Downloaded via 64.124.147.2 on November 17, 2022 at 02:56:51 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

Chapter 1

Foods and Food Ingredients Produced via Recombinant DNA Techniques

An Overview

Karl-Heinz Engel[1], Gary R. Takeoka[2], and Roy Teranishi[2]

[1]Federal Institute for Health Protection of Consumers and Veterinary Medicine, Postfach 330013, D–14191 Berlin, Germany
[2]Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, CA 94710

Commercial products resulting from recombinant DNA techniques in agricultural biotechnology have just entered or are about to enter the market. Some of the progress in improving nutrition and acceptability of foods as well as improving herbicide, insect, and virus resistance of plants are discussed. Enzymes and microorganisms can now be more precisely altered to optimize their roles in food production. Genetic modification of animals is much more complex than that of microorganisms and plants, nevertheless, some interesting progress is being made. The applications of genetic engineering in food production are evoking positive and negative reactions. The concepts presently being developed to assess the safety of foods derived from modern biotechnology are outlined. The aspects involved in creating a framework for regulatory oversight on genetically modified foods are also discussed.

The use of microorganisms in the production of foods and beverages, such as bread, cheese, wine, or beer has a long tradition dating back to ancient days. Industrial biotechnology has its roots in the classical production of fermented foods. Therefore, it is a logical consequence that the application of the so-called "modern biotechnology" does not remain limited to areas such as medicine and pharmaceuticals but is increasingly applied in the production of foods and food ingredients. Developments in genetic engineering have created new dimensions in classical biotechnology. By using recombinant DNA techniques, it has become possible to direct the movements of specific and useful segments of genetic material between unrelated organisms, thereby crossing the barriers between plants, animals, and microorganisms. The scope of "modern biotechnology" goes beyond the traditional area of food fermentation processes. It is now possible to make specific genetic modifications in plants and animals that introduce traits or substances that could not be introduced by traditional methods.

0097–6156/95/0605–0001$12.00/0
© 1995 American Chemical Society

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

After fascinating developments in recent years on the level of basic research, the applications of recombinant DNA techniques in agricultural biotechnology are now heading towards attractive businesses. Commercial products have just entered or are about to enter the market. The spectrum of applications is comprised of genetically modified crops, foods produced from genetically modified microorganisms, as well as food ingredients/additives obtained from genetically modified organisms.

Plants

The enormous advances in genetic engineering of plants are due to progress in both cellular and molecular biology. The first practical technique for introduction of genetic material into plants was based on the use of vectors from *Agrobacterium tumefaciens*. Due to the narrow host range of this soil bacterium, the scope of the method had been limited to dicotyledonous plants. The progress in cell biology enabling the recovery of intact and fertile plants from single cell and protoplast cultures stimulated research activities in the area of direct DNA transfer. Because it is possible to introduce foreign DNA into protoplasts after electroporation or polyethyleneglycol treatment, the range of plants accessible to genetic engineering was increased significantly. A further decisive breakthrough was achieved by the development of the biolistics approach involving bombardment of the plant tissue with DNA-coated metal particles. It is now possible to engineer almost all important legumes and cereals (1-5).

Genetic engineering offers the possibility not only to add new traits to an organism but also to down-regulate the activities of specific endogenous genes. A versatile and useful method for such a blocking of decisive steps in metabolic pathways is the so-called "antisense technique" (6). The methodology is based on the introduction of an oligonucleotide which is transcribed into messenger-RNA (m-RNA) consisting of sequences complementary to the m-DNA produced through the transcription of the endogenous target gene. Due to the interaction (hybridization) of "sense" and "antisense" RNA, the translation of the corresponding enzyme is strongly reduced. The first genetically modified crop approved for food use, the Flavr-Savr™ tomato, is a prominent example of the commercial exploitation of the "antisense" technology (7).

Analogous to the goals of conventional breeding programs, recombinant DNA techniques are applied to improve (i) agronomic characteristics of crops, such as yield and resistance to diseases and pests, (ii) processing parameters, e.g., optimum solids levels or increased shelf life, and (iii) food quality, including factors such as aroma, taste, and nutritional value. Currently, the majority of commercial applications of plant genetic engineering aim at increasing the yield of food crops by influencing their tolerances to specific herbicides and their resistance to insects and diseases.

Herbicide tolerance. One of the agronomically and commercially important applications, and at the same time an application of genetic engineering strongly opposed by consumer advocates, is the attempt to increase crop yields by increasing the tolerance of plants to particular herbicides. Strategies applied to achieve this goal demonstrate the diversity of tools offered by recombinant DNA

Motif Exhibit 1013, Page 2 of 10

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

techniques: (i) increasing the level of the target enzyme for the herbicide by overexpression, (ii) decreasing the sensitivity of the target enzyme by introducing a gene coding for an enzyme resistant to the herbicide either naturally (from a microbial source) or through specific mutation, and (iii) introducing a gene encoding an enzyme metabolizing and thus detoxifying the herbicide (8, 9). Soybeans tolerant to the herbicide glyphosate, oilseed rape tolerant to phosphinotricin, and cotton exhibiting tolerance to bromoxynil are examples of genetically modified crops presently entering the market (3, 10).

Insect resistance. The use of the bacterium *Bacillus thuringiensis* (*B.t.*) to control plant insect pests has long been known (11). The insecticidal properties are due to the biosynthesis of different toxic crystal proteins. These pro-toxins are proteolytically cleaved in the midgut of the insects, bind to specific membrane receptors and finally lead to the death of the insect. The different strains of *B.t.* produce specific toxins differing in their activity against various classes of insects, such as the larval stages of *Lepidoptera* (moths and butterflies), *Diptera* (flies), and *Coleoptera* (beetles). By means of recombinant DNA techniques it is possible to create plants expressing the *B.t.* δ-endotoxins, thus making them resistant to insect damage. This concept has been applied successfully in commercially important crops, such as cotton, potato, tomato, and corn (3).

Virus resistance. Agronomically important results have been achieved by creating plants resistant to viral infections. The most successful approach, the coat protein mediated protection, is based on the long known observation that infection of a plant with a mild strain of a virus protected it from subsequent infection of a more virulent strain. By means of recombinant DNA techniques it has been possible to express the viral coat protein in plants, thus protecting them against viral diseases. From the first demonstration that the expression of the coat protein gene of the tobacco mosaic virus in tobacco conferred resistance to infection by this virus (12), this approach has been successfully applied in a wide spectrum of plant species (13).

Plants can also be genetically engineered to express proteins which increase their resistance to attacks by fungi or bacteria (14). The increasing understanding of the molecular basis for the phenomenon of "systemic acquired resistance" (15) offers promising applications of recombinant DNA techniques. The strategy involving the expression of bactericidal enzymes from heterologous sources in plants is exemplified by the bacteriophage T4 encoded lysozyme reported to give rise to increased resistance of potatoes to *Erwinia carotovora* (16). Transgenic tobacco expressing a stilbene synthase from peanut was more resistant to infections of *Botrytis cinerea* (17).

Food Quality. Texture, taste, and aroma belong to the major criteria determining the acceptance of a food by consumers. For tomatoes recombinant DNA techniques have proven to be suitable to improve these important attributes. By introducing either the "antisense" (7) or a truncated "sense" (18) polygalacturonase gene, the biosynthesis of this cell wall enzyme, responsible for the breakdown of pectin during ripening, is slowed down. In terms of processing

Motif Exhibit 1013, Page 3 of 10

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

characteristics, this results in optimum solids levels and viscosity. The reduced softening process can also be used either to increase the shelf life of the tomato or to improve its flavor properties by allowing the fruit to remain longer on the vine.

The other approach applied to improve the flavor characteristics of tomatoes is to interfere directly with the metabolism of ethylene, the compound which triggers fruit ripening and aroma formation. By applying the "antisense" technology it is possible to inhibit either the 1-amino-cyclopropane-1-carboxylate (ACC) synthase, the rate-limiting enzyme in the biosynthetic pathway of ethylene (19) or the ACC oxidase, the enzyme involved in the conversion of ACC to ethylene (20).

Potential future strategies to influence flavor properties of a plant have been demonstrated by results of the genetic transformation of a scented *Pelargonium* species, referred to as "lemon geranium". Transformation by means of *Agrobacterium rhizogenes* increased the production of essential oil and significantly changed the distribution of monoterpene alcohols (21).

Nutritional Quality. An area which will attract increasing attention in the near future is the improvement of the nutritional value of foods by means of recombinant DNA techniques. Pioneering examples for changing content and composition of the macronutrients (fats, protein and carbohydrates) have already been described. The direction of fatty acid biosyntheses in favor of medium-chain fatty acids can be achieved by expression of a 12:0-acyl-carrier protein thioesterase in transgenic oilseed plants (22). Increasing the level of sulfur-containing amino acids in soybean by introducing a gene from Brazil nut exemplifies the strategy to improve the balance of essential amino acids in important crops (23). However, this project also demonstrates potential limits, such as the influence of the genetic modification on the allergenicity of the host plant (24). The modification of carbohydrate metabolism by recombinant DNA techniques has been demonstrated for starch in potatoes. The composition of this biopolymer, i.e. the ratio of amylose to amylopectin, as well as its amount in the tubers can be influenced (25,26).

Plants are increasingly being considered as "bioreactors" for production of industrially or pharmacologically important substances, such as proteins (27). Compared to microorganisms the eukaryotic plant cells offer the advantage that the post-translational processing necessary for many valuable proteins can be accomplished. Correctly processed human serum albumin could be obtained from transgenic potatoes (28).

Microorganisms

Microorganisms have played an important role in food production for millennia. The transition from the empirical use of microorganisms to an understanding of the underlying scientific principles was initiated by the pioneering discoveries of Pasteur in the middle of the last century. With the increase of knowledge there have always been attempts to optimize and standardize the microorganisms used in order to meet the requirements of food production. The modern food industry can make use of a spectrum of well-defined "starter-

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

cultures" for the production of fermented foods (29). They are the result of mutagenesis and selection techniques based on classical bacteriological and genetic methods. By means of genetic engineering, the properties of microorganisms can be changed more precisely (30). Major goals are optimization of the production process, improvement of product quality, and safety (hygienic status), and enlargement of product diversity.

Strategies applied are based on (i) increasing the copy number of the gene of interest, (ii) coupling genes with strong promoters or other regulatory sequences, and (iii) expressing genes from other sources in microorganisms which are safe for use in food production (GRAS) and which are easily handled under commercial fermentation conditions. There is a broad array of recombinant microorganisms available for industrial and agricultural applications (31).

Because of its importance both as a model organism in basic research and as a production microorganism in the baking and brewing industry, the yeast *Saccharomyces cerevisiae* has attracted considerable attention among molecular biologists (32, 33). In the United Kingdom genetically modified baker's yeast with increased activities of maltase and maltosepermease (34) and a genetically modified amylolytic brewer's yeast (35) have been reviewed for food use.

Lactic acid bacteria play an outstanding role in food fermentation (36). Accordingly, many examples for their genetic engineering have been reported (37, 38). The construction of safe, so-called "food-grade", vectors has been especially and extensively studied with these microorganisms (39).

Enzymes

For many important processes in food production, enzymes rather than intact microorganisms are employed. These biocatalysts possess outstanding properties, such as substrate specificity, regioselectivity, and enantioselectivity. In particular hydrolases which do not require coenzyme regeneration are increasingly being used commercially. Amylases, pectinases or cellulases are applied in the starch and baking industry as well as in fruit juice production. Lipases are employed to modify the properties of triglycerides by hydrolysis, esterification, and interesterification (40).

Enzymes are isolated from animal, plant, and microbial sources. Pure culture fermentations of selected strains of microorganisms are used to obtain enzyme preparations at industrial scale. Genetic engineering offers the possibility to increase the yield of the desired enzyme by introducing multiple copies of the corresponding gene into the production organism or by influencing the regulatory sequences. A major strategy is to introduce the gene encoding the enzyme in safe and efficient microorganisms. Yeasts have been proven to be ideal expression systems for heterologous proteins (41).

One of the most prominent and pioneering examples for an enzyme obtained from genetically modified microorganisms is the milk-clotting protease, chymosin, the first food ingredient produced via recombinant DNA techniques which has been cleared for food use (42).

In the future, applications of recombinant DNA techniques will not be limited to the production of enzymes, which are structurally and functionally identical to their traditional counterparts. Increasing emphasis will be placed on

Motif Exhibit 1013, Page 5 of 10

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

"protein engineering". This allows for the change of the DNA sequence and subsequently the corresponding amino acid at specific positions, thus designing enzymes with new and optimized properties. Adaptation of enzymes to specific conditions, such as pH and temperature, becomes possible. The use of designed proteases and lipases in the detergent industry indicates the future potential of this strategy in food production.

Food Ingredients

Biotechnological fermentation and biotransformation processes have been used for the production of food ingredients, such as vitamins, amino acids, organic acids, or sweeteners (43, 44). By using the strategies described above for the production of enzymes, recombinant DNA techniques can be applied in order to increase the yield and to improve the recovery and the purification of single compounds from fermentation broths (45).

Because of consumers' demand for "natural" flavor, there has been increasing application of biotechnological methods such as fermentations and biotransformations of corresponding precursors in the production of flavor compounds in recent years (46, 47). Therefore, flavor compounds are ideal examples for food ingredients being suitable for the application of recombinant DNA techniques in the course of the production process.

Animals

The commercial use of recombinant bovine somatotropin (rBST), a growth hormone produced from a genetically modified microorganism, is an example for the *indirect* application of recombinant DNA techniques in farm animal production. In contrast to the progress achieved in genetic engineering of microorganisms and plants, *direct* application resulting in transgenic animals will require more years before it reaches the stage of commercialization. Genetic modification of animals is a much more complex task than that of microorganisms or plants (48). Goals of genetic engineering of animals focus on improved growth and on increased resistance to specific diseases (49). Most of the progress has been made with transgenic fish because the efficiency of integration of DNA into fish is much higher than for mammals (50, 51). Goals such as increased production efficiency and improved growth rate have been achieved by introducing mammalian and fish growth hormone genes. The transfer of the antifreeze protein gene from winter flounder to salmon is an example of improving the tolerance of abiotic stresses.

A promising field is the so-called "gene farming", the production of pharmaceutically or nutritionally important proteins in the mammary glands of transgenic animals (52).

Safety Assessment

The outstanding and fast development of genetic engineering and the accelerating transition from basic research to commercial applications provoke both enthusiastic and very strong negative reactions. Today, it is acceptable to produce life-saving pharmaceuticals by means of genetically modified microoganisms. However, the application of this technology in the production of foods is still the subject of controversial discussion.

Motif Exhibit 1013, Page 6 of 10

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

The issues being raised can be divided into three major categories: (i) the fact that this technique touches the fundamentals of life by changing the genetic information of organisms provokes ethical concerns; (ii) the release of viable genetically modified organisms into the environment raises ecological issues; (iii) concerns are expressed whether unexpected or unintentional effects will occur as a result of the genetic modification of crops or microorganisms and whether new substances introduced into food will be safe.

National and international organizations are involved in establishing principles for safety evaluations of foods and food ingredients produced via recombinant DNA techniques. The concept of "substantial equivalence" as developed by the OECD (53) is widely agreed upon. It involves the comparison of a food or food ingredient developed by modern biotechnology to its traditional counterpart. If substantial equivalence can be established, the new food or food component can be treated in a similar manner with respect to safety (54).

Current evaluation procedures of genetically modified foods and food ingredients pay particular attention to the safety implications of (i) intentional changes, (ii) any unintentional changes arising from the genetic modification, (iii) the stability of the genetically modified organism under the intended conditions of use; and (iv) the likelihood of genetic transfer.

There is a general consensus that the applicability of classical toxicological assessment procedures developed for single chemical substances, such as pesticides or food additives, is limited. Safety and wholesomeness studies with whole foods have to be carefully designed in order to avoid nutritional imbalances causing artifacts and uninterpretable results.

Particular attention has been paid to the safety evaluation of marker genes and their respective expression products. Marker genes are needed to identify and select cells which have been successfully transformed at an early stage of the genetic modification process. The most important ones are those conferring resistance to antibiotics and tolerance to herbicides, respectively (10). The safety assessment of the antibiotic resistance marker genes has been the subject of detailed investigations (55); international organizations (10), and national regulatory authorities (56) have discussed this issue.

Another concern being raised is whether there is an increased potential for allergenicity of transgenic foods due to the transfer of new proteins. The above mentioned transfer of a gene from Brazil nut to soybean demonstrates that there are methods available to assess the allergenic potential of proteins derived from sources to which consumers have reacted and for which serum is available (24). However, at present the potential allergenicity of proteins that are derived from sources that are not recognized as allergens cannot be predicted.

Regulatory Aspects

There is a relatively broad consensus about the general scientific principles underlying the safety evaluation of foods and food ingredients produced via recombinant DNA techniques. However, the philosophies about regulatory oversight and the need for legislative restrictions vary in different countries. There are two major approaches: one is based on the assumption that the

Motif Exhibit 1013, Page 7 of 10

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

application of a certain technology, such as genetic engineering, bears the potential for specific risks and therefore requires a corresponding oversight; the second approach primarily focusses on the final product and its safety rather than on the technology applied. These philosophies are reflected in different regulatory frameworks set up around the world, and some of the world leaders discuss their regulatory policies in this book.

Literature Cited:

1. Gasser, C.S; Fraley, R.T. *Science*, **1989**, *244*, 1293-1299.
2. Gasser, C.S; Fraley. R.T., *Scientific American*, **1992**, *6*, 62-69.
3. Beck, C.J.; Ulrich, T. *Bio/Technology*, **1993**, *11*, 895-902.
4. Christou, P. *Agro-Food-Industry-Hi-Tech.*, **1994**, *5*, 17-27.
5. Willmitzer, L., New Developments in Plant Biotechnology. *Lecture Publications*, Vol. 20, **1994**, Ernst Schering Research Foundation.
6. Van Blokland, R.; Van der Geest, N,; Mol, J.N.M.; Kooter, J.M. *The Plant Journal,* **1994**, *6*, 861-877.
7. Sheehy, K.; Kramer, M.; Hiatt, W. *Proc. Natl. Acad. Sci.*, **1988**, *85*, 8805-8809.
8. Mazur, B.; Falco, S. *Ann. Rev. Plant Physiol.* **1989**, *40*, 441-470.
9. Oxtoby, L.; Hughes, M. *Trends in Biotechnology* , **1990**, *8*, 61-65.
10. WHO, Health aspects of marker genes in genetically modified plants. *Report of a World Health Organization Workshop*, **1993**.
11. Peferoen, M. *Agro-Food-Industry-Hi-Tech.* **1991**, *2,* 5-9.
12. Powell-Abel, P.; Nelson, R.S; Be, B.; Hoffmann, N.; Rogers, S.G.; Fraley, R.T.; Beachy, R. *Science*, **1986**, *232*, 738-743.
13. Beachy, R.N. In *Biotechnology in Plant Disease Control*, Wiley-Liss, Inc., **1993**, pp. 89-104.
14. Brears, T.; Ryals, J. *Agro-Food-Industry-Hi-Tech.* **1994**, *5*, 10-13.
15. Lawton, K.; Uknes, S.; Friedrich, L.; Gaffney, T.; Alexander, D.; Goodman, R.; Metraux, J.-P.; Kessmann, H.; Ahl, G.; Gut, R.; Ward, E.; Ryals, J. In *Mechanisms of Defense Responses in Plants*, Fritig, B.; Legrand, M., Editors, Kluwer Academic, Dordrecht, **1993**, pp. 410-420.
16. Düring, K.; Porsch, P.; Fladung, M.; Lörz, H. *The Plant Journal*, **1993**, *3*, 587-598.
17. Hain, R.; Reif, H.-J.; Krause, E.; Langebartels, R.; Kindl, H.; Vornam, B.; Wiese, W.; Schmelzer, E.; Schreier, P.H.; Stöcker, R.H.; Stenzel, K.; *Nature*, **1993**, *361*, 153-156.
18. Smith, C. *Mol. Gen. Genet.*, **1990**, *224*, 477-481.
19. Oeller, P.W.; Min-Wong, L.; Taylor, L.P.; Pike, D.A.; Theologis, A. *Science*, **1991**, *254*, 437-439.
20. Hamilton, A.J.; Lycett, G.W.; Grierson, D. *Nature*, **1990**, *346*, 284-287.
21. Pellegrineschi, A.; Damon, J.-P.; Valtorta, N.; Paillard, N.; Tepfer, D. *Bio/Technology*, **1994**, *12*, 64-68.
22. Voelker, T.A.; Worrell, A.C.; Anderson, L.; Bleibaum, J.; Fan, C.; Hawkins, D.J. Radke, S.E.; Davies, H.M. *Science*, **1992**, *257*, 72-74.

Motif Exhibit 1013, Page 8 of 10

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

23. Townsend, J.A. *Proceedings of the 4th Biennial Conference on Molecular and Cellular Biology of Soybean,* Iowa State University, Ames, IA, **1992**, July 27, p.4.
24. Nordlee, J.A.; Taylor, S.L.; Townsend, J.A.; Thomas, L.A.; Townsend, R. In *OECD Proceedings of a Workshop on Food Safety Evaluation*, Oxford, UK, 12 Sept., **1994**, in press.
25. Müller-Röber, B.; Sonnewald, U.; Willmitzer, L. *EMBO J,* **1992**, *11*, 1229-1238.
26. Stark, D.M.; Timmermann, K.P.; Barry, G.F.; Preiss, J.; Kishore, G.M. *Science*, **1992**, *258*, 287-292.
27. Dale, J.; Belanger, F. *Agro. Food Industry Hi-Tech.* **1993**, *4*, 6-8.
28. Sijmons, P.; Dekker, B.; Schrammeijer, B.; Verwoerd, T.; van den Elzen, P.; Hockema, A. *Biotechnology*, **1990**, *8*, 217-221.
29. *IFBC (International Food Biotechnology Council), Regulat. Toxicol. Pharmacol.* **1990**, *12*, 1-196.
30. Geisen, R; Stander, L.; Leistner, L. *Food Biotechnol.* **1990**, *4*, 497-504.
31. Muraoka, Y.; Imanaka, I., Editors, *Recombinant Microbes for Industrial and Agricultural Applications*, Marcel Dekker, New York, **1993**.
32. Hollenberg, D.P.; Strasser, A.W.M. *Food Biotechnology*, **1990**, *4*, 527-534.
33. Lang-Hinrichs, C.; Hinrichs, J. *Agro. Food Industry Hi-Tech.*, **1992**, *5*, 12-18.
34. *ACNFP (Advisory Committee on Novel Foods and Processes), Report on the use of antibiotic resistance markers in genetically modified food organisms*, **1994**.
35. *ACNFP (Advisory Committee on Novel Foods and Processes), Annual Report*, **1993**, MAFF-Publications, London.
36. Teuber, M. In *Biotechnology, Vol. 1, Biological Fundamentals*, Rehm, H.-J.; Reed, G., Editors, VCH, Weinheim, **1993**, 325-366.
37. De Vos, W.M. In *Harnessing Biotechnology for the 21st Century*, Ladish, M.R.; Bose, A., Editors, American Chemical Society, Washington, DC, **1992**, 524-527.
38. Teuber, M. *Food Reviews International*, **1993**, *9*, 389-401.
39. Venemma, G. *J. Dairy Sci.*, **1993**, *76*, 2133-2144,
40. Graille, J. *Agro-Food-Industry-Hi-Tech., 1991, 6*, 29-35.
41. Gellissen, G. *A. V. Leeuwenhock*, **1992**, *62*, 79-93.
42. Flamm, E.L. *Bio/Technology*, **1991**, *9*, 349-351.
43. Vandamme, E.J., Editor, *Biotechnology of Vitamins, Pigments and Growth Factors,* Elsevier Applied Sciences, London, New York, **1989**.
44. Hodgson, J. *Bio/Technology*, **1994**, *12*, 152-155.
45. Leuchtenberger, A. *Acta Biotechnol.* **1992**, *12*, 57-65.
46. Armstrong, D.A.; Gillies, B.; Yamazaki, H. In *Flavor Chemistry: Trends and Developments*, Teranishi, R.; Buttery, R. G.; Shahidi, F., Editors, ACS Symposium Series 388, **1989**, 105-120.
47. Gatfield, I.L.; Sommer, H. In *Recent Developments in Flavor and Fragrance Chemistry*, Hopp, R.; Mori, K., Editors, VCH, Weinheim, **1993**, 291-304.

Motif Exhibit 1013, Page 9 of 10

Case No.: IPR2023-00321
U.S. Patent No. 10,689,656

48. Pinkert, C.A., Editor, *Transgenic Animal Technology: A Laboratory Handbook*, Academic Press, San Diego, **1994**.
49. Brem, G. Transgenic Animals, In *Biotechnology*, Rehm, H.J.; Reed, G.; Puehler, A.; Stadler, P., Editors, VCH-Verlag, **1993**, 745-832.
50. Chen, T.T.; Lin, C.-M.; Lu, J.K.; Shamblott, M.; Kight, K. In *Science for the Food Industry of the 21st Century, Biotechnology, Supercritical Fluids, Membranes, and other Advanced Technologies for Low Calorie, Healthy Food Alternatives,* Yalpani, M., Editor, ATL Press, **1993**, 145.
51. *OECD Aquatic Biotechnology and Food Safety*, Paris, **1994**.
52. Lee, S.H.; de Boer, H.A. *J. Controlled Release*, **1994**, *29*, 213-221.
53. *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles,* OECD, Paris, **1993.**
54. *Application of the Principles of Substantial Equivalence to the Safety Evaluation of Foods or Food Components from Plants Derived by Modern Biotechnology,* WHO Workshop, WHO, Geneva, in press.
55. Flavell, R.B.; Dart, E.F; Fuchs, R.L.; Fraley,R.T. *Bio/Technology*, **1992**, *10*, 141-144.
56. *Report on the Use of Antibiotic Resistance Markers in Genetically Modified Food Organisms,* Advisory Committee on Novel Foods and Processes, (ACNFP), Annual Report, **1993**.

RECEIVED July 14, 1995

Motif Exhibit 1013, Page 10 of 10