



# Risk Profile for Strawberries

S. NOTERMANS, 1\* J. S. VAN ZANDVOORT-ROELOFSEN, 1 A.W. BARENDSZ, 1 and J. BECZNER2 <sup>1</sup>TNO Nutrition and Food Research, P.O. Box 360, 3700AJ Zeist, The Netherlands <sup>2</sup> KÉKI Central Food Research Institute, Herman Ottó út 15, Budapest, Hungary

#### **SUMMARY**

This document describes a risk profile for strawberries intended for fresh consumption. Attention is paid to the current production methods for strawberries, consumption-related issues, including positive and negative health effects, and contamination with microorganisms and pesticide residues. In addition, pre-harvest and harvesting requirements are described, as well as post-harvest measures, including decontamination and suitable storage conditions. Finally, there is a brief overview of existing regulations.

The study reveals that strawberries intended for fresh consumption have a relatively good safety record. To keep this status and maintain consumer confidence, it is recommended that codes of good agricultural practice be used, for which land and water use, application of organic and inorganic fertilizers, animal exclusion and pest control are described, together with recommended harvesting and cooling practices and measures for ensuring worker health and

#### INTRODUCTION

The consumption of fresh fruits and vegetables is increasing because consumers strive to eat healthful and tasty foods. This applies particularly to strawberries; however, exact consumption data on strawberries are scarce. Data from the United States demonstrate an increase in production of strawberries from 316 million kg in 1970 to 494 million kg in 1993 (5). In that period the consumption of fresh strawberries increased from 850 gram per capita per year in 1970 to 1,750 grams in 1992 (5). In 2000 the consumption increased to 2,700 grams per capita (13).

The consumption of frozen, sliced strawberries in the United States amounts to 600 - 700 grams per capita per year and has not changed significantly over the past 30 years (5). In The Netherlands, data are collected by interviewing 100 families, selected at random, once every three years. In 1999, domestic consumption amounted to 965 grams per person. In 2002 it was 870 grams per person (personal communication, Commodity Board for Fruit and Vegetables, The Netherlands).

Although global trade in strawberries is increasing, the availability of the fruit is still largely seasonal. The price and consumption level depend largely on availability.

#### A peer-reviewed article

\*Author for correspondence: Phone: 31.30.6944943; Fax: 31.30.6944901 E-mail: notermans@voeding.tno.nl

# **PRODUCTION METHODS** FOR STRAWBERRIES

Strawberries are adapted to growing in many different regions. They require well-drained soil with a high concentration of organic matter; a pH between 5.0 and 7.0 is optimum. The majority of strawberries are produced outdoors, for which purpose the use of small protective polythene tunnels is increasing. Indoor production is mostly carried out in greenhouses made of glass. The use of large polythene tunnels is a cheap alternative to the greenhouse.

# **Outdoor production**

Matted row strawberries. The 'matted row' method used by home gardeners and by some commercial growers has been used for many years. Plants are set out in spring or early summer on bare ground and allowed to send out runners. These give rise to daughter plants that also take root and form a wider 'matted' row. The field is allowed to produce fruit for 2-4 years and is then replanted. The production level varies from 6,000 to 8,000 kg per hectare (10,000 m<sup>2</sup>).

Strawberries grown on plastic. Strawberries grow well (and weed-free) through a mulch of polythene. The great majority of strawberries are produced by setting plants out into black plastic in the fall. Irrigation and some fertilizer are supplied through a drip tape laid under the plastic at the time of planting. In late winter and early spring, the plants start to grow in earnest. Growers protect the early flowers from late (night) frosts during March and April by overhead irrigation at night. Although strawberry plants are perennial, those cultured in plastic are grown as

annuals; they are harvested only 7-8 months after planting, and new plants are set out every year. When flowering is finished, the plastic is covered with straw, which protects the berries against contact with sun and soil and avoids mud splashes during periods of rain. Plant density is high (100,000 plants per hectare) and the investment of farmers in the crop is substantial. Production levels vary from 42,000 to 74,000 kg/hectare.

Plastic culture, first developed in the mid-1980s, is now widely used and, in particular, is being adapted for use in colder areas. Also, the use of small, protective polythene tunnels is increasing in the production of plastic-culture strawber-

# **Indoor production**

Hydroponic indoor production. The use of methyl bromide as a soil fumigant for greenhouses has been phased out in The Netherlands and many other countries in Europe, including Germany, Sweden, Switzerland and Denmark, because hydroponics (soil-free culturing) has been developed. This allows strawberries to be grown successfully in greenhouses on hanging shelves. Planting densities in greenhouses have been doubled by the intelligent use of this system. The hydroponic solution (nutrient-rich water) is pumped to the plants by means of a trickle/drip irrigation system. The solution is re-used after being sterilized.

# **CONSUMPTION RELATED MATTERS**

The majority of fresh strawberries are consumed without further processing. At most, they will be washed gently in tap water before being eaten. They may be consumed directly, sometimes in combination with dairy products, such as yogurt and whipped cream, and they may also be used as toppings for pies and desserts.

# **Quality aspects**

An important part of international trade in strawberries is the quality requirements of bodies such as the United Nations Economic Commission for Europe (UNECE) and the European Commission. In, for example, the 'Commission Regulation (EC) No 843/2002 of 21 May 2002,' laying down the marketing standards for strawberries, and the amending Regulation (EEC) No 899/87, the minimum requirements for strawberries are that they should be intact, sound (produce not affected by rotting or deterioration), clean (practically free from any visible foreign matter), fresh in appearance (but not washed), practically free from pests and from damage caused by pests, and with the calyx present (except in the case of wild strawberries). The calyx and the stalk (if present) must be fresh and green, free from any abnormal amount of external moisture, with no foreign smell and/or taste. The strawberries must have been picked carefully. They must be sufficiently developed and display satisfactory ripeness. The development and condition of the fruit must be such that they can withstand transport and handling, and arrive in a satisfactory condition at their final destination.

In the above-mentioned regulation, strawberries are divided into three classes, differing with respect to brightness, color and shape that are characteristic of the variety in question, and aspects related to quality, shelf life and presentation, which are degree of bruising, defects in shape, presence of white patches and amount of any attached soil.

### Consumer data on quality

Consumer data on the identification of quality attributes and acceptance of defects are rarely published. The few studies that have been carried out include investigations of items dealing with, for example, flavor and sweetness; the price, quality relationship; and appearance and color.

Flavor and sweetness are attributes that are becoming increasingly important to the consumer (38). In an Australian study (45), the mean price/quality relationship for strawberries was low, suggesting that consumers do not rely on price as an indication of quality and price does not predict the level of consumption. Quality criteria mentioned by consumers in this study were odor and bruising. For a better understanding of the quality:price relationship, further study is required. In the design of these studies, it is recommended that the quality criteria used be clearly defined. Using quality attributes given by the UM FDA and the Joint Institute for Food Safety and Applied Nutrition in Food, quality criteria could be separated into external features (appearance, color), other sensory attributes (odor, taste) and a third category, including wholesomeness, nutritive value and safety (28).

#### Positive health aspects

Because the health advantages of horticultural products have been proven scientifically, authorities of many countries recommend the consumption of at least 5 portions of a variety of fruits (such as strawberries) and vegetables each day to reduce the risks of cancer and coronary heart disease and many other chronic diseases. For the program introduced in the UK, visit the internet page http:// www.doh.gov.uk/fiveaday/index.htm.

Strawberries are low in calories, are a good source of many bioactive phytochemicals in the human diet, and provide nutrients that a healthy body needs. In addition, they have a good flavor. Strawberries are high in iron and vitamin C and are a good source of folic acid, fiber, potassium and cancer-fighting antioxidants. Eight medium-sized strawberries provide 20% of the recommended daily amount of folic acid (44), which works with vitamins B<sub>6</sub> and B<sub>12</sub> in the body to metabolize homocysteine and bring blood levels down to a safe range. Homocysteine contributes to atherosclerotic plaque formation, which can ultimately lead to a heart attack. In this way, strawberries are a recommended part of a hearthealthy diet (53).

Antioxidants reduce the oxidation of low-density lipoprotein which links to cholesterol to form LDL-cholesterol. It has been demonstrated that decreased oxidation of LDL-cholesterol greatly diminishes the development of atherosclerosis (55). The antioxidant property depends on the food matrix, as has been shown recently in studies demonstrating that strawberries have more antioxidant activity than apple, apricot, peach or kiwi fruit (48).

It has been claimed that antioxidants present in strawberries, including flavanoids, anthocyanidin, ellagic acid and other phenolic acids that may have also anti-inflammantory properties, reduce the risk of developing several forms of cancer (http://www.labspec.co.za/l\_fruit2. htm# Strawberry).

#### **Negative health aspects**

Hazards associated with fresh produce include biological, chemical and physical agents. A general overview of these hazards that can also be associated with strawberries is presented in Table 1.

Microbiological pathogens such as bacteria, parasites and viruses are part of the environment. Many of them reside in the intestinal tract of animals and humans. They can contaminate strawberries

**TABLE 1.** A general overview of the hazards that can be associated with fruits and vegetables, including strawberries (6, 7, 20, 22, 29, 41, 43)

I Microbiological hazards

**Bacteria** Salmonella Shigella

Escherichia coli (pathogenic)

Campylobacter

Listeria monocytogenes

Vibrio spp.

Parasites Cryptosporidium

Cyclospora
Giardia
Entamoeba
Toxoplasma
Nematodes
Plathelminthes

Viruses Hepatitis

Norovirus Rotavirus Enterovirus

II Chemical hazards

Natural chemical hazards

allergens

mycotoxins

**Extraneous chemical hazards** 

Polychlorinated biphenyls

Agricultural chemicals

- pesticides
- fertilizers

Toxic elements and compounds

- lead
- zinc
- cadmium
- mercury
- arsenic
- cyanide

#### Other contaminants

- lubricants
- cleaners
- disinfectants
- coatings
- refrigerants
- pest control chemicals

# From packaging materials

- plasticizers
- venyl chloride
- adhesives

III Physical hazards

Foreign bodies Filth, foreign matter like soil

through infiltration of sewage waters into fields, irrigation with contaminated water, presence of animals in the field or use of inappropriately composted organic fertilizers. Especially during the growing period, many of these microorganisms can come into contact with strawberries. This can also occur during harvest, storage and handling. Microorganisms with infectious properties are of special concern because they are able to cause disease.

Strawberries may also contain hazardous chemical agents. They may be present naturally (allergens) or be added, deliberately or inadvertently, during agricultural production, post harvest handling and other operations. An important source of contamination is the use of plant protection agents (pesticides) and fertilizers.

In addition to microbiological and chemical hazards, strawberries may be contaminated with filth and other foreign matter.

A literature survey on the association between strawberries and adverse health effects has demonstrated that during the last 10 years, strawberries have only incidentally been involved in acute disease outbreaks. In addition to microbial diseases, strawberries might be involved sporadically in allergic reactions, such as rashes. This information is presented below.

Disease outbreaks In the period 1998 – 2000, the US Centers for Disease Control and Prevention (CDC) reported a total of 1,135 foodborne disease outbreaks. In 43% of the outbreaks, a suspected vehicle was indicated. For this same period, a total of 4 outbreaks implicated strawberries as the possible vehicle (Table 2). In 3 cases the agent was hepatitis A virus and in 1 case a Norwalk-like virus. Water is a very common vector of these viruses. In one case the outbreak was caused by frozen sliced strawberries.

In 1997 two large outbreaks of hepatitis A associated with consumption of frozen strawberries occurred in the United States (2, 27).

In an outbreak that occurred in Canada, the disease agent was the parasite *Cyclospora cayetanensis*. The incriminated food was a mixture of different types of berries, and uncertainty exists regarding which of the berries were contaminated. *C. cayetanensis* is a human parasite that can be transmitted to other people via water contaminated with human feces. Both food and water may act as transmission vectors (42, 50).

In the period 1993–1998, the WHO Surveillance Program for Control of Foodborne Infections and Intoxications in

TABLE 2. Strawberryborne outbreaks reported to the Centers for Disease Control and Prevention with strawberries as the suspected vehicle (period 1998-2000) (http://www. cdc.gov/foodborneoutbreaks). Figures for Canada, Australia and in Europe (period 1993–1998) are also included

| Year  | Etiological agent       | Nr ill | Suspected vehicle  | Ref.       |  |  |  |
|---|-------------------------|--------|--|------------|--|--|--|
| US Centers for Disease Control and Prevention (1998–2000) |                         |        |  |            |  |  |  |
| 1998  | Hepatitis A             | 29     | Frozen strawberries  | *          |  |  |  |
| 1998  | Hepatitis A             | 41     | Strawberries,<br>honeydew melon                                    | *          |  |  |  |
| 1999  | Norwalk-like virus      | 63     | Pasta salad,<br>strawberries                                       | **         |  |  |  |
| 2000  | Hepatitis A             | 8      | Strawberries   | ***        |  |  |  |
| Canada  | (1998–2000)             |        |  |            |  |  |  |
| 1999  | Cyclospora cayatenensis | 94     | Blackberries, raspberr<br>strawberries                             | ries,<br>3 |  |  |  |
| Europe (  | Europe (1993–1998)      |        |  |            |  |  |  |
| 1997  | Hepatitis A             | > 8000 | Frozen creams with strawberries                                    | 46         |  |  |  |
| Australia, 200 l  |                         |        |  |            |  |  |  |
| 2001  | S. Typhimurium          | 5      | Pastry-filled custard<br>tart topped with<br>strawberries in jelly | 37         |  |  |  |

<sup>\*</sup> Internet page: http://www.cdc.gov/foodborneoutbreaks/us\_outb/ fbo I 998/viral98.htm

# \*\*\* Internet page: http://www.cdc.gov/foodborneoutbreaks/us\_outb/ fbo2000/viral00.htm

Europe (59) registered a total 22,386 outbreaks in which the food vehicle was identified. Fruit, vegetables and spices were involved in 261 (1.2%) of these outbreaks. In none of these were strawberries involved. In 1997 there was a large outbreak of hepatitis A both in the Czech Republic and in Slovakia (46). This outbreak could be traced back to the consumption of frozen-moderated strawberries. In Slovakia, more than 8,000 of the cases were hospitalized. It was suspected that the strawberry field was irrigated with contaminated water a few days before the fruit was picked, washed and frozen.

In Australia, an outbreak of Salmonella Typhimurium was linked to a pastryfilled custard tart topped with fresh strawberries in jelly (37) and five cases were reported. However, it has not been es-

tablished if the strawberries were the source of the outbreak.

Sporadic cases. In most countries, it is mandatory to report outbreaks of foodborne disease only. As a consequence, there is not much data concerned with sporadic cases. Some countries (USA, UK and The Netherlands), however, collect foodborne infection data based on sentinel studies of the general population. A comparison of data from The Netherlands reported to the authorities and data collected by sentinel studies reveals that the large majority (> 90%) of foodborne diseases are, by definition, sporadic cases (23). Because of the way strawberries become contaminated (for example, through fecal droppings of birds), it might be expected that many of the disease incidents will be sporadic.

Recalls. Another source of information on strawberry-borne diseases is recall action. Examination of the 'recall' internet sites of the US Food and Drug Administration (FDA) and Health Canada for the period 2000-2002 revealed only two such events.

Recall 1: On April 28, 2000, New West Foods, in conjunction with the FDA, initiated a nationwide recall of frozen strawberries (http://www.fda.gov/oc/po/ firmrecalls/strawberries.html). The recall was in response to an outbreak of hepatitis A in early February among seven laboratory workers at Boston's Brigham and Women's Hospital. Following an investigation by the FDA, ice cream on which frozen strawberries were served as one of the toppings, was the most suspected food. After this recall, many others followed, involving frozen strawberry products that were associated with the same outbreak.

Recall 2: On May 23, 2000, the FDA announced that Expo Fresh LLC had recalled fresh strawberries, in bulk cardboard cartons, because the product was contaminated with Salmonella (http:www. safetyalerts.com/recall/f/00/460.htm).

Allergies. In allergic reactions, which are immunologically mediated, IgE plays an important role. Allergic reactions caused by strawberries are very rarely observed and when they are observed, the symptoms are relatively mild. The allergic reactions from eating strawberries are usually caused by the small hairs on the surface of the fruit.

Strawberries are a common cause of skin rashes, which are common symptoms in individuals that react to strawberries. These reactions are not immunologically mediated and are induced by aromatic and colored substances found in strawberries (http//www.labspec.co.za/ l\_fruit2.htm#Strawberry).

#### CONTAMINANTS

#### Microbiological contamination

A survey of the scientific literature for microbiological analyses of strawberries revealed that only a small number of studies have been carried out. In recent years, there were only two such studies.

In 2000, the FDA surveyed domestic fresh produce, including strawberries, for microbiological quality (http://vm.cfsan. fda.gov/~dms/prodsu10.html). A total of 136 samples of strawberries were investigated for the presence of Salmonella, Shigella and Escherichia coli O157. Each

<sup>\*\*</sup> Internet page: http://www.cdc.gov/foodborneoutbreaks/us\_outb/ fbo I 999/viral99.htm

sample consisted of 454 grams which was rinsed with a buffer solution. The rinse was tested for the presence of pathogens. No *Salmonella*, *Shigella* or *E. coli* O157:H7 were detected in any of the samples.

In a follow-up study, 143 samples of imported strawberries were tested for the presence of *Salmonella*, *Shigella* and *E. coli* O157:H7. Only in one sample were pathogenic organisms detected (http://vm.cfsan.fda.gov/~dms/prodsu6.html).

Johannessen et al. (28) tested the microbiological status of 120 samples of strawberries obtained from Norwegian markets. Samples were analyzed for thermotolerant coliform bacteria (an indicator of fecal contamination) and for the pathogens E. coli O157:H7, Salmonella, Listeria monocytogenes, Staphylococcus aureus and Yersinia enterocolica. Thermotolerant coliform bacteria were found in only a small proportion of the samples; Salmonella, Yersinia enterocolica and E. coli O157 were not found in any. L. monocytogenes was found in one sample, while S. aureus was found more frequently.

Based on the above investigations, it may be concluded that strawberries are not often contaminated with human pathogens. However, the limited numbers of samples tested do not provide a guarantee of safety.

## Pesticide residues

In The Netherlands, data are obtained from the pesticide residue monitoring program 'Programme for the quality of agricultural products'. These data are available only for the years before 1998. The results are presented in Table 3. Detailed information is available in the internet site http://library.wur.nl/cgi-bin/WebQuery/kapgf.

Also presented in Table 3 are data from the European monitoring program on pesticide residues in products of plant (source: http://europa.eu.int/comm/food/fs/inspections/fnaoi/reports/annual\_eu/monrep\_2001\_en.pdf).

In 2001, five commodities (strawberries, apples, tomatoes, lettuces and table grapes) were tested for 36 different pesticides. Residues of pesticides at or below the Maximal Residue Levels (MRL) were found most often in table grapes (60%), followed by strawberries (51%), lettuce (49%), apples (47%) and tomatoes (33%). Residues exceeding the MRL were found most often in lettuce (3.9%) followed by strawberries (3.3%), table grapes (1.8%), tomatoes (1.5%) and apples (1.1%).

Chronic exposure assessments demonstrated that Acceptable Daily Intake (ADI) values were not exceeded for the pesticide/ strawberry combination. The finding indicates that there is no acute risk in this case.

# PRE-HARVEST AND HARVEST

#### Pre-harvest

Land and water use. Soil is a rich environment for a variety of microorganisms. The non-pathogenic flora is important for the mineralization of plants and animals after their death, but the tissuedegrading properties of the microorganisms that contaminate fruits and vegetables may cause damage to the produce during transport and storage. Subsequently, the products are exposed to further microbial attack. In addition, soil is a reservoir of foodborne pathogens, such as Bacillus cereus, Clostridium botulinum, and Clostridium perfringens (35). Listeria monocytogenes has been isolated from non-cultivated soil. Pathogenic microorganisms from human/animal reservoirs can be found in the soil because of irrigation with contaminated water, fertilization with manure and sewage sludge, or droppings of animals in the farming area.

Water is often used for irrigation of plants. Its quality will vary depending on whether it is surface water or potable water. Water may be a source of contaminating microorganisms Surface water from streams and lakes may be contaminated with pathogenic protozoa, bacteria and viruses. The occurrence of L. monocytogenes, Salmonella and viruses in water has been reported (10, 41, 42). The transfer of foodborne pathogens from irrigation water to fruits and vegetables will depend on the irrigation technique and the nature of the produce being grown (39). Spray irrigation would be expected to increase the risk of contamination, in comparison with drip irrigation or flooding. Leafy vegetables provide large surfaces for contact with water and for the attachment of microorganisms.

In hydroponic systems, water is used for the transport of nutrients into the plant. Water from sewage plants can be used for this purpose. However, in the absence of prior treatment, it may represent a risk to crop contamination. There is a similar concern over the use of recycled water. Recycling of water for agricultural purposes is carried out in several countries, such as Australia, Germany, Israel, Spain,

The Netherlands and USA (10). The safety of treated sewage water depends on the efficacy and reliability of the treatments used to inactivate pathogens.

Organic fertilizers. Sewage, manure, slurry, sludge and compost of human and animal origin are commonly used as organic fertilizers for fruit and vegetable production, particularly for organic produce. The fecal origin of these fertilizers, however, represents a potential risk of contamination by viruses, bacteria and parasites pathogenic to humans.

Members of the family Enterobacteriaceae, such as Salmonella, Shigella, Yersinia, and E. coli, as well as Campylobacter spp. can be found in the intestinal tracts of a wide range of domestic, wild and companion animals. In Belgium and Finland, L. monocytogenes was found in 6.7 to 20% of the fecal samples analyzed (26, 56), and also in sewage sludge (52). De Luca et al. (15) found Listeria in sewage sludge and concluded that fertilizing land with this material for vegetable farming could present potential health risks. In Italy and The Netherlands, L. monocytogenes has been detected in sewage treatment-plant effluents (4, 16). In the UK, in 1992, 1,029,555 tons (dry solids) of sewage sludge were generated, and over 460,000 tons of it were applied to agricultural land (36). Even greater amounts of farm animal waste are applied to land. In the UK, some 21 million tons (dry solids) of farm animal waste are spread annually on the land (39, 40).

In some foodborne outbreaks linked to the consumption of raw fruits and vegetables, epidemiological investigations have identified manure as the source of contamination, as in the case of *L. monocytogenes* on cabbage in Canada, and *Salmonella* and *E. coli* O157:H7 on apples used to make apple juice in the USA (42, 54). The occurrence of *E. coli* O157:H7 on fresh produce may also result from field contamination, because of water run-off from nearby cow pastures or exposure to droppings from wild animals (24, 47).

The microbiological processes during composting or aeration of manure are not well understood. Important factors are the increase in temperature to 50–60°C and the treatment time. If the composting process is managed carefully, it will kill those foodborne pathogens that do not form spores (52). However, the adequacy of existing methods of composting and the relevant regulations need to be reviewed (53). In general, increasing the delay between the application of organic fertiliz-

TABLE 3. Pesticide residue levels for (1) Dutch strawberries (domestic and imported) and (2) strawberries monitored in the European monitoring program for pesticide residues in products of plant origin (see for source under pesticide residues)

### (I) Dutch strawberries (domestic and imported)

| Year | The Netherland | łe |
|------|----------------|----|
| rear | i ne nemenanc  | 12 |

|      | Domestic       | 3             |                               |                  | Imported       | 1             |                               |                 |
|------|----------------|---------------|-------------------------------|------------------|----------------|---------------|-------------------------------|-----------------|
|      | Nr. of samples | No<br>residue | Residue<br>< MRL <sup>1</sup> | Residue<br>> MRL | Nr. of samples | No<br>Residue | Residue<br>< MRL <sup>1</sup> | Residue<br>>MRL |
| 1993 | 381            | 30%           | 67%                           | 2.9%             | 51             | 28%           | 55%                           | 17.7%           |
| 1994 | 493            | 38%           | 53%                           | 8.1%             | 87             | 17%           | 72%                           | 10.3%           |
| 1995 | 547            | 48%           | 48%                           | 4.0%             | 97             | 19%           | 71%                           | 10.3%           |
| 1996 | 697            | 33%           | 64%                           | 2.6%             | 60             | 30%           | 47%                           | 23.3%           |
| 1997 | 900            | 47%           | 51%                           | 3.3%             | 89             | 23%           | 63%                           | 14.6%           |

#### (2) European strawberries

| Year | Commodities  | European Union |               |                  |                  |
|------|--|----------------|---------------|------------------|------------------|
|      |  | Nr. of samples | No<br>residue | Residue<br>< MRL | Residue<br>> MRL |
| 2001 | Strawberries   | 1652           | 46%           | 51%              | 3.3%             |
| 2001 | Strawberries, apples, tomatoes, lettuces, table grapes | 9868           | 51%           | 47%              | 2.2%             |
| 2000 | Strawberries, apples, tomatoes, lettuces, table grapes | 3737           | 80%           | 17%              | 2.7%             |
| 1999 | Strawberries, apples, tomatoes, lettuces, table grapes | 4707           | 69%           | 22%              | 8.7%             |
| 1998 | Strawberries, apples, tomatoes, lettuces, table grapes | 3836           | 66%           | 32%              | 2.0%             |
| 1997 | Strawberries, apples, tomatoes, lettuces, table grapes | 6021           | 65%           | 34%              | 1.1%             |

ers and harvest could reduce the occurrence of foodborne pathogens on fruits and vegetables. More evidence is needed to establish the minimum delay necessary for pathogens to be completely eliminated.

Usually, vegetative pathogenic bacteria and viruses decline in numbers within a few days of their introduction into the soil (17, 22, 51, 56, 58) or presence on plant surfaces (32, 43) although they may survive (in low numbers) for several weeks or months (1, 9, 17, 58). Survival in the soil is influenced by several factors, e.g. soil type, humidity, temperature and competing microflora (7, 17, 51). E. coli O157:H7 has been found to survive in bovine and ovine manure for periods from several weeks up to 12 months, depending on environmental conditions (21, 33).

Plant protection products. Chemical biocides are generally used to protect plants against pests and disease agents. Even though substances authorized for this purpose have undergone extensive safety evaluations, there is consumer concern about their need and safety. These substances are not authorized for use in organic production of fruits and vegetables and this has stimulated the development of alternative control methods based on microorganisms or their metabolites. A wide range of microorganisms are used in biological control, including members of Bacillaceae, Micrococcaceae, Streptomyces, Trichoderma, fungi, viruses, Lactobacillaceae, and the Pseudomonas group (31). Strains of Bacillus thuringiensis, or its bioactive crystalline protein, have been used for the control of insects (57). B. thuringiensis is also permitted in organic production of fruits and vegetables, and the gene for the active protein has been inserted into GM-plants for insect control. The genomic structure of B. thuringiensis is similar to that of Bacillus cereus, and discrimination between these organisms is largely based on the possession by B. thuringiensis of the crystalline protein. B. thuringiensis strains used for pest control have been found to express an enterotoxin similar to that of B.cereus (14).

Viruses also have a long tradition for controlling pests and mites. A well-known example is the use of Baculo viruses against arthropods (25).

Antibiotic substances such as kasugamycin, octhilinone, oxytetracycline, validamycin, polyoxin, and streptomycin are used for plant protection in some countries. The emerging risk related to this practice was discussed in an opinion of the EU Scientific Steering Committee on antibiotic resistance (49). Their advice was not to use antibiotics as plant production agents.

#### **Harvest**

Strawberries are mainly harvested by manual picking. However, mechanical picking has been considered. For example, a mechanical harvester for strawberries was developed in Sweden between 1986 and 1990 (http:// www.actahort.org/books/348/ 348\_35.htm). The harvester was based on experience of similar harvesters in the United States and Canada. The entire plant is cut at ground level and the leaves are removed inside the machine with the help of cross-flow fans. In a second step, the clusters of fruit become separated when the stems are raised in an air flow and hedgers cut them off. The harvester is designed to work in solid-bed plantations. In commercial trials, ten tons of fruit per hectare have been harvested. In some years, it has been possible to harvest 80% of the ripe fruit. The development of graymold fruit rots affects the quality and quantity of fruit harvested. In dry years, it has been possible to start harvest more than ten days after the primary berries are ripe. The harvested product can then be used for juice with no further sorting. Processors of quality products prefer the unripe berries to be removed. For jam or similar products, it is necessary to decap the berries. At this time, mechanical decapping is not practiced.

Fruits and vegetables can become contaminated with pathogenic microorganisms during harvesting through fecal material, human handling, harvesting equipment, transport containers, wild and domestic animals, air, transport vehicles, ice or water (6). In an investigation of several foodborne illnesses associated with fresh produce (NACMCF, 1999a), agricultural workers were often the most likely source of contamination. Lack of suitable sanitary hand-washing facilities in the production area can create a potential hygienic problem. This appears to be particularly important in the transmission of enteric viruses, such as hepatitis virus. Beuchat (6, 7) referred to outbreaks of illness due to Shigella flexneri and hepatitis A, which could be traced to infected people working in the fields or the packaging facility.

Harvesting at the appropriate time and keeping the harvested product under controlled environmental conditions will help retard growth of post-harvest spoilage organisms and pathogens.

#### **POST-HARVEST MEASURES**

Post-harvest treatment of fruits and vegetables includes handling, storage, transportation and cleaning. During these practices, conditions may arise that lead to cross contamination of the produce from other agricultural materials or from the workers. Environmental conditions and transportation time will also influence the hygienic quality of the produce prior to processing or consumption.

Poor handling can damage fresh produce, rendering the product susceptible to the growth and/or survival of spoilage and pathogenic microorganisms. This damage can also occur during packaging and transport. The presence of cut and damaged surfaces provides an opportunity for microbial contamination and growth, as well as ingress of microbes into plant tissues (20).

#### Decontamination

Strawberries are characterized by a very short post-harvest life because of fungal decay and deterioration in appearance and texture. In order to prolong postharvest shelf life and the quality of these fruits, several decontamination techniques have been tested. They include washing with a solution containing disinfectants, gamma irradiation and modified atmosphere packaging.

Natural survival. Survival of Escherichia coli O157:H7 was studied on strawberries, together with the effect of washing with disinfectants (30). Strains inoculated onto the surfaces of strawberries did not multiply during subsequent storage at ambient temperatures. Actually, a small decrease in numbers was observed.

To ascertain the potential for pathogenic enteric viruses to survive on strawberries, the fruit was inoculated with poliovirus and tested for survival (34). It took a storage period of up 8.4 days before a one-log<sub>10</sub> reduction was observed.

Disinfectants. Dipping of inoculated strawberries in water alone reduced the levels of pathogens by approximately 0.8 log, units. None of the disinfectant compounds used (NaOCl, acetic acid, Na,PO, and H<sub>2</sub>O<sub>2</sub>) reduced the numbers of *E. coli* O157:H7 by more than  $2 \log_{10} \text{ units } (30)$ .

El-Ghaouth et al. (18) studied the effect of chitosan coating. Strawberries were inoculated with a mixture of spores of Botrytis cinerea and Rhizopus stolonifer and then coated with chitosan solution (10 and 15 mg/ml respectively). After storage for 14 days at 13°C, the coating markedly reduced decay by both mold species. It was concluded that the reduction in decay was related to the fungistatic properties of chitosan.

Irradiation. Brecht et al. (8) investigated the effects of gamma irradiation (100 and 200 krad). Irradiation did not influence the color of strawberries. Treatment delayed decay by Rhizopus and Botrytis molds, but resulted in a clear softening of

Modified atmosphere packaging Modified atmosphere packaging (MAP) (7% O, and 20% CO, ) did not influence the color of strawberries. However, there was little delay in spoilage by several strawberry pathogens (8). The effects of different atmospheres (low O2 and high CO2) on biological changes and growth of fungal pathogens were also studied by Chambroy and colleagues (11). At 20°C, control of fungal development was impossible, regardless of the composition of the surrounding atmosphere. At 10°C and with CO<sub>2</sub> concentrations of >10%, a reduction in the development of mold decay was observed. Under these conditions, the strawberries had a better appearance and firmer texture.

Despite all the attempts made to decontaminate strawberries, no practical control measure has been devised. One of the main reasons is that strawberries are so sensitive to damage during treatments such as washing and irradiation. There is also a consumer preference for 'natural' produce that has received minimal treatment. This means that Good Agricultural Practice (GAP) must be the main factor in controlling contamination. Cooling of the fruit is actually the only acceptable means of increasing shelf life.

# Cooling

Immediately after harvest, strawberries must be chilled by forced-air cooling to a temperature of 7°C or less. Hydrocooling (flooding them with chilled water) is not recommended because wet berries are much more susceptible to decay. Cooling with crushed or 'liquid' ice would be even worse because, in this case, the berries are likely to sustain physical damage.

It is never sufficient simply to place the packaged strawberries in a chill room and allow them to cool gradually. For palletized loads, the cooling process would take too long, so that fruits at the center of the pallet would not be adequately cooled and would start to decay. Without forced movement of the cooling air, the heat from plant respiration would destroy the fruit.

#### LAWS AND REGULATIONS

Codex Alimentarius. Good Hygienic Practice (GHP) as defined in the Codex document on "General Principles of Food Hygiene," in combination with HACCP, as the basis for safe food production (12). A code of "Hygienic Practices for Fresh Fruits and Vegetables" including an Annex on "Ready-to-Eat Fresh Pre-cut Fruits and Vegetables" has been elaborated by the Codex Alimentarius Committee on Food Hygiene.

The Codes were initiated in response to the growing concern about fresh fruits and vegetables being a possible source of foodborne pathogens. They address Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) that will help to control microbial, chemical and physical hazards at all stages of the production of fresh fruits and vegetables, from primary production to packing. The following areas are acknowledged to be important in connection with microbial safety: environmental hygiene, hygienic production (water, manure, soil, agricultural chemicals, biological control, indoor facilities and personal hygiene), handling, storage, transport, cleaning, maintenance and sanitation.

The draft code follows the format of the Codex code on General Principles of Food Hygiene. It addresses hazards to be managed by the producer according to GAP and GMP. It does not generally define measures to be taken or criteria to be observed.

Food and Drug Administration (FDA) and United States Department of Agriculture (USDA). As guidance for the US industry (19), the FDA and USDA define procedures to be followed with respect to microbial food safety hazards and good agricultural and management practices common to growing, packaging and transport of fruits and vegetables. The guide focuses on the quality of water used for different purposes, manure and municipal biosolids, worker health and hygiene, field sanitation, packaging-facility sanitation, transportation and traceability.

European Union (EU). The European Commission has produced several directives, regulations and recommendations related to the production and consumption of fresh strawberries. They include:

> Pesticide residues in foodstuffs of animal origin: Council Directive 86/363/EEC, Official

- Journal No L 221, 07/08/1986 p. 0043 - 0047
- Fixed maximum levels for pesticide residues in and on products of plant origin: Council Directives 86/362/EEC, Official Journal No. L 221, 07/08/1986 p. 0037 - 0042 Amended Council Directives 90/642/EEC, Official Journal No. L 350, 14/12/1990 p. 0071 - 0079
- Inspections and monitoring: Council Directive 89/397/EEC, Official Journal No. L 186, 30/06/ 1989 p. 0023 - 0026
- Additional measures concerning the official control of foodstuffs: Council Directive 93/99/ EC, Official Journal No. L 290, 24/ 11/1993 p. 0014 - 0017
- Sampling: Commission Directive 79/700/EEC, Official Journal No. L 207, 15/08/1979 p. 0026 - 0028
- Specific EU coordinated monitoring programme: Commission Recommendation 2001/42/ EC, Official Journal No. L 11, 16/ 01/2001 p. 0040 - 0045
- Requirement of Member States to report to the Commission the results of the monitoring programme for pesticide residues carried out: Article 7 of Council Directive 86/362/EEC and Article 4 of Council Directive 90/ 642/EEC, as amended by Council Directive 97/41/EC, Official Journal No. L 184, 12/07/1997 p. 0033 - 0049
- Detailed implementing rules for the monitoring provisions: Commission Regulation (EC) No. 645/2000, of 28 March 2000, Official Journal No. L 78, 29/03/ 2000, p. 0007 - 0009
- Laying down the marketing standard for strawberries and amending Regulation (EEC) No 899/87: Commission Regulation (EC) No. 834/2002 of May 21, 2002, Official Journal No. L 134,24 22/05/2000, p. 0024 -0028.

#### **CONCLUSIONS**

The results of this risk profile reveals that strawberries have a healthful image and are appreciated by consumers. The consumption rate of strawberries continues to rise. The results also indicate that strawberries intended for fresh consumption have a relatively good safety record. Only a few strawberry-related outbreaks have been reported, and most of them are associated with consumption of frozen strawberries. Nevertheless, the quality and consumer aspects need attention and further studies are required.

Monitoring data for pesticide residues show that the majority of strawberries tested do not contain detectable residues. Only a small percentage exceed the Maximum legally permissible Residue Level (MRL). Acceptable Daily Intakes (ADI) values were not exceeded, indicating that there is no acute risk.

Microbiological examination of fresh strawberries reveals that pathogenic infectious organisms are rarely present. Nevertheless, in various stages of the production process of strawberries, contamination with pathogenic microorganisms may occur. Because no practical decontamination methodologies are available, attention needs to be paid to preventing contamination. Therefore, it is recommended that well-developed codes of good agricultural practices be used, in which land and water use, application of organic and inorganic fertilizers, and animal and pest control are described, together with recommended harvesting and cooling practices and measures for ensuring worker health and safety.

#### **REFERENCES**

- I. Al-Ghazali, M. R., and S. K. Al-Azawi. 1990. Listeria monocytogenes contamination of crops grown on soil treated with sewage sludge cake. J. Appl. Bacteriol. 69:642-647.
- 2. Anonymous. 1997. Hepatitis A associated with consumption of frozen strawberries-Michigan, March 1997. MMWR weekly. 1997. 46 (13)288-295.
- 3. Anonymous. 1998. Outbreak of Cyclosporiasis —Ontario, Canada, May 1998. MMWR weekly, 1998, 47(38)806-809.
- 4. Bernagozzi, M., F. Bianucci, R. Sachetti, and P. Bisbini. 1994. Study of the prevalence of Listeria spp. in surface water. Zbl. Hyg. 196:237-244.
- 5. Bertelson, D. 1995. The US strawberry industry. Statistical Bulletin Number 914, United States Department of Agrigulture, Economical Research Service. http://www.nal. usda.gov/pgdic/Strawberry/ers/ ers.htm.

- 6. Beuchat, C. R. 1995. Pathogenic microorganisms associated with fresh produce. J. Food Prot. 59:204-216.
- 7. Beuchat, L. R. 1998. Surface decontamination of fruits and vegetables eaten raw: A review. Food Safety Unit, World Health Organization WHO/FSF/FOS/98.2.
- 8. Brecht, J. K., S.A. Sargent, J.A. Bartz, K. V. Chau, and J. P. Emond. 1992. Irrdiation plus modified atmosphere for storage of strawberries. Proceedings of the Florida State Horticultural Society 105:97-100.
- 9. Bryan, F. L. 1977. Diseases transmitted by foods contaminated by wastewater. J. Food Prot. 40:45-56.
- 10. Castillo Martín, A., J. Cabrera Jordán, M. Fernández Artigas, B. García Villanova Ruiz, J. Hernandez Ruiz, J. Laguna Sorinas Nogales, R. Vargas-Machuca, and J. Picazo Muñoz. 1994. Criterios para la evaluación sanitaria de proyectos de reutilización directa de aguas residuales urbanas depuradas. Junta de Andalucía, Sevilla.
- 11. Chambroy, Y., M. H. Guinebreterie, G. Jacquemin, M. Reich, L. Breuils, and M. Souty. 1993. Effects of carbon dioxide on shelf-life and post harvest decay of strawberries fruit. Science des Aliments 13:409-423.
- 12. Codex Alimentarius, 1997, Food Hygiene-Basic Texts-General Principles of Food Hygiene, HACCP Guidelines, and Guidelines for the Establishment of Microbiological Criteria for Foods 1997. ISBN 92-5-104021
- 13. Cook, R. 2002. Strawberry production in the United States-1990-2000. Department of Agriculture and Resource Economics, UC Davis June 2002. (http://postharvest. ucdavis.edu/pubs/strawberriesfinal | Sept02.pdf.
- 14. Damgard, P.H. 1995. Diarrhoreal enterotoxin production by strains of Bacillus thuringiensis isolated from commercial Bacillus thuringiensisbased incectecides. FEMS Immun. Med. Microbiol. 12:245-250.
- 15. De Luca, G., F. Zanetti, P. Fateh-Moghadm, and S. Strampi. 1998. Occurrence of Listeria monocytogenes in sewage sludge. Zent. Bl. Hyg. umweltmed. 201:269-177.
- 16. Dijkstra, R. 1989. Ecology of Listeria. Microbiol. Alim. Nutr. 7:353-359.
- 17. Dowe, M. J., E. D. Jackson, J. G. Mori, and C. R. Bell. 1997. Listeria mono-

- cytogenes survival in soil and incidence in agricultural soils. I. Food Prot. 60:1201-1207.
- 18. El-Ghaouth, A., J. Arul, J. Grenier, and A. Asselin. 1992. Antifungal activity of chitosan on two postharvest pathogens of strawberry fruit. Phytopathology 82:398-402.
- 19. FDA. 1998. Guidance for Industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (http://vm.cfsan.fda.gov/ ~lrd/fr981029.html).
- 20. Francis, G. A., C. Thomas, and T.O'Beirne. 1999. The microbiological safety of minimally processed vegetables. Internat. I. Food Sci. and Technol. 34:1-22.
- 21. Fukushima, H., K. Hoshina, and M. Gomyoda. 1999. Long-term survival of shiga-toxin producing Escherichia coli O26, O111, and O157 in bovine faeces. Appl. Environ. Microbiol. 65:5177-5181.
- 22. Geldreich, E. E., and R. H. Bordner. 1971. Fecal contamination of fruits and vegetables during cultivation and processing for market. A review. J. Milk Food Technol. 34:184–195.
- 23. Health Council of the Netherlands. 2000. Foodborne infections nr 2000/09. Dutch with executive English summary (for ordering order@gr.nl).
- 24. Hilborn, E. D., J. H. Mermin, P. A. Mshar, J. L. Hadler, A. Voetsch, C. Wojtkunski, M. Swartz, R. Mshar, J. A. Lambert-Fair, M. Farrar, M. K. Glynn, and L. Slutsker. 1999. A multistate outbreak of Escherichia coli O157:H7 infection associated with consumption of mesclun lettuce. Arch. Intern. Med. 159:1758-1764.
- 25. Hunter-Fujita, F. R., P. F. Entwistle, H. F. Evans, and N. E. Crook. 1998. Insect, viruses and pest management. John Wiley & Sons. Inc. New York.
- 26. Husu, J. R. 1990. Epidemiological studies on the occurence of Listeria monocytogenes in the faeces of dairy cattle. J. Vet. Med. B. 37:276-282.
- 27. Hutin, Y. J. F., V. Pool, H. Elaine, E. H. Cramer, O. V. Nainan, J. Weth, I. T. Williams, S. T. Goldstein, K. F. Gensheimer, B. P. Bell, C. N. Shapiro, M. J. Alter, and H.S. Margolis. 1999. A multistate, foodborne outbreak of hepatitis A. New Eng. J. of Medicine 340:595-602.

- 28. Improving the safety and quality of fresh fruits and vegetables: A training manual for trainers http:// ucce.ucdavis.edu/freeform/UC\_ GAPs/documents/Other\_ Training\_Resources2659.pdf.
- 29. Johannessen, G.S., S. Loncarevic, and H. Kruse. 2002. Bacteriological analysis of fresh produce in Norway. Inter. J. Food Microbiol. 77:199-204.
- 30. Keshun, Yu, M. C. Newman, D. D. Archbold, and T. R. Hamilton-Kemp. 2002. Survival of Escherichia coli O157:H7 on strawberry fruit and reduction of the pathogen population by chemical agents. J. Food Prot. 65:1334-1340.
- 31. Kirschbaum, J.B. 1985. Potential implication of genetic engineering and other biotechnologies to insect control. Ann. Rev. Entomol. 30:51-
- 32. Kott, H., and L. Fishelson. 1974. Survival of enteroviruses on vegetables irrigated with chlorinated oxidation pond effluents. Israel J. Technol. 12: 290-297.
- 33. Kudva, I.T., K.Blanch, and C. J. Hovde, 1998. Analysis of Escherichia coli O157:H7 survival in ovine and bovine manure and manure slurry. Appl. Environ. Microbiol, 64: 3166-3174.
- 34. Kurdziel, A. S., N. Wilkinson, S. Langton, and N. Cook. 2001. Survival of poliovirus on soft fruit and salad vegetables. J. Food Prot. 64: 706-709.
- 35. Lund, B. M. 1986. Anaerobes in relation to foods of plant origin. pp. 351-372. In Anaerobic bacteria in habitats other than man (eds E.M. Barnes and G.C. Mead). Blackwell Scientific Publications, Oxford.
- 36. Maule, A. 2000. Survival of verocytotoxigenic Escherichia coli O I 57 in soil, water and on surfaces. J. Appl. Microbiol. 88: 71S-78S.
- 37. Milazzo, A., and N. Rose. 2001. An outbreak of Salmonella Typhimurium phage type 126 linked to a cake shop in South Australia. Communicable Diseases Intelligence, 25, No. 2, April 2001.
- 38. Modise, D. M., C. J. Wright, and R.Watson. Regulation of strawberry fruit quality through water stress; Division of Agriculture & Horticulture, School of Biological Sciences, University of Nottingham. (http://

- www.nottingham.ac.uk/biosciences/ah/posters/pdf/strawberry. pdf).
- 39. NACMCF. 1999a. National Advisory Committee on Microbiological Criteria for Foods. Microbiological safety evaluations and recommendations on fresh produce. Food Control 10:117-143.
- 40. NACMCF. 1999b. National Advisory Committee on Microbiological Criteria for Foods. Microbiological safety evaluations and recommendations on sprouted seeds Internat. I. Food Microbiol. 52:123-153.
- 41. Nguyen-the, C., and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. Crit. Rev. Food Sci. Nutr. 34:371-401.
- 42. Nguyen-the, C. and F. Carlin. 2000. Fresh and processed vegetables. (pp. 620-684). In The microbiological safety and quality of foods. B.M. Lund, T.C. Baird-Parker and G.W. Gould (eds). Aspen Pub. Gaithersburg.
- 43. Nichols, A.A., P.A. Davies, and K.P. King. 1971. Contamination of lettuce irrigated with sewage effluent. |. Hort. Sci. 46:425-433.
- 44. Nutrition facts: Strawberies: County of Los Angeles Public Health Nutrition Program. Division of Health Promotion and Binational/Border Health.www.lapublichealth.org/nutrition.
- 45. Owen, K., V. Wright, and G. G. Griffith. 2000. Quality, uncertainty and consumer valuation of fruits & vegetables. Australian Agribusiness review, 8, paper 4, ISSN 1442-6951
- 46. ProMED Mail, April 7, 1997. Archive number 19970407.0731

- 47. Rice, D. H., D. D. Hancook, and T. E. Besser. 1995. Verotoxigenic E. coli O157 colonisation of wild deer and range cattle. Vet. Rec. 137:524.
- 48. Scalzo, J., F. Capocasa, A. Aplandrani, B. Mezzetti, and M. Battino. 2003. Quality and nutritional value in strawberry breeding and variety evaluation. COST 836 FINAL WORKSHOP. Towards an Organization of the Integrated Research in Berries: Model for a Strawberry of Quality, in Respect with the Environment Rules and Consumers' Requirements' Euro berry symposium, Ancona, Italy, October 9 to 11, 2003.
- 49. SSC (Scientific Steering Committee) 1999. Opinion on antimicrobial resistance expressed on 28 May 1999. European Commission, Brussels.
- 50. Sterling, C. R., and Y. N. Ortega. 1999. Cyclospora: An enigma worth unraveling. Emerging Infectious Diseases, volume 5 number | (http:// www.cdc.gov/ncidod/eid/vol5no1/ sterling.htm).
- 51. Tierney, J.T., R. Sullivan, and E.P. Larkin. 1977. Persistence of Poliovirus I in soil and on vegetables grown in soil previously flooded with inoculated sewage sludge or effluent. Appl. Environ. Microbiol. 33:109-113.
- 52. Strauch, D. 1991. Survival of microorganisms and parasites in excreta, manure and sewage sludge. Rev. Sci. Tech. Off. Int. Epiz. 10:816-846.
- 53. Tanne, D., M. Haim, U. Goldbourt, V. Boyko, R. Doolman, Y. Adler, D. Brunner, S. Behar, and B.-A. Sela. 2003. Prospective study of serum homocysteine and risk of ischemic

- stroke among patients with preexisting coronary heart disease. Stroke 34:632-636.
- 54. Tauxe R. 1992. Epidemiology of Campylobacter jejuni infections in the United States and other industrialized nations, p. 9-19. Nachamkin I., M. J. Mlaser, and L. S. Tompkins. Campylobacter jejuni: Current status and future trends. Washington, D.C. Am. Soc. Microbiol.
- 55. Unwin, N., R. Thomson, A. M. O'Byrne, M. Laker, and H. Armstrong. 1998. Implications of applying widely accepted cholesterol screening and management guidelines to a British adult population: cross sectional study of cardiovascular disease and risk factors. British.
- 56. Van Renterghem, B., F. Huysman, R. Rygole, and W. Verstraete. 1991. Detection and prevalence of Listeria monocytogenes in the agricultural ecosystem. J. Appl. Bacteriol. 71:211-217. Medical Journal, 317: 1125-1130.
- 57. Veaeck, M., A. Reynaerts, H. Höfte, S. Jansens, de M. Beuckeleer, C. Dean, M. Zabeau, M. van Montagu, and I. Leemans. 1987. Transgenic plants protected from insect attack. Nature 328:33-36.
- 58. Watkins, J., and K. P. Sleath. 1981. Isolation and enumeration of Listeria monocytogenes from sewage, sewage sludge and river water. J. Appl. Bacteriol. 50:1-9.
- 59. WHO Surveillance Program for Control of Foodborne Infections and Intoxications in Europe, 2001. Seventh report 1993-1998. Published by Bundesinstitut für Risiko Bewertung, Diedersdorferweg 1 D-Berin, Germany.