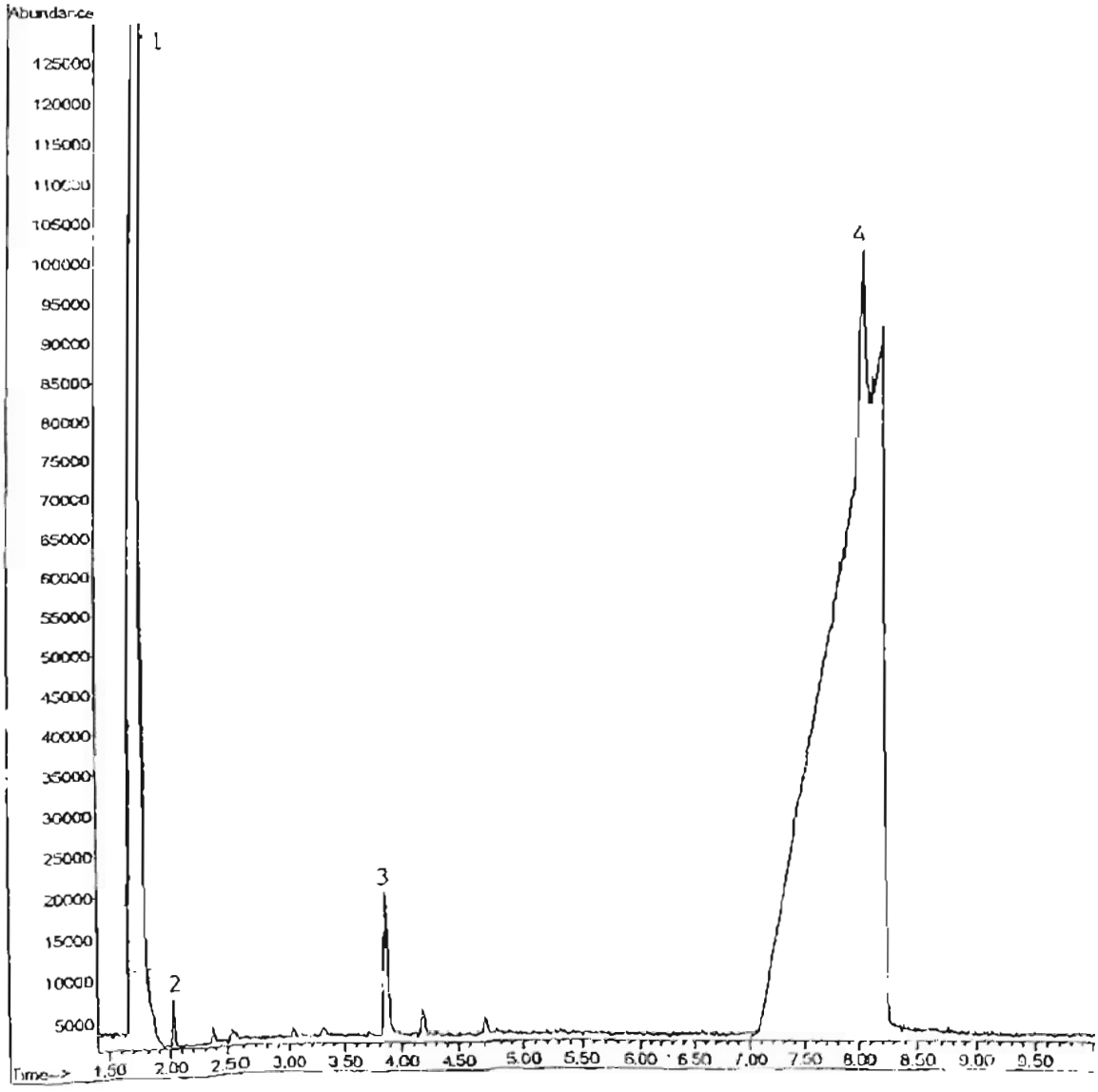

Figure 10. Capillary GC chromatogram of the headspace volatiles detected from soymilk control sample using the mass spectrometer detector for identity of major peaks. Peaks: 1, air; 2, acetaldehyde; 3, ethanol; 4, hexanal.

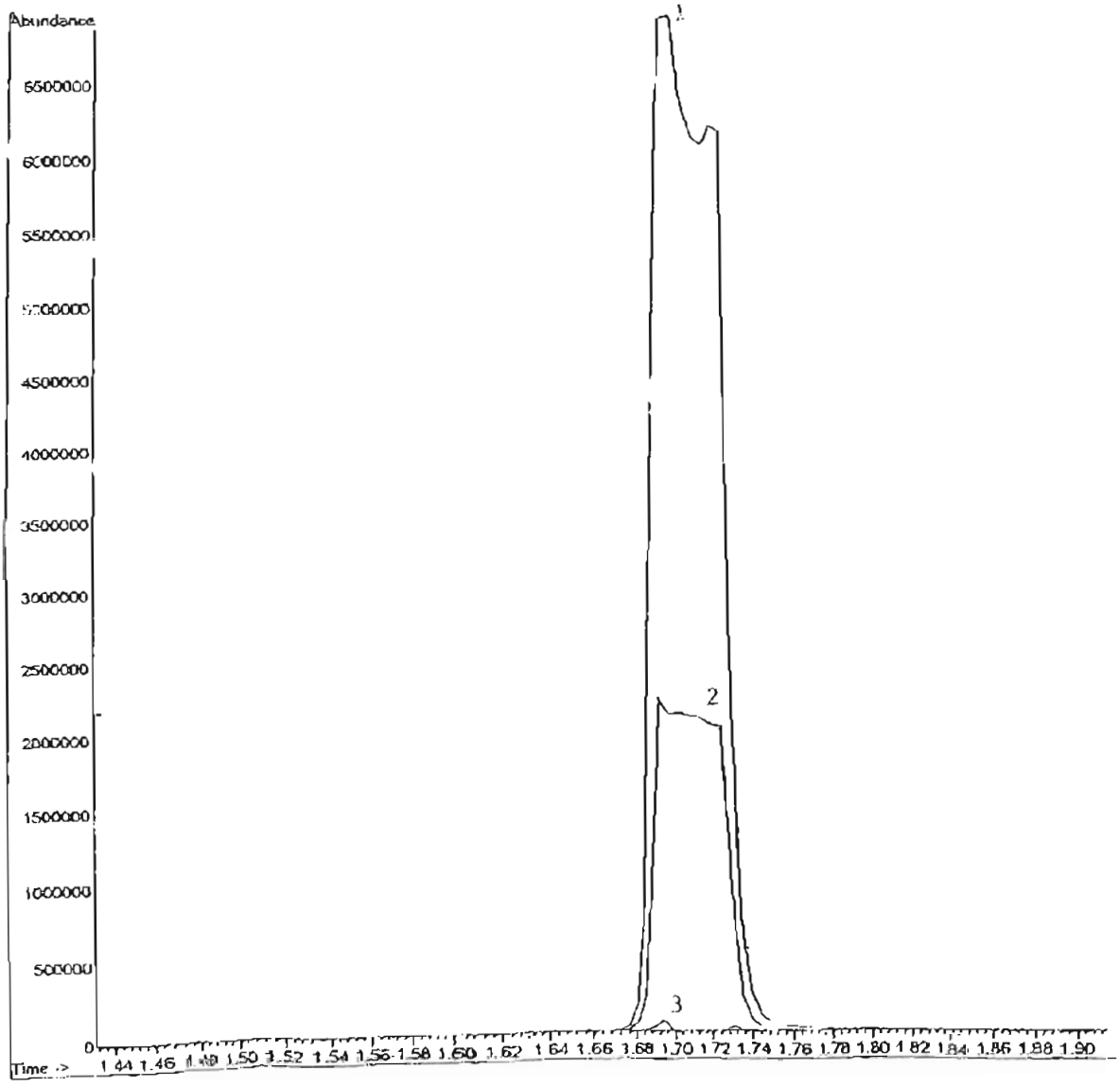

Figure 11. Extracted ion chromatogram of the air peak detected from soymilk control sample using the mass spectrometer detector identity of compound fragments present. Peaks: 1, nitrogen; 2, oxygen; 3, methanol.

APPENDIX I

CONFIRMATION THAT THE PEAKS DETECTED USING THE MS DETECTOR WERE THE SAME PEAKS DETECTED USING THE FID

Samples were analyzed using the mass spectrometer detector with a flow rate of 0.9 mL/minute and an average velocity of 34 cm/second. The samples analyzed in earlier experiments were examined the FID with a flow rate of 0.4 mL/minute and an average velocity of 13 cm/second. This change in flow rates altered the retention time of the major peaks found in the soymilk samples. To confirm that the peaks seen under both conditions were the same compound we analyzed the soymilk samples using the FID with the set of flow rates that matched those used with the mass spectrometer. The following calculations are a ratio of the peak areas for each of the four major peaks detected in the soymilk from the two different conditions analyzed using the FID.

	FID flow rate = 0.9 mL/minute		FID flow rate = 0.4 mL/minute	
Compound	Retention Time	Peak area	Retention Time	Peak area
Methanol	**4.61**	**2806103**	**1.76**	**2772543**

METHANOL RATIO: 2806103 \ 2772543 = 1.0

	FID flow rate = 0.9 mL/minute		FID flow rate = 0.4 mL/minute	
Compound	Retention Time	Peak area	Retention Time	Peak area
Acetaldehyde	**5.29**	**774771**	**2.02**	**786095**

ACETALDEHYDE RATIO: 774771 \ 786095 = 1.0

	FID flow rate = 0.9 mL/minute		FID flow rate = 0.4 mL/minute	
Compound	Retention Time	Peak area	Retention Time	Peak area
Ethanol	**9.51**	**4419452**	**3.83**	**4292471**

ETHANOL RATIO: 4419452 \ 4292471 = 1.0

	FID flow rate = 0.9 mL/minute		FID flow rate = 0.4 mL/minute	
Compound	Retention Time	Peak area	Retention Time	Peak area
Hexanal	**15.01**	**1695076**	**7.99**	**1755011**

HEXANAL RATIO: 1695076 \ 1755011 = 1.0

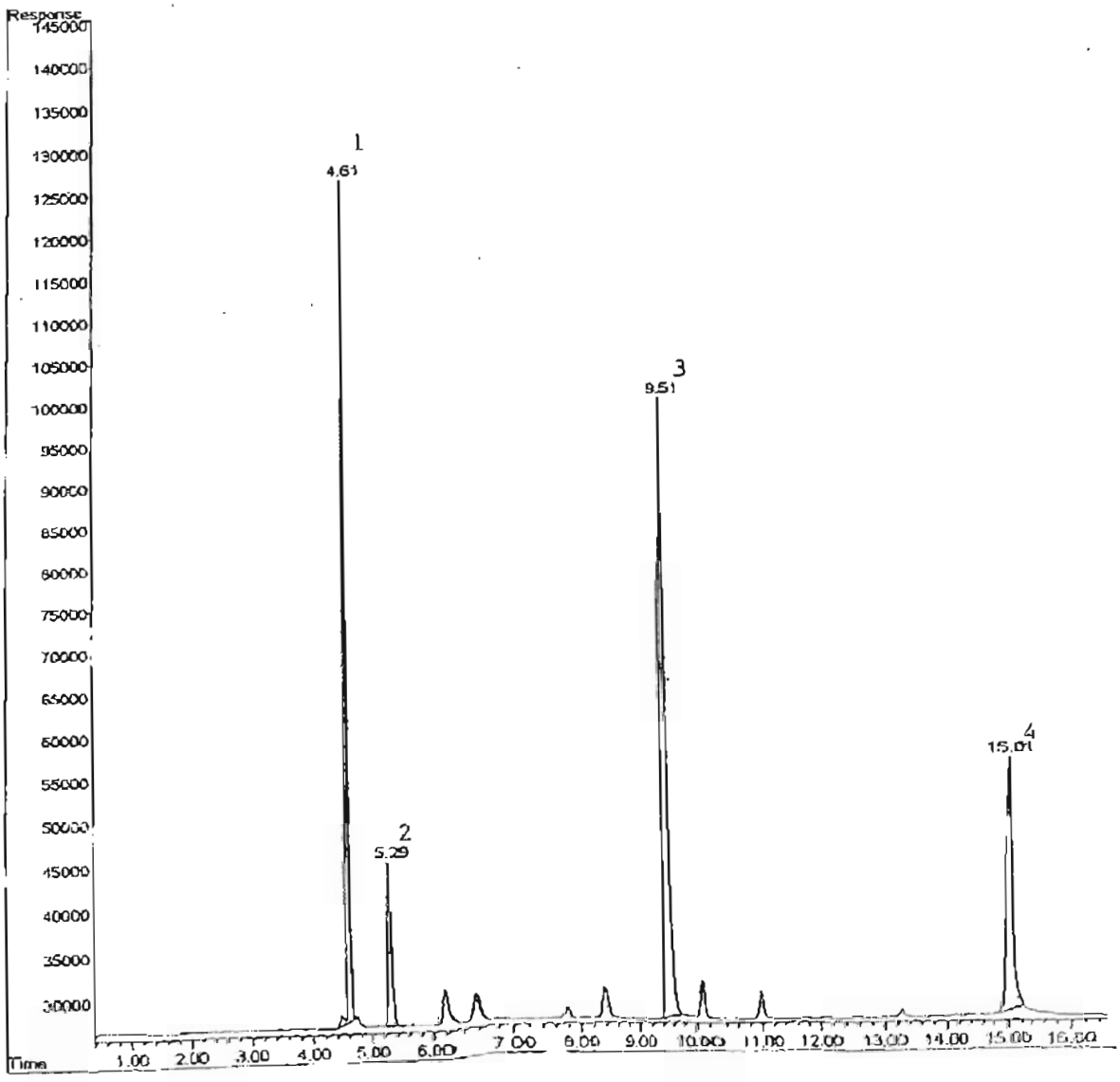

Figure 12. Capillary GC chromatogram of the headspace volatiles of the soymilk contr
sample detected through use of the FID with the slower flow rates. Peaks. 1, methanol
2, acetaldehyde; 3, ethanol; 4, hexanal.

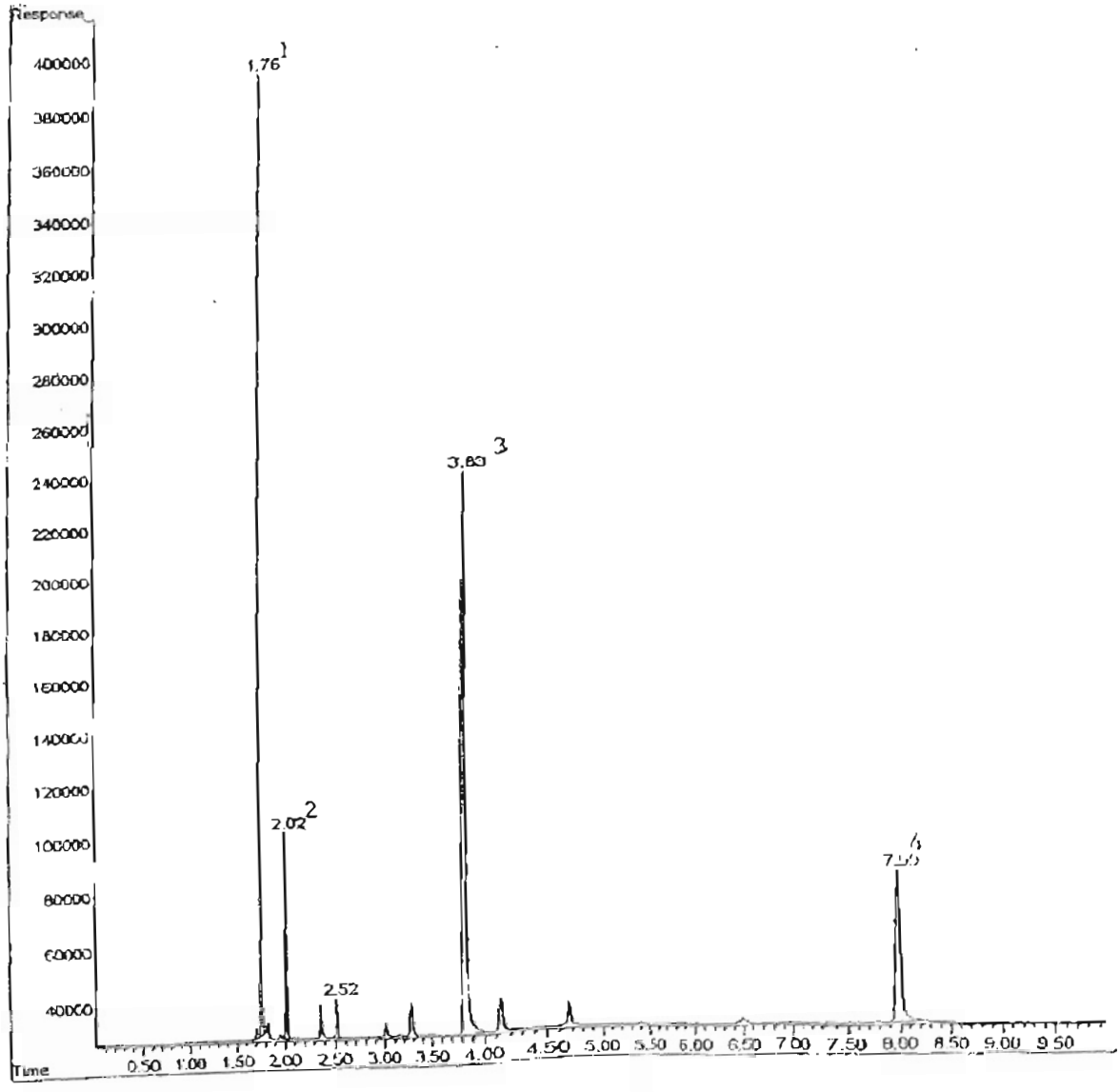

Figure 13. Capillary GC chromatogram of the headspace volatiles of the soymilk contro sample detected through use of the FID with the faster flow rate. Peaks: 1, methanol; 2 acetaldehyde, 3, ethanol; 4, hexanal.

VITA

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Master of Science

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