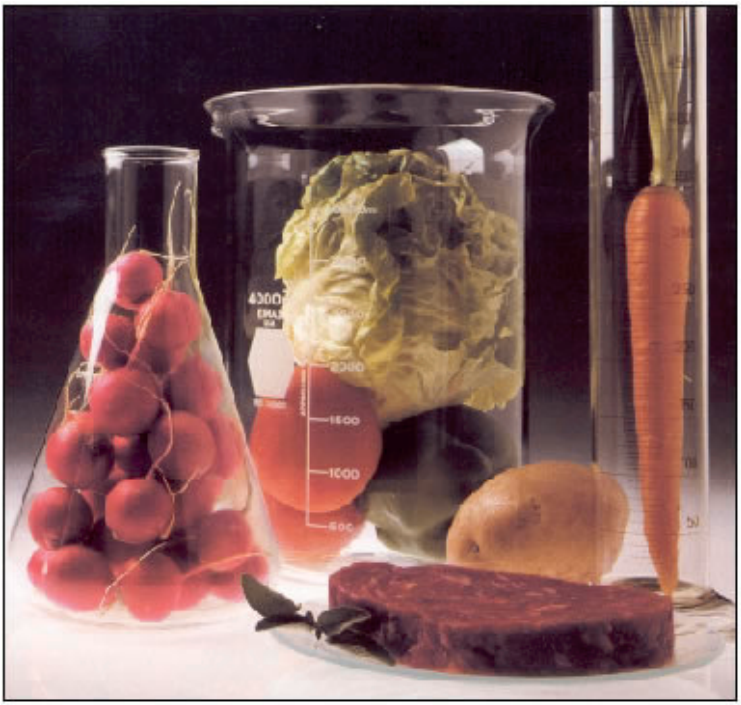
Selected Articles in Food Science & Technology for College Graduate Students

Perkins Muredzi



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Epigraph

"If you are planning for a year, sow rice;
if you are planning for a decade, plant trees;
if you are planning for a lifetime, educate people."

Chinese Proverb

Dedicated to Mrs Dorothy Stembile Muredzi,
Sean Muredzi, Sheldean Muredzi

and

In loving memory of
Callisto Wunganayi Temba, Anastasia Rudo Bwanya,
and John Tanjani

Preface

Food Science represents the application of the basic sciences, biotechnology, and engineering to the production, processing, packaging, distribution, and evaluation of foods. Food science and food technology complement production agriculture by developing methods that minimize waste and improve the quality, utility, safety, attractiveness, and shelf life of foods. Food scientists strive to improve the efficiency of food processing while ensuring high quality, nutritious, safe, and convenient food products. To this end, they employ the principles of chemistry, physics, biochemistry, microbiology, engineering, nutrition, and management in an integrated manner. Food scientists require specialized education and technical training.

Advanced studies in food science and technology taken by graduate students provide a broader, more varied education than is possible in the other study programmes. Graduate students are more often expected to take courses in food chemistry, food engineering and processing, food microbiology, nutrition, and food marketing as well as in the supporting disciplines and commodity areas in their special interests. Graduate students therefore require exposure to information regards immerging and critical topics in the various areas offered in their studies. To complement taught courses some programmes offer seminars on advanced topics or food research areas. This collection of articles serves to give the graduate student a varied portfolio of articles as reference material for the various disciplines of food science and technology under study thus enriching knowledge in selected critical topics.

Acknowledgement

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Use of Biotechnology in Traditionally Fermented African Foods

1.1 Introduction

The relationship between biotechnology and traditionally fermented foods has not been discussed widely in the literature hence the writer's choice of the topic. It should be noted however that biotechnology can be of help in the improvement of fermented foods at three levels:

Firstly - raw materials. Fermented foods are produced from either animal or plant starting materials, and the availability of these substrates will aid in the production of fermented foods. Biotechnological methods to improve animal and plant production have been dealt with by experts in those fields on many occasions. It should be noted that certain wild plants and marginalised crops (the so called lost crops of Africa should not be neglected e. g., sorrel and okra) Attempts to restore the forest cover should give some attention to trees that bear fruits used during famines or even trees that host caterpillars.

The second level is fermentation engineering. Recent developments in biotechnology have given rise to great innovations in bioreactor designs. Most of these designs deal with liquid reaction media, but it should not be forgotten that a great number of fermented foods are produced through a solid-substrate fermentation in which the fermenting paste is frequently hand mixed. Bioreactors to simulate such a process are needed for the modernization of such traditional fermented foods.

The third level is microbiology and enzymology. There are many opportunities for biotechnological innovations in the microbiology of fermented foods. First, all the microorganisms involved should be isolated, characterized, and preserved as a germplasm collection. Second, the metabolic role of each of the strains involved should be clearly identified, and their full potential, even in

other fields of biotechnology, should be studied. The powerful technique of monoclonal antibodies for the characterization of different strains of the same species can be of great help in this area.

Many of these organisms have the enzyme complement to produce vitamins and amino acids in fermented foods. This potential can be improved through the technique of recombinant DNA technology to produce strains that are capable of producing and releasing the required amino acid or vitamin into the food. To avoid food losses due to spoilage-causing organisms and to avoid possible development of food-poisoning microbes, it is possible to genetically engineer a strain required for a process as a pure culture. Such a strain may bring about all the changes required in the food and grow at a convenient temperature.

The general aims of food technology are to exploit natural food resources as efficiently and profitably as possible. Adequate and economically sound processing, prolongation of shelf life by preservation and optimization of storage and handling, improvement of safety and nutritive value, adequate and appropriate packaging, and maximum consumer appeal are key prerequisites to achieving these aims. Fermentation is one of the oldest methods of food processing. The history of fermented foods has early records in Southeast Asia, where China is regarded as the cradle of mould-fermented foods, and in Africa where the Egyptians developed the concept of the combined brewery bakery. The early Egyptian beers were probably quite similar to some of the traditional opaque sorghum, maize, or millet beers found in various African countries today (Hesseltine, C. W; 1981) In technologically developed regions, the crafts of baking, brewing, wine making, and dairying have evolved into the large-scale industrial production of fermented consumer goods, including cheeses, cultured milks, pickles, wines, beers, spirits, fermented meat products, and soy sauces. The introduction of such foreign “high-tech” fermented products to tropical

countries by early travellers, clergymen, and colonists was followed by an accelerated demand during the early post in dependence period. Their high price ensured status, and their refined quality guaranteed continued and increasing consumption. In contrast, many of the traditional indigenous foods lack this image; some may even be regarded as backward or poor people's food. Factors contributing to such lack of appeal include inadequate grading and cleaning of raw materials, crude handling and processing techniques, and insufficient product protection due to lack of packaging. Such unhygienic practices are easily translated into a fear of food borne diseases. From a nutritionist's point of view, many traditional starchy staples are deficient in energy, protein, and vitamins. Variable sensory characteristics (quality) and lack of durability (shelf life) reduce convenience to the consumer: time needs to be spent selecting products of adequate quality, whereas perishable products require frequent purchasing and result in increased wastage. In addition, ungraded heterogeneous products, inconvenient unpacked bulk foods, or unattractive presentation inhibit consumers to develop regular purchasing attitudes.

Below is a table of current information available on African Foods and their methods of inoculation.

Table 1.1 *African fermented foods and their methods of inoculation - compiled from information obtained from Odunfa and Oyewole (1997).*

Raw Material	Local Product Name	Region /country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development[2]
(A)	Fermented starchy staples					
cassava	Gari	West and Central Africa	Solid state	*Corynebacterium mannihot, Geotrichum* species, *Lactobacillus plantarium, Lactobacillus buchnerri, Leuconsostoc* species, *Streptococcus* species.	Natural/ chance	1, 2, 5, 7, 8
	Fufu	West Africa	Submerged	*Bacillus* species, *Lactobacillus* species *such as Lactobacillus plantarum; Leuconostoc*	Natural / chance	1, 2, 5, 6

Raw Material	Local Product Name	Region /country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development[2]
				mesenteroides; *Lactobacillus cellobiosus*; *Lactobacillus brevis*; *Lactobacillus coprophilus*; *Lactobacillus lactis*; *Leuconostoc lactis* and *Lactobacillus bulgaricus*, *Klebsiella* species, *Leuconostoc* species, *Corynebacterium* species and a yeast of the *Candida species.* *Bacillus* species, *Klebsiella* species, *Candida* species, *Aspergillus* species*;*		
	Lafun / Konkote	West Africa	Submerged	*Leuconostoc mesenteroides*, *Corynebacterium manihot, Lactobacillus plantarum, Micrococcus luteus and Geotrichum candidum*	Spontaneous	1, 2, 5, 6
		Africa / Zaire	Solid state	*Corynebacterium, Bacillus, Lactobacillus, Micrococcus,*	Spontaneous	1, 2, 7
	Chikwangue	Central Africa / Zaire	Solid state		Spontaneous	1, 2, 7
	Cingwada	East and Central Africa	Solid state	*Pseudomonas, Acinetobacter* and *Moraxella* *Corynebacterium, Bacillus, Lactobacillus, Micrococcus,*	Spontaneous	1, 2
(B)	Gruels and Beverages					
maize	Ogi	West Africa / Nigeria	Submerged	*Lactobacillus plantarum, Corynebacterium specie, Aerobacter*, yeasts *Candida mycoderma*, *Saccharomyces cerevisiae* and *Rhodotorula* and molds *Cephalosporium*, *Fusarium, Aspergillus* and *Penicillium*	Appropriate starters produced by back-slopping	1, 2, 3, 4, 5, 7
Sorghum	Abreh	Sudan	Solid state and Submerged	*Lactobacillus plantarum.*	Appropriate starters produced by back-slopping	1, 2
Millet	Uji	East Africa/	Submerged	*Leuconostoc mesenteroides,*	Appropriate starters	1, 2

Raw Material	Local Product Name	Region /country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development[2]
		Kenya		*Lactobacillus plantarum.*	produced by back-slopping/inoculation belt	
maize	Kenkey/ Koko/ Akasa	West Africa / Ghana	Solid state	*Enterobacter cloacae, Acinetobacter sp., Lactobacillus plantarum, L. brevis, Saccharomyces cerevisiae, Candida mycoderma*	Spontaneous	1, 2
(C) Alcoholic Beverages						
Palm	Palm wine / Emu	West Africa	Submerged	*Saccharomyces cerevisiae, Schizosaccharomyces pombe, Lactobacillus plantarum, L. mesenteroides*.	Spontaneous	1, 2, 7
Various types of African cereal grains (Maize, Sorghum, Millet-used)	Busa	East Africa / Kenya	Submerged	*Saccharomyces. Cerevisiae, Schizosaccharomyces pombe, Lactobacillus plantarum, L. mesenteroides*.	Spontaneous	1, 2, 7
	Mbege	Tanzania	Submerged	*Saccharomyces cerevisiae, Schizosaccharomyces pombe, Lactobacillus plantarum, L. mesenteroides*.	Spontaneous	1, 2
	Burukutu	West Africa	Submerged	*Saccharomyces cerevisiae, S. chavelieri, Candida sp* and, *Leuconostoc meseteroides*. *Acetobacter sp*.	Spontaneous	1, 2
	Pito	West Africa	Submerged	*Geotrichum candidum, Lactobacillus* sp. and *Candida* sp.	Natural / chance Inoculation belt	1, 2,
(C)	Acid Leavened Bread / Pancakes					
Various types of African cereals grains	Kisra	Sudan	Submerged		Appropriate starters produced by back-slopping	
	Enjera / Tef Injera	Ethiopia	Submerged		Appropriate starters produced by back-slopping	

Raw Material	Local Product Name	Region /country	Type of fermentation	Micro-organisms associated with the fermentation process	Methods of inoculation	State of development[2]
(D)	Legumes					
Locus bean / Soybeans	Iru, Dawadawa/ Etchum, Kal Soumbara, Chu	West Africa		*Bacillus subtilis, B. pumilus, B. licheniformis* and *Staphylococcus saprophyticus*	Spontaneous	1, 2, 3, 6, 7
African oil bean	Ugba			*Bacillus subtilis, B. pumilus, B. licheniformis* and *Staphylococcus saprophyticus*	Spontaneous	
Melon Seeds, castor oil seeds, pumpkin bean, sesame	Ogiri / Ogili	West, East and Central Africa		*Bacillus subtilis, B. pumilus, B. licheniformis, Staphylococcus saprophyticus, Lactobacillus plantarum*	Spontaneous	1, 2
Cotton seed	Owoh	West Africa		*Bacillus subtilis, B. pumilus, B. licheniformis, Staphylococcus saprophyticus*	Spontaneous	1
(E)	Animal Products					
Goat milk	Ayib	East and Central Africa		*Canida* spp., *Saccharomyces* spp., *Lactobacillus* spp., *Leuconostoc* spp.,	Spontaneous	1, 2
Cow milk	Leben / Lben	North, East Central Africa Afi		*Candida* spp., *Saccharomyces* spp., *Lactobacillus* spp., *Leuconostoc* spp.,	Spontaneous	1, 2, 3

**Key to codes for the 'state of development'

1: Micro-organisms involved known

2: Roles of individual micro-organisms known

3: Genetic improvement carried on organisms.

4: Starter cultures available for the fermentation

5: Varieties of raw materials that are best suited for the product known

6: Improved technology available and adopted

7: Pilot Plant production

8: Industrial Plant production

If blank, then information is not available

[2]State of development of each fermented product. It is the personal assessment of data, literature, internet search and other information by O. B. Oyewole as at March 2009.

1.2 Description

The fermentation bioprocess is the major biotechnological application in food processing. It is often one step in a sequence of food-processing operations, which may include cleaning, size reduction, soaking, and cooking. Fermentation bioprocessing makes use of microbial inoculants for enhancing properties such as the taste, aroma, shelf-life, safety, texture, and nutritional value of foods. Microbes associated with the raw food material and the processing environment serve as inoculants in spontaneous fermentations, while inoculants containing high concentrations of live micro-organisms, referred to as starter cultures, are used to initiate and accelerate the rate of fermentation processes in non-spontaneous or controlled fermentation processes. Microbial starter cultures vary widely in quality and purity.

Starter culture development and improvement is the subject of much research both in developed and in developing countries. While considerable work on GM starter culture development is on-going at the laboratory level in developed countries, relatively few GM micro-organisms have been permitted in the food and beverage industry globally. In 1990, the United Kingdom became the first country to permit the use of a live genetically modified organism (GMO) in food. It was a baker's yeast, engineered to improve the rate at which bread dough rises by increasing the efficiency with which maltose is broken down. This modification was done by using genes from yeast and placing them under a strong constitutive promoter. The United Kingdom has also approved a GM brewer's yeast for beer production. By introducing a gene encoding glucoamylase from yeast, better utilization of carbohydrate present in conventional feedstock can be obtained, resulting in increased yields of alcohol and the ability to produce a full-strength, low-carbohydrate beer. More recently,

two genetically modified yeast strains were authorized for use in the North American wine industry (Bauer *et al*., 2007).

Current literature documents volumes of research reports on the characterization of microbes associated with the production of traditional fermented foods in developing countries. Relatively few of these studies document the application of the diagnostic tools of modern biotechnology in developing and designing starter cultures. The development and improvement of microbial starters has been a driving force for the transformation of traditional food fermentations in developing countries from an "art" to a science. Microbial starter culture development has also been a driving force for innovation in the design of equipment suited to the hygienic processing of traditional fermented foods under controlled conditions in many developing countries.

Starter culture improvement, together with the improvement and development of bioreactor technology for the control of fermentation processes in developed countries, has played a pivotal role in the production of high-value products such as enzymes, microbial cultures, and functional food ingredients. These products are increasingly produced in more advanced developing economies, and are increasingly imported by less advanced developing countries, as inputs for their food processing operations.

1.2.1 Spontaneous Inoculation of Fermentation Processes

In many developing countries, fermented foods are produced primarily at the household and village level, using spontaneous methods of inoculation. Spontaneous fermentations are largely uncontrolled. A natural selection process, however, evolves in many of these processes which eventually results in the predominance of a particular type or group of micro-organisms in the

fermentation medium. A majority of African food-fermentation processes make use of spontaneous inoculation (Table 1). Major limitations of spontaneous fermentation processes include their inefficiency, low yields of product and variable product quality. While spontaneous fermentations generally enhance the safety of foods owing to a reduction of pH, and through detoxification, in some cases there are safety concerns relating to the bacterial pathogens associated with the raw material or unhygienic practices during processing.

1.2.2 "Appropriate" Starter Cultures as Inoculants of Fermentation Processes

"Appropriate" starter cultures are widely applied as inoculants across the fermented food sector, from the household to industrial level in low-income and lower-middle-income economies. These starter cultures are generally produced using a back slopping process which makes use of samples of a previous batch of a fermented product as inoculants (Holzapfel, 2002). The inoculation belt (Holzapfel, 2002) used in traditional fermentations in West Africa serves as a carrier of undefined fermenting micro-organisms, and is one example of an appropriate starter culture. It generally consists of a woven fibre or mat or a piece of wood or woven sponge, saturated with "high"- quality product of a previously fermented batch. It is immersed into a new batch, in order to serve as an inoculant. The inoculation belt is used in the production of the indigenous fermented porridges, "*uji"* and "*mawe*," as well as in the production of the Ghanaian beer, "*pito*" (Table 1).

Iku, also referred to as iru, is yet another example of an "appropriate" starter culture produced by back slopping. This starter culture is produced from concentrated fermented *dawadawa* (a fermented legume product), mixed with ground unfermented legumes, vegetables such as pepper, and cereals, such as

ground maize. It is stored in a dried form and is used as an inoculant in *dawadawa* fermentations in West Africa (Holzapfel, 2002). A range of appropriate starter cultures, either in a granular form or in the form of a pressed cake is used across Asian countries as fermentation inoculants. These traditional mould starters are generally referred to by various names such as *marcha* or *murcha* in India, *ragi* in Indonesia, *bubod* in the Philippines, *nuruk* in Korea, *koji* in Japan, *ragi* in Malaysia and *Loog-pang* in Thailand. They generally consist of a mixture of moulds grown under non-sterile conditions.

1.2.3 Defined Starter Cultures as Inoculants of Fermentation Processes

Few defined starter cultures have been developed for use as inoculants in commercial fermentation processes in developing countries. Nevertheless, the past ten years have witnessed the development and application of laboratory-selected and pre-cultured starter cultures in food fermentations in a few developing countries. “Defined starter cultures” consist of single or mixed strains of micro-organisms (Holzapfel 2002). They may incorporate adjunct culture preparations that serve a food-safety and preservative function. Adjunct cultures do not necessarily produce fermentation acids or modify texture or flavour, but are included in the defined culture owing to their ability to inhibit pathogenic or spoilage organisms. Their inhibitory activity is due to the production of one or several substances such as hydrogen peroxide, organic acids, diacetyl and bacteriocins (Hutkins, 2006). By and large, defined cultures are produced by pure culture maintenance and propagation under aseptic conditions. They are generally marketed in a liquid or powdered form or else as a pressed cake.

Defined starter cultures are also widely imported by developing countries for use in the commercial production of dairy products such as yogurt, kefir,

cheeses, and alcoholic beverages. Many of these cultures are tailored to produce specific textures and flavours. In response to growing consumer interest in attaining wellness through diet, many yogurt cultures also include probiotic strains. Probiotics are currently produced in India for use as food additives, dietary supplements and for use in animal feed (e.g. www.prnewswire.co.uk/cgi/news/release?id=262320). Methodologies used in the development and tailoring of these starters are largely proprietary to the suppliers of these starters. Monosodium glutamate and lactic acid, both of which are used as ingredients in the food industry, are produced in less-advanced developing countries using defined starter cultures.

1.2.4 Defined Starter Cultures Developed Using the Diagnostic Tools of Advanced Biotechnologies

The use of DNA-based diagnostic techniques for strain differentiation can allow for the tailoring of starter cultures to yield products with specific flavours and/or textures. Random amplified polymorphic DNA (RAPD) techniques have been applied in, for example, Thailand, in the molecular typing of bacterial strains and correlating the findings of these studies to flavour development during the production of the fermented pork sausage, nham The results of these analyses led to the development of three different defined starter cultures which are currently used for the commercial production of products having different flavour characteristics (Valyasevi and Rolle, 2002).

1.2.5 GM Starter Cultures

To date, no commercial GM micro-organisms that would be consumed as living organisms exist. Products of industrial GM producer organisms are, however, widely used in food processing and no major safety concerns have

been raised against them. Rennet which is widely used as a starter in cheese production across the globe is produced using GM bacteria.

1.3 General Analysis

Socio-economic factors have played a major role in the adoption and application of microbial inoculants in food fermentations. In situations where the cost of food is a major issue, uptake and adoption of improved biotechnologies has been generally slow. Demand for improved inoculants and starter culture development has been triggered by increasing consumer income, education, and new market opportunities.

1.3.1 Socio-Economics of the Consumer Base

The consumer base of traditionally fermented staple foods in most developing countries is largely poor and disadvantaged. Price, rather than food safety and quality, is therefore a major preoccupation of this group when purchasing food. Fermented foods provide that target group with an affordable source of food, and make a substantial contribution to their food and nutritional security. These foods are generally produced under relatively poor hygienic conditions at the household and village level. Fermentation processing is practised largely as an art in such contexts. Interventions designed to upgrade processes used in the production of these traditionally fermented staples have been largely carried out through donor-funded projects and have focused primarily on reducing the drudgery associated with the fermentation processes. Improvements have also targeted the up gradation of hygienic conditions of fermentation processes and the introduction of simple and “appropriate” methodologies for the application of inoculants, such as the use of back slopping. While the uptake of simple back slopping technologies at the

household level has, in general, been very good by that target group, the uptake of defined starter cultures has been less successful, owing to cost considerations.

With growing incomes and improved levels of education in urban centres across a number of developing countries, dietary habits are changing and a wider variety of foods is being consumed. Fermented foods are no longer the main staples, but are still consumed as side dishes or condiments by that target group. The demand of that target group for safe food of high quality has begun to re-orient the traditional fermented food sector, and led to improvements in the control of fermentation processes through the development and adoption of defined starter cultures, the implementation of GHPs and HACCP in food fermentation processing, and the development of bioreactor technologies, coupled with appropriate downstream processing to terminate fermentation processes and thus extend the shelf-life of fermented foods. The packaging of fermented products has also improved.

1.3.2 Changing Consumer Demand Trends

Apart from their changing dietary patterns and their demand for safety and quality, higher-income consumers demand convenience and are increasingly concerned about deriving health benefit from the foods they consume. Many of these consumers also show a preference for shopping in supermarkets. Consumer demand for deriving wellness through food consumption has stimulated the development of industrial fermentation processes for the production of functional ingredients such as polyunsaturated fatty acids and pro-biotic cultures for use as food ingredients in developing countries. These functional ingredients are currently applied in the fortification of fermented foods as well as in the production of dietary supplements in countries such as India. The growth of supermarkets in developing countries has promulgated the

need for standardized products of a reasonable shelf-life that meet safety and quality criteria. Packaged fermented products such as kimchi, miso, and tempeh, for example, are widely available in supermarkets across Asia. The production of traditional beer in a powdered format and in ready-to-drink containers in Zambia is a very good example of product development that has taken place in response to consumer demand for convenience, both in local and export markets. Shifting consumer preferences in South Africa, away from basic commodity wine to top-quality wine, is yet another example of how market demand has led to research and biotechnological innovation in the wine industry. Biotechnological innovations in that country are currently focused on the improvement of *Saccharomyces cerevisiae* strains to improve wholesomeness and sensory quality of wines.

1.3.3 The Enabling Environment for Starter Culture Development

A considerable amount of research in developing countries has focused on the identification of starter micro-organisms associated with the fermentation of these staple foods. The greatest strides in starter culture development have, however, been realized in countries that have prioritized the development of technical skills, the infrastructural support base and funding support for research into the up gradation of fermentation processes. Linkages between research institutions and the manufacturing sector have also been critical to the successful introduction of starter cultures. Collaborative initiatives among research institutions have also had a major positive impact on biotechnological developments in developing countries**.** Collaboration among African institutions and their counterparts in the North has greatly facilitated improvements in biotechnological research and capacity development in the area of food biotechnology on the continent. One major success story in this regard has been collaborative projects involving Burkina Faso, Ghana and institutions in the

Netherlands. This programme facilitated the typing and screening of microbial cultures associated with fermented African foods as a basis for starter culture development. Results of this work (Mengu, 2009) led to improvements in the production of gari, a fermented cassava product and dawadawa, a fermented legume product. Issues related to the protection of intellectual property rights (IPR) are of growing concern with respect to starter culture development.

1.3.4 Proactive Industrial Strategies

Biotechnology developments have been most successful in areas where proactive approaches are taken by industry. The Thai food industry successfully creates perceived quality by launching new product logos and associating these new products with biotechnology or with the fact that they were developed using traditional biotechnology, such as starter cultures. The goal of the industry is to project an image of itself as producing products of superior quality and safety that represent progressiveness based on a higher level of technology.

1.3.5 Export Opportunities for Fermented Products

Increasing travel due to globalization has changed the eating habits of consumers across the globe. Export markets for fermented foods have grown out of the need to meet the requirements of developing country diaspora in these markets as well as to satisfy growing international demand for niche and ethnic products. Indonesian *tempe* and Oriental soy sauce are well known examples of indigenous fermented foods that have been industrialized and marketed globally. The need to assure the safety and quality of these products in compliance with requirements of importing markets has been a driving force for the upgrading of starter cultures as well as for diagnostic methodologies for verification of their quality and safety.

Growing interest and trade in fermented food products is also likely to lead to the greater use of the DNA barcode for identifying the origins of specific fermented food products produced in developing countries.

1.4 Actualisation: Case Study of Flavour Production from Alkaline-Fermented Beans (West Africa)

This Case Study on the indigenous fermentation of the locust bean, dawadawa (fermented locust bean), is a classic example of how traditional fermentations can be exploited for the production of high-value products such as flavour compounds. The work, however, was undertaken by a large cooperation with little involvement of local researchers. Returns on commercial successes derived from this study did not go back to the people who invented the traditional method of producing this indigenous fermented food. This Case Study, therefore, serves to highlight the critical issue of IPR of traditional production systems. Dawadawa is produced by alkaline fermentation of the African fermented locust bean (Steinkraus, 1995). It is an important condiment in the West/Central African Savannah region (Odunfa and Oyewole, 1986). Similar fermented food products can be found throughout Africa, with regional differences in the raw materials used as processing inputs or in post processing operations. Similarly fermented products are referred to as “kinda” in Sierra Leone, “iru” in coastal Nigeria, “soumbara” in Gambia and Burkina Faso, and “kpalugu” in parts of Ghana (Odunfa and Oyewole, 1986). Foods produced by alkaline fermentation in other parts of the world include “natto” in Japan, “*thua noa*” in Thailand and “kinema” in India (Tamang, 1998). These are mainly used as culinary products to enhance or intensify meatiness in soups, sauces and other prepared dishes. The production of dawadawa involves extensive boiling and dehulling of the beans, followed by further boiling to facilitate softening. Spontaneous fermentation of the softened

beans is subsequently allowed to take place over 2–4 days. Micro-organisms associated with the fermentation include *Bacillus subtilis* (Ogbadu and Okagbue, 1988), *B. pumilus* (Ogbadu and Okagbue, 1988), *B. licheniformis* (Ogbadu, Okagbue and Ahmad, 1990), and *Staphylococcus saprophyticus* (Odunfa, 1981). During the fermentation process, the pH increases from near neutral to approximately 8.0, temperature increases from 25 ℃ to 45 ℃ and moisture increases from 43% to 56% (Odunfa and Oyewole, 1986). At the same time, a five-fold increase in free amino acids takes place, and glutamate, a flavour enhancer, increases five-fold during the process. Mechanisms of flavour production during the fermentation process, as well as flavour principles generated during dawadawa fermentation processing, have been studied by international food manufacturers and been used as a basis for the development of flavours for incorporation in bouillon-type products (Beaumont, 2002).

1.5 Discussion

Fermented food products play a significant socio-economic role in African countries and the developing world. The importance of traditional fermented foods has been reviewed. These products also contribute to the protein requirements of the indigenous consumers [Achi OK., 2005]. Lowering the pH of food products through fermentation is a form of food preservation [Ananou S, Maqueda M, Mart ńez-Bueno M and Valdivia E; 2007]. This is a self-limiting process in that further reduction of pH may be inimical to the producing organisms. As a result the pH normally stays just below 5. Other benefits of fermentation include improvement of food quality through food digestibility to increase essential amino acids, vitamins and protein in the era of diminishing food quality, fermentation can play a role in complementing the food fortification programme instituted by the WHO. Cereal grains are also susceptible to

contamination by naturally occurring mycotoxins both on the fields and during storage. The fermentation of maize meal has been demonstrated to detoxify these toxins making maize meal safer for human consumption. Children are the hardest hit segment of the population when a nation faces food crisis. Donor countries would normally supply mainly maize to fight off hunger. Fermentation of maize meal makes the final product suitable to serve as weaning food since the bacteria responsible for fermentation also produce vitamins and amino acids during their growth and serve as single cell proteins after cooking Moreover, maize is more likely to be readily available in a poor setting where a balanced diet is not available. Therefore, fermentation technology is of great importance in ensuring food safety, preservation and food flavouring [Nout MJR and Motarjemi Y.; 1997]. Fermentation is also known to soften food texture and alter its composition in such a way that it will require minimal energy both in cooking and preservation process. Thus, less fuel will be used for cooking and eliminates the need of preservation as fermentation increases the shelf life of food. These advantages make fermentation a highly desirable technique in the rural communities of the third world where resources for cooking and preservation are scarce. Fermentation technology also has the potential of meeting the world's food supply demand if adequately developed into the industrial scale [Nout MJR and Motarjemi Y.; 1997]. In a Joint FAO/WHO Workshop held in Pretoria, South Africa, the importance of antibiotic activity and nutritional benefits of LAB was revisited [Motarjemi Y and Nout MJ.; 1996]. In this workshop, a gap of knowledge was identified and the participants were unanimous that further research was required to further expand the usefulness of food fermentation especially its antibiotic activities against parasites, viruses, and bacteria. Additionally, assessment of physicochemical effects of fermented foods on consumers and the establishment of starter cultures for commercial market were identified as priorities [Motarjemi Y and Nout MJ.; 1996]. The handlers of

traditional foods also need to be educated on food hygiene, as there are many instances where food is contaminated by bad handling after cooking. LAB fermentation fits into primary care initiatives and can reduce child mortality by supplying the minimum required nutrients [Motarjemi Y.; 2002]. In addition to its potential use to tackle malnutrition, the technology is a low cost means of food preservation.

There is need to educate the African citizens on the need of consuming fermented foods and food safety. Although fermented foods are generally safe, and in the view that certain antimicrobial factors are present, lack of standardization in the methods used, the environment and the hygiene of the people that prepare them, will determine the quality of the product. Safety is of paramount importance. Personal hygiene should be practiced to complement the overall benefits of fermented foods. The greatest drawback in the development of fermented food products in Africa is that many products are produced under primitive conditions, resulting in low yield and poor quality, including short shelf-life [Achi OK; 2005]. Other problems include the lack of appeal in the presentation and marketing of the food products, as well as the fact that the processes are often laborious and time-consuming [Nout MJR, Kok B, Vela E, Nche PF and Rombouts FM; 1995]. The technology needs to be improved through research to advance its potential for food safety and nutritional value. Imported products should not stifle the development of traditional food products at a national and international level. With the current technologies, it should be possible to be innovative about many of the foods produced using fermentation and indigenous knowledge systems. The challenge is to ensure that technology is used to add value to such products, such as increased shelf-life, flavour and appealing packaging and labelling. Old ferments are not an efficient way of preserving the LAB probiotic organisms as poor survival has been reported in these products. Microencapsulation technology is a new technique which can be

used to preserve and propagate LAB cultures for mass production of fermented foods [Kailasapathy K; 2002]. Current research conducted by our group include the isolation and identification of the microorganisms associated with *amahewu* and *incwancwa* production. This is hoped to preserve the cultures for future use as starter cultures and as a base to extend the product-range of fermented foods. In addition, antimicrobial peptides produced by some LAB can be used as lead compounds in drug discovery. It is also important to document these traditional indigenous technologies in order to preserve them for future generations, as the old days practices keep changing from time to time. This will also create a reference database for future generations of food research scientists, nutritionists and food regulatory bodies and policy makers in different ladders of government.

1.6 General Recommendations

It is important that countries recognize the potential of fermented foods and prioritize actions to assure their safety, quality, and availability. A number of specific options can be identified for developing countries to help them make informed decisions regarding adoption of biotechnologies in food processing and in food safety for the future.

1.6.1 Regulatory and Policy Issues

Governments must be committed to protecting consumer health and interests, and to ensuring fair practices in the food sector. There has to be consensus at the highest levels of government on the importance of food safety, and the provision of adequate resources for this purpose. Government policy that is based on an integrated food-chain approach is science-based, transparent and includes the participation of all the stakeholders from farm to table must be put in place. The importance of the regional and international dimensions of the use

of biotechnologies in food processing and safety must be recognized. Priority must be accorded to promoting fermented foods in the food-security agendas of countries. Governments must also provide an enabling environment that is supportive of the growth and development of upstream fermentation processes such as the production of high-value fermented products, such as enzymes, functional-food ingredients, and food additives.

1.6.2 International Cooperation and Harmonization

The organization and implementation of regional and international fora are critical requirements for the enhancement of national organizational capability and performance and for the facilitation of international co-operation. Further, the setting up of administrative structures with clearly defined roles, responsibilities, and accountabilities could efficiently govern processed foods and safety issues.

Biotechnology-based Standard Operating Procedures (SOPs) for food safety should also be documented for use in authorized laboratories. National food control databases for the systematic collection, reporting, and analysis of food-related data (food inspection, analysis, etc.) with set regulations and standards based on sound science and in accordance with international recommendations (Codex) are key requirements.

1.6.3 Education Policy

While the consumption of fermented foods is growing in popularity among higher-income consumers thanks to increasing interest in wellness through diet, the consumption of fermented foods by lower-income consumers in many developing countries is perceived to be a backward practice.

Strategies should therefore be developed for the dissemination of knowledge about food biotechnology and, particularly, fermented foods. Targeted consumer education on the benefits of consuming fermented food products and on applying good practice in their production is required.

Food biotechnology should be included in educational curricula in order to improve the knowledge base in countries on the contribution of fermented foods to food and nutritional security and to generate awareness of the growing market opportunities for fermented foods and high-value products derived from fermentation processes.

1.6.4 Information-Sharing

Access to specialized technical information on biotechnology and biotechnological developments in the food processing sector are critical and necessary inputs and support systems for guiding and orienting the research agendas of countries. The necessary information systems should therefore be developed to facilitate rapid access to information on biotechnological developments across both the developed and the developing world.

1.6.5 Legislation and Policy on Technologies

Expertise in legislation and technology licensing, as well as knowledge about how to nurture innovation and turn it into business ventures, are critical requirements for developing countries. Successful technology transfer requires all of these elements and an environment that is conducive to innovation. Government policy in developing countries should therefore prioritize technology transfer that helps create new business ventures, an approach that requires government support such as tax incentives and infrastructure investment.

1.6.6 Intellectual Property Rights (IPR)

Many of the traditional fermentation processes applied in developing countries are based on traditional knowledge. Enhanced technical and scientific information is required in order to claim ownership of the traditional knowledge of the craft of indigenous fermented foods. Lack of technical knowledge has resulted in the failure to realize the benefits of the industrialization of indigenous fermented foods by individuals who are the rightful owners of the technology. Greater focus is required on issues of relevance to IPR and on the characterization of microbial strains involved in traditional fermentation processes. Emphasis must be placed on IPR education for scientists. National governments should put in place the requisite infrastructure for IPR to facilitate the process. At the institutional level, this infrastructure would include technology management offices for assisting scientists in procedures relating to intellectual property matters. The processes used in the more advanced areas of agricultural biotechnology are generally covered by IPR, and the rights are generally owned by parties in developed countries.

1.6.7 Communication and Consumer Perceptions

Communication between various stakeholders is critical in proactively engaging with consumers. Communication must be established with the public at large on processed food and associated hazards. Communication gradually builds confidence and will be critical to advancing the application of biotechnologies in food processing and safety. The primary role stakeholders are incorporated in the discussion and decision-making process. The need for specific standards or related texts and the procedures followed to determine them should also be clearly outlined. The process, therefore, should be transparent.

Public awareness and education are critical to the success of food bioprocessing and food safety in developing countries.

Greater attention must be directed toward understanding consumer and producer (processor) perceptions on food safety and quality in developing countries.

If foods are to be promoted as being safe and healthy, their nutritional and safety attributes must be transparently demonstrated by presenting scientific data to substantiate the nutritional and health benefits and by applying good manufacturing/hygiene practice and HACCP as safety measures to ensure that issues of consumer concern are addressed.

1.6.8 Technical Capacities and Technology Transfer

Traditional fermented foods should be viewed as valuable assets. Governments should capitalize on these assets and add value to them by supporting research, education, and development, while building on and developing the indigenous knowledge base on food fermentations.

Government agencies in developing countries should focus on the development of technical capacities to deal with emerging technical issues.

The technical capacities of academic and research institutes should be strengthened in the fields of food biotechnology, food processing, bioprocess engineering, and food safety through training and exchange programmes for researchers. Such programmes should emphasize collaboration with both developed and developing country institutions engaged in work on food biotechnology, starter culture development, bioprocess engineering, and food safety.

Training capabilities in food biotechnology and food safety should be developed within developing country institutions through the introduction of degree courses in order to broaden the in-country technical support base for food bioprocess development. Given the similarities among fermentation processes across regions, an inventory of institutions engaged in food biotechnology in developing countries would be an asset in facilitating networking among institutions. Food processors, policy-makers, and equipment manufacturers should also be integrated into the networking activities.

The development of appropriate levels of bioreactor technology with control bioprocess parameters will be necessary to improve the hygienic conditions of the fermentation processes.

Research and infrastructural development to enable the cost-effective production of defined starter cultures in a stable format (i.e. cultures which do not require refrigeration and have prolonged shelf-life under ambient conditions) should be prioritized.

Infrastructure development to facilitate the transfer and adaptation of fermentation technologies developed at the laboratory level to the household and village and, where necessary, the enterprise level should be prioritized.

Appropriate levels of equipment will also be required to facilitate the downstream processing of these products.

Traceability systems that facilitate the differentiation and identification of food products should be prioritized in order to broaden market opportunities for these products.

A food-chain approach to assuring food safety should be prioritized by governments.

Food safety management systems should be strengthened by implementing systematic food safety measures such as GHP, GMP, and HACCP in food fermentation operations. Diagnostic kits are important tools for monitoring and verifying the level of sanitation in processing plants.

Highly sensitive and rapid diagnostic kits are invaluable for monitoring and rapidly detecting chemical and microbiological hazards that pose a threat to human health, with high precision and sensitivity. The development of low-cost diagnostic kits suitable for use by small processors would greatly facilitate food-safety monitoring. Development should target the realization of multiplex diagnostic systems with the capacity to detect several pathogens or many chemical contaminants using a single diagnostic kit. The development of diagnostic kits at a national level could further reduce their cost of production. Given the regional specificity of bacterial pathogens at the species and subspecies levels, such diagnostic kits should be developed with specificity and sensitivity to the species or subspecies that are prevalent in a specific region. Investment is therefore needed for the development of expertise, facilities, and the infrastructure for the mass production of antibodies, cell culture technology and for the formation of technical know-how on assembling the requisite components of diagnostic kits.

The development of national hazard -profile databases that document the prevalent pathogens in different regions will be critical. Such information would be useful for further research into the development of diagnostic kits with high precision and sensitivity and in implementing HACCP as well as risk assessment research. The culture collection of identified infectious agents in the hazard profiles could play an important role for specific antibody production for use in the development of immunoassay diagnostic kits.

For developing countries to make full use of the available biotechnologies in their traditional food fermentations, an understanding and acquisition of expertise in the following areas are essential:

1.6.9 Art of Fermentation

A clear understanding by the master brewer of every step used in the fermentation is needed. This is the art of fermentation. Although the master brewers might not have scientific backgrounds, they could normally ensure a proper fermentation as a result of years of experience. Without knowledge of the art of traditional food fermentation, a scientist cannot provide a scientific explanation for the process and proceed to provide assistance in improvement of the process.

1.6.10 Microbiology

It is essential to know which microorganisms involved in food fermentations are useful and how the physiology and metabolism of these microbes are affected by the physical and chemical environments of fermentations, as well as how their microbial activities in turn affect the fermentation processes. Microorganisms normally break down carbohydrates, proteins, and lipids present in the raw materials to be fermented by releasing enzymes into the medium. As the raw materials are hydrolysed, the environment is changed, as sometimes reflected by a drop in pH value. Moreover, the breakdown products such as peptides and amino acids can be further converted into smaller volatile molecules that are odoriferous and hence improve the flavour characteristics of the fermented foods.

1.6.11 Upstream and Downstream Processing

Normally raw materials are pre-treated before fermentation. It is important to comprehend how such pre-treatment could affect the fermentation process. In soy sauce fermentation, whole soybeans are steamed to make the soy protein more easily hydrolysable by the proteases of *Aspergillus oryzae*. In so doing, too much moisture is introduced and wheat flour must be added to lower the moisture content to a level that does not favour early bacterial growth and hence prevents spoilage of the fermentation. Downstream processing does not affect the bioprocess involved. However, it could alter the normal organoleptic properties of the product, especially when downstream processing involves heating, such as in the pasteurization of soy sauce. Heating causes a change in the flavour of soy sauce due to nonenzymic browning reactions, which could result in the production of pyrazine compounds.

1.6.12 Biochemistry

An understanding of the biochemical activities of the microbes actively participating in the fermentation could help to explain the change in the texture of the raw material as well as the origin of flavouring substances often present in fermented foods. Flavour and texture are important properties of fermented foods. Elucidation of flavour production in such fermentations could result in the development of processes for producing of flavouring materials by fermentation, as in the production of cheese flavours by *Penicillium roquefortii.*

1.6.13 Fermentation Equipment and Techniques

Practical experience in the use of both solid-state and submerged culture fermentation equipment is very useful. Normal training includes submerged

culture bioreactors but not solid-state fermenters. It is useful to know both types of fermentations because traditional food fermentations often involve solid state fermentation. In soy sauce fermentation an initial solid-state fermentation is followed by a submerged fermentation step. Systems that measure and control pH, dissolved oxygen, temperature, and moisture help to make these bioprocesses more efficient and reduce the time required for production of a quality product.

1.7 Conclusion

Fermentation of traditional foods, as a hurdle technology, is profitable in terms of food quality, preservation, and decontamination of toxins, often found in food. Together with food safety, the nutritional and flavour profile of the products need to meet the expectations of modern consumers. Education of communities about benefits of consuming fermented foods needs to be part of health education. This technology needs to be further developed to enhance safety and ease of application in a rural poor-resource setting. Development of convenient starter cultures and processing methods will ensure that many people in Africa will reap the benefits of indulging in fermented foods and beverages both during cultural ceremonies and during their normal daily activities.

With the rapid progress in the biological sciences, both basic and applied aspects, it has been possible to gain a better understanding of the mystery that has surrounded fermentation processes. The types of microorganisms involved has been isolated and identified, and the physiology and metabolism of these organisms have been studied. Hence, traditional fermented foods can now be made better, faster, and more economically. The application of available knowledge to improve traditional food fermentations in developed countries has far outpaced that in developing countries. The terms “old biotechnology” and “new biotechnology” have been used- “old” to mean the undirected manipulation

of microorganisms and plants, such as by mutagenesis and selection of the better strains. In this old biotechnology it is prudent to include directed control of the physical and chemical environments of the fermentation process, which could result in better performance of the useful microbes. Though mutation increases the ability to select better strains, there can, of course, be little directed alteration of genetic material. The new biotechnology, such as recombinant DNA techniques, overcomes this problem. The new biotechnology can, of course, be of tremendous help in producing super strains of microbes that could enable acceleration of fermentation processes, provide more efficient utilization of raw materials, and produce better-quality products. How best can African nations apply these biotechnologies to traditional fermented foods? Should it be application of the "old" before the "new", "new" without the "old", or "old" and "new" simultaneously? In their enthusiasm to promote the new biotechnology for traditional fermented food applications, scientists from developed countries should not forget the different environments that exist in developed and developing countries. In developed countries the old biotechnology is already well understood and practiced efficiently in fermented food industries. African countries may need to acquire a better understanding of the old biotechnology before efficiently absorbing and implementing the new biotechnology to its fullest.

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2

Applying Cleaner Production and Optimising Heat Process Operations in Dairy Processing Plants

2.1 Introduction

It is often claimed that Cleaner Production techniques do not yet exist or that, if they do, they are already patented and can be obtained only through expensive licences. Both statements are true, and this belief wrongly associates Cleaner Production with 'clean technology'. Firstly, Cleaner Production depends only partly on new or alternative technologies. It can also be achieved through improved management techniques, different work practices and many other 'soft' approaches. Cleaner Production is as much about attitudes, approaches and management as it is about technology. Secondly, Cleaner Production approaches are widely and readily available, and methodologies exist for its application. While it is true that Cleaner Production technologies do not yet exist for all industrial processes and products, it is estimated that 70% of all current wastes and emissions from industrial processes can be prevented at source by the use of technically sound and economically profitable procedures (Baas et al., 1992).

Dairy processing occurs world-wide; however the structure of the industry varies from country to country. In less developed countries, milk is generally sold directly to the public, but in major milk producing countries most milk is sold on a wholesale basis. In Ireland and Australia, for example, many of the large-scale processors are owned by the farmers as co-operativers, while in the United States in individual contracts are agreed between farmers and processors. Dairy processing industries in the major dairy producing countries have undergone rationalisation, with a trend towards fewer but larger plants operated by fewer people. As a result, in the United States, Europe, Australia, and New Zealand most dairy processing plants are quite large. Plants producing market milk and products with short shelf life, such as yoghurts, creams and soft cheeses, tend to be located on the fringe of urban centres close to consumer

markets. Plants manufacturing items with longer shelf life, such as butter, milk powders, cheese and whey powders, tend to be located in rural areas closer to the milk supply. The general tendency world-wide, is towards large processing plants specialising in a limited range of products. There are exceptions, however. In Eastern Europe for example, due to the former supply-driven concept of the market, it is still very common for 'city' processing plants to be large multi-product plants producing a wide range of products. The general trend towards large processing plants has provided companies with the opportunity to acquire bigger, more automated and more efficient equipment. This technological development has, however, tended to increase environmental loadings in some areas due to their requirement for long-distance distribution. Basic dairy processes have changed little in the past decade. Specialised processes such as ultra-filtration (UF), and modern drying processes, have increased the opportunity for the recovery of milk solids that were formerly discharged. In addition, all processes have become much more energy efficient and the use of electronic control systems has allowed improved processing effectiveness and cost savings.

Since the production of safe products is becoming increasingly important, predictive models for product contamination greatly benefit the food industry, especially if it is possible to optimize the process operation in relation to the desired product quality and safety. In general, three types of predictive models are necessary for optimization and improvement of food heat treatments:

- Model Type I: Process models that describe the production chain in terms of model reactors. In general, process models are based on energy and mass balances of the liquid phase and not on the food components or contaminants. For example, a plate heat exchanger can be described by at least four plug flow reactors in series: upstream regenerative section,

heater, holding tube and downstream regenerative section. All the plug flow reactors must have the same volume and specific surface areas the equipment itself (De Jong, 1996). The main output of such models is a temperature-time history of the food product. In cases where water is removed (e.g. evaporating, drying), the local water content is important, since an additional concentration change of food components and contaminants is introduced.

- Model Type II: Kinetic models that predict the transformation of food components and contaminants related to the food properties recognized by the consumer. These models include, for example, the de-naturation and aggregation of proteins, the inactivation of enzymes, bacteria and spore inactivation, contamination and the formation of reaction products (pigments, (off-) flavours). In some cases, the models are quite complex. For example, to predict the contamination of bacteria in the production chain, a predictive model for the concentration of microorganisms as a result of growth, adherence, release, and inactivation is needed.

- Model Type III: Predictive kinetic models for estimation of the operating costs related to process operation. In many processes, the operating costs are governed by microbial and physical fouling. In cases where it is possible to predict the amount of protein and mineral deposits and the number of adhered and growing bacteria, it is relatively simple to estimate the operating costs.

In order to simulate a heat treatment in the food production chain with respect to food properties and operating costs, the model types II and III are integrated with the process model (type I). All three types of kinetic models have been developed and validated for industrial application. In this section, a general procedure for optimization of the heat treatment in the food production chain is

described. The operating costs of many food production chains primarily depend on microbial and physical fouling of the equipment In general, process operating times at relatively low temperatures (<70 ℃) are due to adherence and growth of bacteria. The operating time of equipment at temperatures above 80 ℃ is determined largely by the deposition of protein and minerals. The amount of fouling can be related to the costs due to cleaning, changeover (rinsing losses), depreciation, energy, operator, pollution, and product losses (De Jong, 1996).

2.2 Description

2.2.1 Impact of Dairy Processing - The Need for Cleaner Production

For many other food processing operations, the main environmental impacts associated with all dairy processing activities are the high consumption of water, the discharge of effluent with high organic load and the consumption of energy. Noise, odour, and solid wastes may also be concerns for some plants. Dairy processing characteristically requires very large quantities of freshwater. Water is used primarily for cleaning process equipment and work areas to maintain hygiene standards. The dominant environmental problem caused by dairy processing is the discharge of large quantities of liquid effluent. Dairy processing effluents generally exhibit the following properties:

- high organic load due to the presence of milk components;
- fluctuations in pH due to the presence of caustic and acidic cleaning agents and other chemicals;
- high levels of nitrogen and phosphorus;
- fluctuations in temperature.

If whey from the cheese-making process is not used as a by-product and discharged along with other wastewaters, the organic load of the resulting effluent is further increased, exacerbating the environmental problems that can result. In order to understand the environmental impact of dairy processing effluent, it is useful to briefly consider the nature of milk. Milk is a complex biological fluid that consists of water, milk fat, a number of proteins (both in suspension and in solution), milk sugar (lactose), and mineral salts. Dairy products contain all or some of the milk constituents and, depending on the nature and type of product and the method of manufacturing, may also contain sugar, salts (e.g. sodium chloride), flavours, emulsifiers, and stabilisers. For plants located near urban areas, effluent is often discharged to municipal sewage treatment systems. For some municipalities, the effluent from local dairy processing plants can represent a significant load on sewage treatment plants. In extreme cases, the organic load of waste milk solids entering a sewage system may well exceed that of the township's domestic waste, overloading the system. In rural areas, dairy processing effluent may also be irrigated to land. If not managed correctly, dissolved salts contained in the effluent can adversely affect soil structure and cause salinity. Contaminants in the effluent can also leach into underlying groundwater and affect its quality. In some locations, effluent may be discharged directly into water bodies. However this is generally discouraged as it can have a very negative impact on water quality due to the high levels of organic matter and resultant depletion of oxygen levels. Electricity is used for the operation of machinery, refrigeration, ventilation, lighting and the production of compressed air. Like water consumption, the use of energy for cooling and refrigeration is important for ensuring good keeping quality of dairy products and storage temperatures are often specified by regulation. Thermal energy, in the form of steam, is used for heating and cleaning. As well as depleting fossil fuel resources, the consumption of energy causes air pollution and greenhouse gas emissions,

which have been linked to global warming. Dairy products such as milk, cream, and yogurt are typically packed in plastic-lined paperboard cartons, plastic bottles and cups, plastic bags or reusable glass bottles. Other products, such as butter and cheese, are wrapped in foil, plastic film, or small plastic containers. Milk powders are commonly packaged in multi-layer Kraft paper sacs or tinned steel cans, and some other products, such as condensed milks, are commonly packed in cans. Breakages and packaging mistakes cannot be totally avoided. Improperly packaged dairy product can often be returned for reprocessing; however the packaging material is generally discarded. Emissions to air from dairy processing plants are caused by the high levels of energy consumption necessary for production. Steam, which is used for heat treatment processes (pasteurisation sterilisation, drying etc.), is generally produced in on-site boilers, and electricity used for cooling and equipment operation is purchased from the grid. Air pollutants, including oxides of nitrogen and sulphur and suspended particulate matter, are formed from the combustion of fossil fuels, which are used to produce both these energy sources. In addition, discharges of milk powder from the exhausts of spray drying equipment can be deposited on surrounding surfaces. When wet these deposits become acidic and can, in extreme cases, cause corrosion. For operations that use refrigeration systems based on chlorofluorocarbons (CFCs), the fugitive loss of these gases to the atmosphere is an important environmental consideration, since CFCs are recognised to be a cause of ozone depletion in the atmosphere. For such operations, the replacement of CFC-based systems with non- or reduced-CFC systems is thus an important issue. Some processes, such as the production of dried casein, require the use of hammer mills to grind the product. The constant noise generated by this equipment has been known to be a nuisance in surrounding residential areas. The use of steam injection for heat treatment of milk and for the creation of reduced pressure in evaporation processes also causes high noise levels. A substantial

traffic load in the immediate vicinity of a dairy plant is generally unavoidable due to the regular delivery of milk (which may be on a 24-hour basis), deliveries of packaging, and the regular shipment of products. Noise problems should be taken into consideration when determining plant location. Hazardous wastes consist of oily sludge from gearboxes of moving machines, laboratory waste, cooling agents, oily paper filters, batteries, paint cans etc. At present, in Western Europe some of these materials are collected by waste companies. While some waste is incinerated, much is simply dumped.

2.2.2 Cleaner Production Assessment

Table 2.1 *Methodologies for undertaking a Cleaner Production Assessment.*

Organisation	Document	Methodology
UNEP, 1996	*Guidance Materials for the UNIDO/UNEP National Cleaner Production Centre*	1. Planning and organisation 2. Pre-assessment 3. Assessment 4. Evaluation and feasibility study 5. Implementation and continuation
UNEP, 1991	*Audit and Reduction Manual for Industrial Emissions and Wastes. Technical Report Series No. 7*	1. Pre-assessment 2. Material balance 3. Synthesis
Dutch Ministry of Economic Affairs, 1991	*PREPARE Manual for the Prevention of Waste and Emissions*	1. Planning and organisation 2. Assessment 3. Feasibility 4. Implementation
USEPA, 1992	*Facility Pollution Prevention Guide*	1. Development of pollution prevention programme 2. Preliminary assessment

A Cleaner Production assessment is a methodology for identifying areas of inefficient use of resources and poor management of wastes, by focusing on the environmental aspects and thus the impacts of industrial processes. Many organisations have produced manuals describing Cleaner Production assessment methodologies at varying levels of detail. However, the underlying strategies are much the same. The basic concept centres on a review of a company and its production processes in order to identify areas where resource consumption,

hazardous materials and waste generation can be reduced. Table 1.0 lists some of the steps described in the more well-known methodologies.

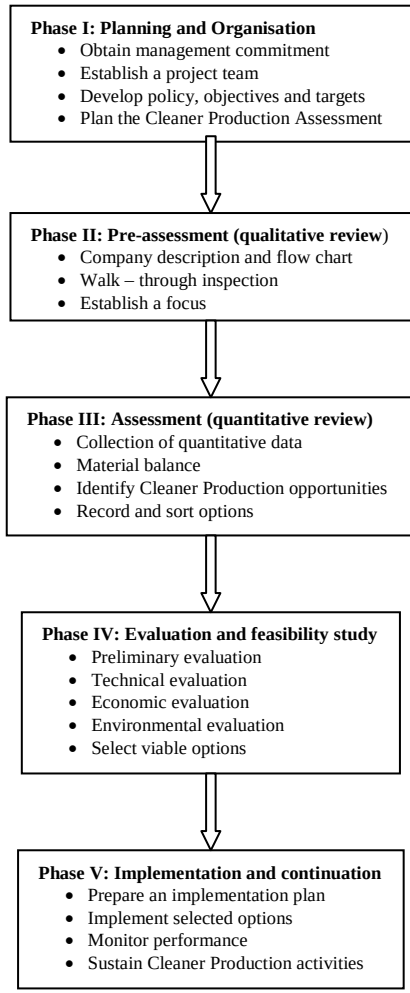

Figure 2.1 *Overview of the Cleaner Production assessment methodology (UNEP, 1996).*

2.2.3 Approach in Optimising Heating Processes

For the application of models in the food industry, an approach is needed that integrates the three types of models (process, product and costs model). Another need is kinetic data. The increasing availability of predictive kinetic models and necessary kinetic data has stimulated a reaction engineering approach to obtain optimal product quality (0). The functional properties of the product and the operating costs of the equipment are largely determined by conversion of so-called key components in the raw materials processed. The main control factors for product and process optimization are the temperature-time relationship and the configuration of the processing equipment. In order to determine the optimal values of the control variables, a general objective function is used:

$$F(u,x) = a\ c(u,x) + bc(u)\ quality\ operation \tag{1.8}$$

where u is a vector of process control variables (e.g. temperature, flow), and x is a vector of desired product properties related to food quality and safety. The value of c quality depends on the outcomes of the predictive models for contamination and transformation of food components cooperation is related to the operating costs. The optimal configuration and operation of a production chain are achieved by minimization of the objective function. To avoid trivial and undesired solutions, the weight factors α and β are introduced. These weight factors give the relative importance of each term of the objective function. For example, too high a value of β may result in a very clean and cheap production process but an inferior product quality. Figure 2.0 shows a general approach for process and product development by use of predictive kinetic models. In order to optimize a production chain, first the available raw materials and ingredients, the desired product properties and a general process description should be known. Based on the desired product properties, the desired conversion of key components is determined. Embedding the predictive models for product properties (II) and for

physical and microbial fouling (III) into the process model (I), the values of *c* quality and cooperation can be calculated. Next, the evaluation of objective function results in improved conditions (i.e. control factors) for the production chain and the evaluation of the predictive models are repeated. This process goes on until the minimum of the objective function is obtained, i.e. the optimal conditions are found. Before the optimization results are applied, some validation experiments can be performed. Some examples of recent industrial applications that accelerated the process and product development are:

- improvement of the performance (extended operating time) of a cheese milk pasteurizer; examination of two evaporator designs with respect to bacterial growth;
- determination of critical points in the downstream processing of whey;
- extended operating time (200%) of a production chain for baby food.

For the Dutch dairy industry, it has been calculated that in terms of energy, there reduction of fouling and contamination by predictive models has already a savings potential of 90 million m3 of gas (De Jong and Van der Horst, 1997).

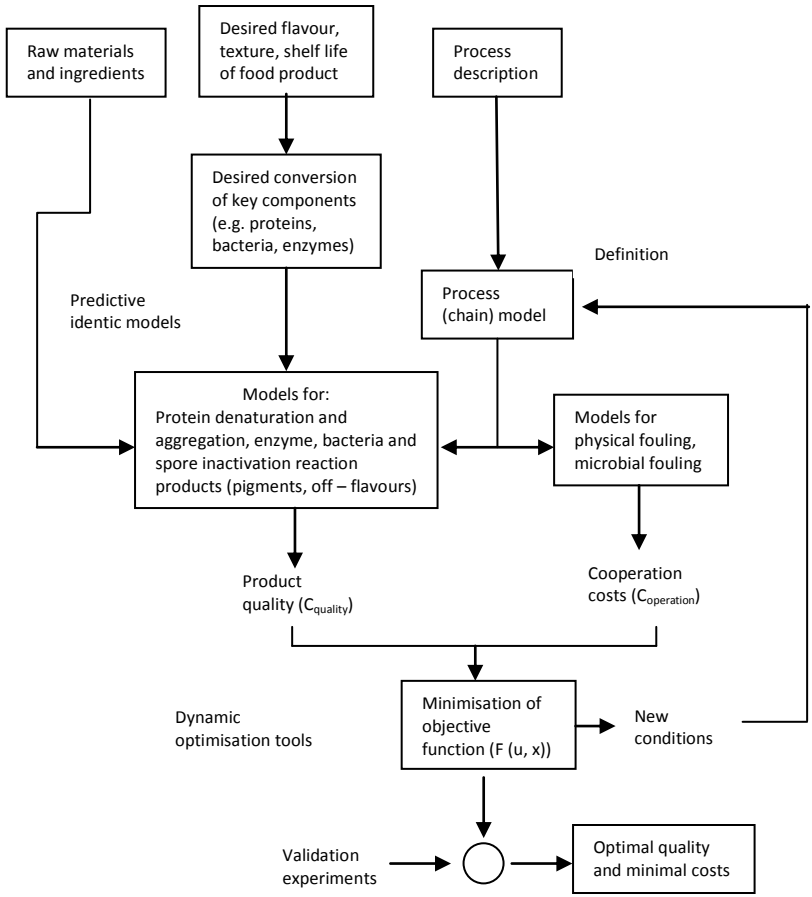

Figure 2.2 *Schematic representation of process and product development of heated milk products using predictive kinetic models. Source: Britz. J. T and Robinson. R. K. 2008.*

2.3 General Analysis

2.3.1 Identifying Cleaner Production Opportunities for Production Processes

An analysis of the production processes in Milk and Dairy processing reveals the following:

(a) Receipt and Storage of Milk

Cleaner Production opportunities in this area focus on reducing the amount of milk that is lost to the effluent stream and reducing the amount of water used for cleaning. Ways of achieving this include:

- avoiding milk spillage when disconnecting pipes and hoses;
- ensuring that vessels and hoses are drained before disconnection;
- providing appropriate facilities to collect spills;
- identifying and marking all pipeline to avoid wrong connections that would result in unwanted mixing of products;
- installing pipes with a slight gradient to make them self-draining;
- equipping tanks with level controls to prevent overflow;
- making certain that solid discharges from the centrifugal separator are collected for proper disposal and not discharged to the sewer;
- using ‘clean-in-place’ (CIP) systems for internal cleaning of tankers and milk storage vessels, thus improving the effectiveness of cleaning and sterilisation and reducing detergent consumption;
- improving cleaning regimes and training staff;
- installing trigger nozzles on hoses for cleaning;
- reusing final rinse waters for the initial rinses in CIP operations;
- collecting wastewaters from initial rinses and returning them to the dairy farm for watering cattle.

(b) Separation and Standardisation

Cleaner Production opportunities specific to this area are related to reducing the generation of separator sludge and optimising its collection and disposal. Ways of achieving this include:

- reducing the frequency with which centrifugal separators are cleaned, by improving milk filtration at the receiving stage or by clarification of the raw milk;
- collecting the sludge and disposing of it along with other waste solids.

Also of importance is the optimisation of cleaning processes, to make them water and energy efficient.

(c) Pasteurisation and Homogenisation

Cleaner Production opportunities in this area focus on improving energy efficiency. Ways of achieving this include:

- replacing batch pasteurisers with continuous process incorporating plate heat exchanger (PHE) pasteurisers, where feasible. PHE pasteurisers are more energy efficient than batch pasteurisers because the heat from the pasteurised milk can be used to preheat the incoming cold milk (regenerative counter current flow);
- installing new manufacturing equipment, which will result in less waste of milk products than the equipment currently used in many dairies;
- avoiding stops in continuous processes. The more constant the production, the less milk will be lost, since most waste comes from cleaning of batch

process equipment. In the event of upgrades to process equipment, high-volume pasteurising units should be considered;

- reducing the frequency of cleaning of the pasteuriser. Particularly for small dairies, optimising the size of balance tanks before and after the pasteuriser will allow continuous operation of the pasteuriser and reduce cleaning frequency;
- planning production schedules so that product change-over's coincide with cleaning regimes;
- collecting and recovering the milky wastewater generated at start-up of pasteurisation and supplying it to farmers as animal feed.

Also of importance is optimisation of cleaning processes, to make them water and energy efficient. To make possible the reprocessing of excess milk returned from the market, dairy plants may wish to consider developing policies which allow for the reprocessing of milk without affecting the quality of the freshly pasteurised product. The introduction of poorer quality milk into the pasteurisation process can result in milk scale and coagulation problems due to higher acidity. This may cause higher milk losses in the pasteuriser due to the need for more frequent cleaning in order to remove milk scale. These issues should be weighed against the benefits of reprocessing returned milk. The controlled return and reprocessing of milk from the market may require training of sales representatives. Alternatively, penalties could be applied for inappropriate ordering, or bonuses paid for extended periods of no market returns.

(d) Deodorisation

Water used for the vacuum pump can be recirculated to reduce or eliminate the necessity to discharge it.

(e) Storage and Packaging

Cleaner Production opportunities in this area focus on improving the energy efficiency of refrigeration systems and optimising CIP processes to reduce both water use and the organic load discharged into the effluent stream. Ways of achieving this include:

- clearing milk residues from the pipes using compressed air before the first rinse;
- collecting the more highly concentrated milk wastewater at start-up and shut-down for use as animal feed;
- optimising the accuracy of filling operations. This will not only result in improved efficiency, but will also reduce potential for waste and spillage. Minor variations in filling performance can have significant cumulative effects particularly for small unit fill quantities;
- improving procedures for recovering milk from wrongly filled containers;
- emptying and collecting product from wrongly filled containers for use as animal feed;
- reducing energy consumption through improved insulation, closing of doors to cold areas, good maintenance of room coolers and regular defrosting;
- using direct ammonia-based cooling systems instead of CFC-based systems.

(f) Butter Production

Cleaner Production opportunities in this area focus on reducing water use and loss of product. Ways of achieving this include:

- minimising the number of times the pasteuriser is cleaned. Particularly in small butter dairies, optimising the size of balance tanks before and after the pasteuriser will allow it to operate continuously, resulting in less need for cleaning;

- installing modern pasteurising equipment. This will reduce waste of cream in many dairies, because improvements in plate design now give a more gentle and constant heat treatment. This decreases the build-up of overheated solids on heating surfaces. In the event of upgrades to process equipment, high-volume pasteurising units should be considered;

- collecting the more highly concentrated milk wastewater generated when starting up the pasteuriser, for use as animal feed.

(g) Butter Churning

Cleaner Production opportunities in this area focus on reducing loss of product. Ways of achieving this include:

- ensuring that the buttermilk is collected separately and hygienically so that it can be used in other processes, such as a base for low fat spreads;

- collecting all first rinses, and separating the residual fat for use in other processes;

- preventing the build-up of milk scale deposits;

- maintaining butter makers on a regular basis;

- avoiding spills by ensuring that the buttermilk collection facilities are large enough to hold all the liquid.

(h) Butter Packaging

Cleaner Production opportunities in this area focus on reducing water use and loss of product. Ways of achieving this include:

- collecting first rinses while still warm and separating the milk fat residues for use in other processes;
- reducing the disposal of packaging material by having personnel constantly optimising operation of the packaging machines.

(i) Butter Storage

Cleaner Production opportunities in this area focus on improving the energy efficiency of refrigerated storage. Ways of achieving this include:

- installing insulation;
- keeping doors closed in cold areas;
- undertaking regular defrosting of cold rooms and regular maintenance of refrigeration systems;
- avoiding refrigerants that contain CFCs. Refrigeration systems based on ammonia cooling are preferred.

(j) Cheese Production

A number of opportunities exist for the recovery of the valuable high-grade protein from sweet whey. However it has only been in recent years that they have become technically and economically viable. The method used is ultra-filtration (UF), followed by spray drying of the protein. This process is costly, so is only worthwhile when large quantities of fresh whey are available. Spray-

dried whey powder contains between 25% and 80% protein and is used in food products, where it performs a similar function to egg proteins. Whey powder is highly soluble, even at high acidity, and is capable of forming stable foams and gels when heated. Whey protein powder is therefore used in the manufacturing of bakery and meat products, where its gelatinous properties are particularly useful. Other options available for whey utilisation are:

Evaporation Followed by Spray Drying to Produce Whey Powder

One of the problems associated with this solution is that the lactose tends to caramelise, making any heating process difficult. Unless special precautions are taken, the resulting product is very hygroscopic due to the high concentrations of lactose (70–75%). Whey powder in this form is not suitable for use as a food ingredient because it is very sticky and absorbs moisture during storage, forming hard lumps. Non-hygroscopic whey powder can be produced by recrystallizing the lactose before drying. In this way, most of the lactose is present in the alpha-crystalline form, which is no hygroscopic. Higher-quality whey powder can be produced by incorporating a secondary crystallisation step after spray drying. Powder is removed from the drying chamber at 8–14% moisture. The moisture remaining in the powder permits almost complete crystallisation of the lactose and the residual moisture can then be removed in a secondary drying system (e.g. a fluid bed) before the powder is cooled and packaged.

Feeding It to Animals

In most countries where this is practised, the whey is normally fed to pigs or cows. This is a low-cost solution but the price obtained for whey, after transport costs are considered, is often only a very small fraction of the cost of the original milk. The advantages are that there are no capital costs and no effluent charges.

Demineralisation or Reduction of the Mineral Content of Whey

This increases the range of opportunities for its use as a food ingredient. Ion exchange treatment or electro dialysis is used in the demineralisation process, and demineralised whey is spray-dried in the same way as whey powder. The main use of demineralised whey powder is in the manufacture of infant milk formulations, where it is used in combination with skimmed milk powder to give similar composition to that of human milk. Another use of demineralised whey powder is in the manufacture of chocolate. Electro dialysis, or ion exchange technology, is comparatively expensive but it does give an end product with a higher value.

Anaerobic Digestion and Fermentation

Whey can be anaerobically digested to produce methane gas, which can be captured and used as a supplementary fuel on site. Whey can also be fermented to produce alcohol. In addition, there are a number of Cleaner Production opportunities for reducing the loss of product from the process, which include:

- preventing the loss of curds by not overfilling cheese vats;
- completely removing whey and curds from the vats before rinsing;
- segregating all whey drained from the cheese;
- sweeping up pressings instead of washing them to drain;
- screening all liquid streams to collect fines.

(k) Cheese Packaging

All cheese scraps should be collected separately from other waste and either used as raw material for processed cheese manufacturing (where possible) or

sold as animal feed. Liquid wastes should be treated, together with other effluent streams.

(l) Cheese Storage

Methods for reducing energy consumption and minimising the impacts of refrigerant use are:

- installing good insulation;
- keeping doors to cold rooms closed;
- undertaking regular defrosting and maintenance of refrigeration systems;
- avoiding refrigerants that contain CFCs. Refrigeration systems based on ammonia cooling are preferred.

(m) Evaporation in Evaporated and Dried Milk Production

Cleaner Production opportunities in this area focus on ensuring the efficient operation of the evaporators, including:

- maintaining a liquid level low enough to prevent product boil-over;
- using entrainment separators to avoid carry-over of milk droplets during condensation of evaporated water;
- recirculating low concentration milk and other feed stocks until required concentration is reached;
- prior to scheduled shut-downs, processing rinse waters with solids content greater than 7% or evaporating them during the next run rather than discharging them to the effluent stream;

- draining equipment thoroughly before starting rinsing and washing;
- collecting the first rinse water for animal feed;
- reducing the frequency of cleaning operations as much as possible;
- reusing condensate as cooling water after circulation through cooling tower, or as feed water to the boiler.

(n) The Drying Process

Methods for avoiding the release of fine milk powder to surrounding areas include:

- minimising emissions to air by using fabric filters or wet scrubbers;
- undertaking wet cleaning only when absolutely necessary, and plan for it to coincide with a change of product;
- controlling air emissions and taking corrective action if levels are beyond acceptable limits.

(o) Packaging and Storage of Milk Powder

The Cleaner Production opportunities in this area focus on the prevention of emissions of milk powder dust, including:

- ensuring the proper management of storage operations;
- installing exhaust ventilation to minimise dust in the work place.

(p) Cleaning

For dairies without CIP systems, consideration should be given to their installation. CIP systems make the recovery and reuse of cleaning solutions possible, and systems equipped with in-line monitoring can control the quality of cleaning solutions, thereby maximising the use of detergents, and minimising water use. For dairies with CIP equipment, it's important to determine and maintain optimum operational settings to reduce the consumption of both water and detergents. Further water reductions can be achieved by providing facilities for the collection of final rinse waters so that they can be reused as the initial rinse water in the next CIP cycle. Detergents and disinfectants can be significant sources of pollution if too much is used. It is very important, therefore, to monitor their consumption. An optimum detergent concentration for cleaning should be determined. Operators should ensure that tanks, pipes, and hoses are as completely empty as possible before they are cleaned. Empty pipelines can be blown with compressed air before cleaning in order to reduce any milk film that may have adhered to the walls of vessels and pipelines. Cleaning of floors and equipment often consumes large quantities of water, due to the traditional cleaning method in which the operator directs a jet of water from a hose onto equipment and floors until the milk and solids float down the drain. Solid wastes, such as curd particles in the cheese making process, can be collected using a brush or broom rather than being rinsed down the drain. The use of pigging systems to remove product residues from the internal surfaces of pipeline prior to cleaning can help to reduce the pollutant load of cleaning wastewaters and also allow for product recovery. Spray nozzles are subject to wear that causes deterioration of the orifice and distortion to the spray pattern. This results in an increased flow rate of water and reduced effectiveness. In general, 10% nozzle wear will result in a 20% increase in water consumption (McNeil and Husband, 1995).

(q) Crate Washing

Cleaner Production opportunities in this area therefore focus on reducing the consumption of water. Ways of achieving this include:

- optimising water consumption by monitoring the water pressure and the condition of the water spray nozzles;
- installation of spray nozzles of the optimum dimensions;
- fixing leaks promptly;
- turning off the crate washer when not in use;
- recirculating wash water through a holding tank.

(r) Refrigeration and Cooling

CFC-based refrigerants should be replaced by the less hazardous hydrogenated chlorofluorocarbons (HCFCs) or, preferably, by ammonia. In the long run both CFCs and HCFCs should be replaced by other refrigerants according to the Montreal Protocol. Replacing CFCs can be expensive, as it may require the installation of new cooling equipment. Minimising the ingress of heat into refrigerated areas can reduce energy consumption. This can be accomplished by insulating cold rooms and pipes that contain refrigerant, by closing doors and windows to cold areas, or by installing self-closing doors.

If water and electricity consumption in the cooling towers seems high, it could be due to algal growth on the evaporator pipes. Another reason could be that the fans are running at too high a speed, blowing the water off the cooling tower. Optimising the running of the cooling tower can save a lot of water.

2.4 Actualisation

2.4.1 Case Study of Pasteurisation (Optimisation of Heating Processes)

To illustrate the application of the described procedure for optimizing food production chains, the following case study has been performed. A heating process with a capacity of 40 tonnes of skim milk per hour consists of a regenerative section, a heating section, two holder sections, and a cooler. In Fig. 3.0., the scheme of the process is shown with some preliminary temperatures and residence times. In order to have a process model, the equipment is transformed to a cascade of model reactors. Details of the characteristic dimensions are given in the literature (De Jong *et al.*, 2002b). The objective is to develop a process that meets the specifications as given in Table 2.1. The objective function is defined as:

$$F(u,x) = \sum_{i=1}^{3} a_i \left(\frac{x_{ides} - x_i(u)}{x_{ides}} \right)^2 + F_{cost} \qquad \text{(i)}$$

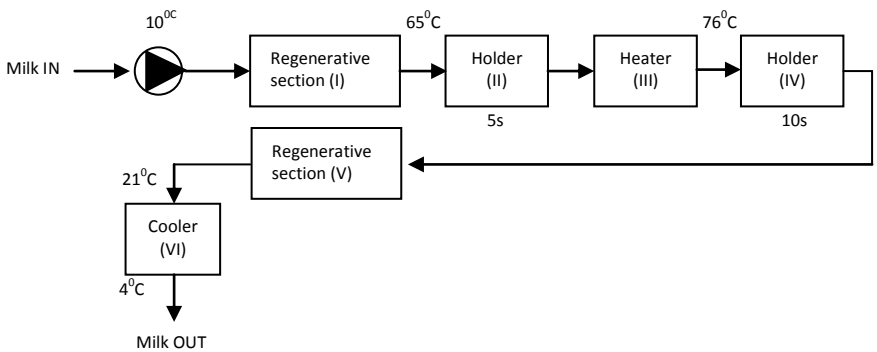

Figure 2.3 *Scheme of the pre-design of the pasteurization process.*

Table 2.2 *Product and process specifications of pasteurization (process variables). Source: Britz. J. T and Robinson. R. K. 2008.*

Process variable	**Desired value (*xi*, des)**	**Weight factor ($\alpha_{1)}$**
β-Lactoglobulin denaturation	72–80	4–8
Decimal reduction *Strep thermophilus*	130–145	75–90
Production costs	142–150	60–85

Where

$$F_{cost} = \frac{c_{operation} t_{operation} + c_{solids} t_{production} \int\int_{x,i} J_{x,i} dtdx}{t_{production} \varnothing} \quad \text{(ii)}$$

where the integral term is the total amount of deposits after 1 h of production, and φ is the capacity of the process in tonnes per hour and:

$$t_{production} = \frac{t_{operation} t_{run}}{t_{run} + t_{cleaning}} \quad \text{(iii)}$$

and

$$t_{run} = t \text{ if } (C_{\text{Strep. thermophiles}} > 0.001 \text{cfu ml}^{-1)} \quad \text{(iv)}$$

V values of several constants are given in the literature (De Jong *et al.*, 2002b). The weight factor α_1 is introduced to avoid trivial and undesired solutions; for example, a low-cost process resulting in an inferior product quality. The chosen values of the weight factors are determined by the relative importance of the different product properties. However, since the relationship between the weight factor values and the optimization results are not clear beforehand, the determination of the weight factor value is an iterative process in consultation with industrial experts. In this case, the control variables (*u*) are limited to two, the heating temperature, and the residence time at this temperature in the second holder section. With two control variables, surface plots can present the results of the computer model simulations. Figure 2.4 shows the results of the model

evaluations. According to Equation ii, in this case it is assumed that the maximum run time is limited by contamination with *Strep. the rmophilus* and not limited by the deposition of proteins and minerals on the wall surface. At a temperature lower than 79 ℃ and run times shorter than 30 h, the deposition layer does not result in insufficient heat transfer. Related to that, the objective function accounts for the increasing amount of product losses. According to Fig. 4 d, the operating costs per tonne of heated product decrease with temperature and residence time. This is due to the increased operating time resulting in an extended annual production time. However, at higher temperatures and longer residence times, the amount of denatured proteins exceeds the desired value of 2.5% resulting in a substantial contribution to the objective function. Catalase activity was not a key parameter in the temperature and residence time region applied. At a temperature of 78.5–79.0 ℃ and a residence time of 1 s or longer, the activity was <0.1%. In Table 2.2, the optimal values of the control variables and the related process variables are listed. Compared with the initial preliminary design (10 s, 76 ℃), the operating costs could be decreased by 14%. At an annual production time of 4700 h, this means an estimated cost saving of €58 000.

Table 2.3 *Optimization of the pasteurization process Source: Britz. J. T and Robinson. R. K. 2008.*

Variable	Reference	Optimal
Control variable	760	78.7
Heating temperature (℃)	10	3
Residence time (s)		
Process variable		
Catalase activity (%)	1.6	0.01
β-Lactoglobulin denaturation (%)	0.84	2.46
Decimal reduction Streptococcus thermophilus	6	6
Production costs (€ ton $^{-1}$)	2.16	1.86

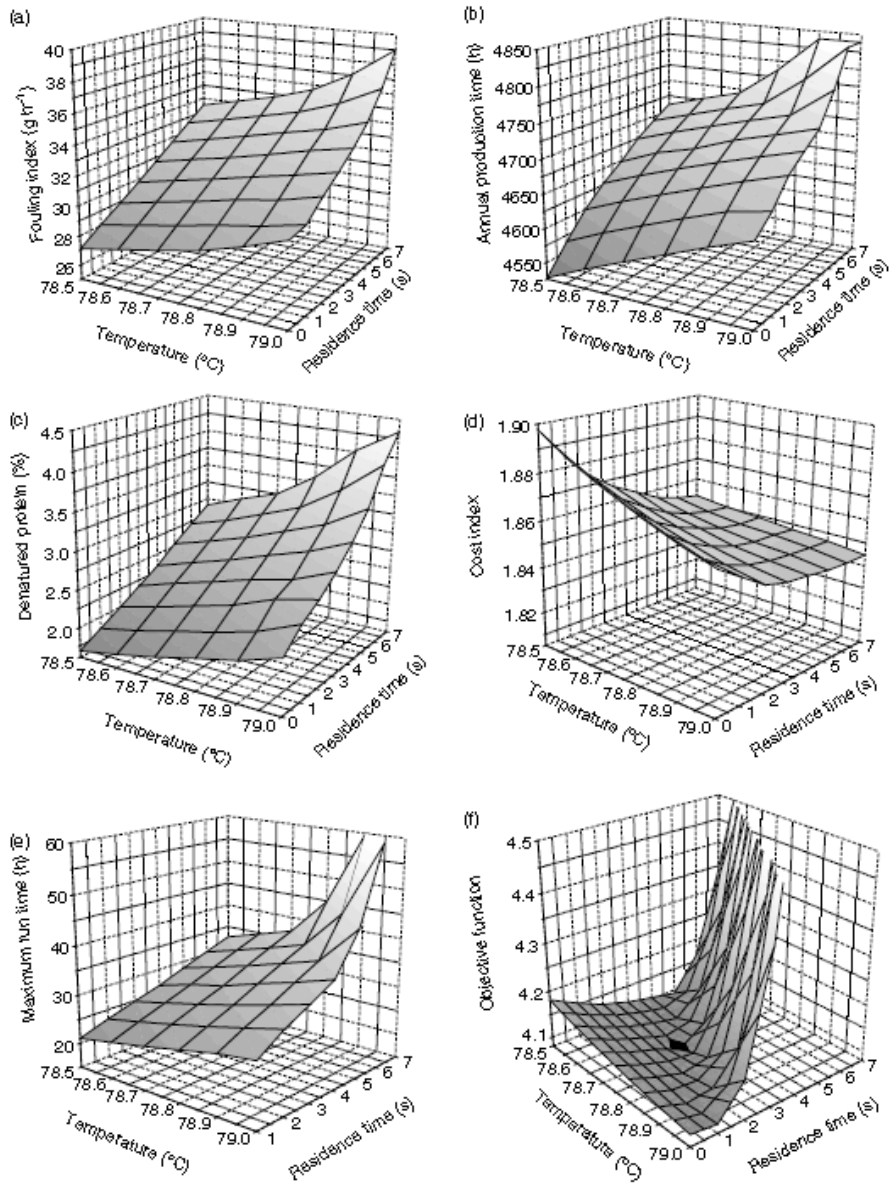

Figure 2.4 *Results of the optimization for different aspects of the objective function as a function of the control variables: (a) fouling index; (b) annual production; (c) protein denaturation; (d) cost index (€ ton−1); (e) maximum run time; (f) objective function evaluation. Source: Britz. J. T and Robinson. R. K. 2008.*

2.4.2 Case Study of Cleaner Production - Campina Melkunie Maasdam - The Netherlands

The Cleaner Production assessment for this Dutch company was carried out as part of the PRISMA project (Dutch Ministry of Economic Affairs, 1991). The project identified five Cleaner Production opportunities:

- better emptying of production tanks;
- elimination of rinsing between yogurt batches;
- reduced rinsing at product change-over;
- optimisation of cleaning operations;
- recovery of low-grade heat.

This case study demonstrates that even when considerable effort has already been made to improve the environmental performance of accompany, it may still be possible to identify additional Cleaner Production opportunities through a formal Cleaner Production assessment process.

(a) Company Description

Campina Melkunie Maasdam is part of the Campina Melkunie Holland cooperative. The company employs 170 people, who work two shifts. The company produces a wide range of milk, custard, and yogurt products. In total l05 million litres of milk is processed per year; 92 million litres for market milk and 13 million litres for other dairy products.

(b) Process Description

The milk is delivered to the plant in milk tankers, after which it is separated. Depending on the required end product, the milk may then be mixed with non-

separated milk to obtain the correct fat content. The milk is pasteurised and homogenised, and packed into cardboard or glass packaging. A proportion of the milk is processed further into yogurt, custard, and buttermilk. During the production process, product clings to the internal surfaces of pipes and equipment, which can lead to reduced product quality. To avoid this, the entire process is cleaned and sanitised after each production day, and specific pieces of equipment may also be cleaned throughout a production day. Cleaning agents containing, among other things, sodium hydroxide, and hydrogen peroxide and per acetic acid are commonly used.

(c) Environmental Aspects

Like all dairy processing plants, the company generates a warm, liquid effluent stream containing milk constituents and cleaning and sanitising agents. The quantity of effluent discharged per year is 130,000 L. The organic loading of this wastewater averages about 1240 mg COD/L, which is equivalent to 3600 pollution units (pu), where 1 pu equals the organic pollution load generated by one person. The company is not connected to a wastewater treatment plant and therefore discharges treated effluent directly to surface water. The cost for discharging effluent is calculated according to the Dutch Pollution of Surface Water Act and amounts to US$120,000 per year, based on US$33 per pu. Emissions to air principally result from the combustion of fossil fuels in the boiler for steam generation. Pollutants emitted include NO_2, CO, CO_2 and PAHs, but the quantities have not been measured. The company has three chemical waste streams: ink, solvents, and laboratory waste. About 10 litres per year of each of these wastes are generated. This is taken away to the small municipal chemical waste depot. By far the largest proportion of the company's solid waste stream is of packaging materials, particularly the cardboard containers used to package milk. Approximately 125,000 containers are lost as waste per

year, which represents approximately 0.25% of the total number of cartons consumed. The value of this waste stream has been estimated to be about US$6000. Paper wastes are reused off site wherever possible and reject glass bottles are also recycled off site. The company generates its own steam in an on-site boiler for heating and processing, and other energy needs are met using electricity. Prior to the PRISMA project waste prevention measures had already been taken by the company, driven by financial and efficiency considerations. A lot of energy was used for the production of milk products. With the high energy prices of the 1970s it was cost-effective to take energy-saving measures. A lot of water was also used. For the production of 1 litre of milk ten years ago, 10 litres of water were needed. This has since been reduced to 1.4 litres of water. The preventive measures taken primarily involved reuse options, such as using the cooling and rinse water several times before discharging it. Another measure, to reduce the effluent charge, is to return waste product to the production process or collect it separately and take it away as cattle feed. Only if this is not possible is the product discharged.

(d) The Cleaner Production Assessment

Based on previous studies of product losses undertaken by the company, it was possible to identify areas where relatively large amounts of waste and emissions were being produced. The primary sources of pollution load are product loss to the effluent stream and the use of cleaning agents. This is caused by, among other things, batch production processes, which lead to the need for frequent cleaning and subsequent losses during start-up and shut-down.

Another area of concern was the high energy consumption for heating and cooling. To reduce the pollution load fourteen preventative measures were drawn up. Since then, eight of them have been implemented. Three options still

have to be looked at more closely and three have been found to be impracticable for various reasons. The result has been as follows:

- a reduction in product loss by 24,000 litres (3.4% reduction);
- a 23% saving in consumption of chemicals;
- a reduction in pollution load by 198 pu./yr. (a 5.5% reduction);
- a 138,000 m3/yr. saving in natural gas consumption.

Total savings have amounted to US$68,000 per year, and possibly an additional US$26,000 in reduced effluent charges. This was achieved by a single investment of US$32,000.

Table 2.4 *Identified Cleaner Production options.*

	Projects implemented	**Projects still to be implemented**	**Feasibility study required**
Loss of product	Improvements to procedures Improvements to tank emptying practices	Replacement of cooling installation	
Cleaning operations	No rinsing between yogurt batches Optimisation of cleansing process Reduced rinsing		Substitution of cleansing agents Reuse of sour products
Energy	Pre-heating milk for buttermilk Custard heating	Pre-heating milk for yogurt production	
Miscellaneous	Replacement of ink injector		

2.5 Discussion

The life cycle of milk and milk products commences with the production of fresh cow's milk on dairy farms. Milk is then processed to produce pasteurised and homogenised market milk, butter, cheese, yogurt, custard, and dairy desserts etc. It may also be preserved for a longer shelf life in the form of long-life (UHT), condensed, evaporated, or powdered milk products. The various products are packaged into consumer portions and distributed to retail outlets. For fresh dairy products, refrigerated storage is required throughout the life of

the products to maintain eating appeal and prevent microbiological spoilage. Following use by the consumer, packaging is either discarded or recycled.

The processing of milk to produce dairy products is a significant contributor to the overall environmental load produced over the life cycle of milk production and consumption. Therefore the application of Cleaner Production in this phase of the life cycle is important. As in many food processing industries, the key environmental issues associated with dairy processing are the high consumption of water, the generation of high-strength effluent streams, the consumption of energy, and the generation of by-products. For some sites, noise and odour may also be concerns. Investing in Cleaner Production, to prevent pollution and reduce resource consumption is more cost effective than continuing to rely on increasingly expensive 'end-of-pipe' solutions. When Cleaner Production and pollution control options are carefully evaluated and compared, the Cleaner Production options are often more cost effective overall. The initial investment for Cleaner Production options and for installing pollution control technologies may be similar, but the on-going costs of pollution control will generally be greater than for Cleaner Production. Furthermore, the Cleaner Production option will generate savings through reduced costs for raw materials, energy, waste treatment, and regulatory compliance. The environmental benefits of Cleaner Production can be translated into market opportunities for 'greener' products. Companies that factor environmental considerations into the design stage of a product will be well placed to benefit from the marketing advantages of any future Eco labelling schemes.

2.6 General Recommendations

Recommendations regards Cleaner Production are derived from perceived environmental impacts associated with each production process and they

represent a range of available options, from profitable activities that require no investment to other activities that may increase the production plant's costs:

2.6.1 Water Pollution

Site small dumps or waste treatment sites far away from surface or groundwater water sources.

Separate harmful chemical waste from organic waste, and use more care in handling chemical waste. Dispose of chemical waste in methods in a manner that prevents chemicals from leaching into ground or surface waters (such as clay- or concrete-lined pits).

If the enterprise stores waste temporarily before transporting it to a treatment facility or landfill, make sure it is not leaking into the ground.

2.6.2 Working Conditions

Maintain safety equipment and reinforce safety training. Safety measures may already be in place, but workers should be reminded often; designate one person as the safety trainer and have that person train others. Check existing safety equipment regularly, and replace elements like dust filters frequently.

Create a prevention strategy. Sometimes small changes such as buying a face mask or rubber gloves can dramatically reduce incidences of harm to workers. Find ways of preventing accidents.

Find ways of reducing harmful by-products. For example, clean the floors in between production cycles to get rid of excess dust, or install drip trays to catch acidic fruit juice.

2.6.3 Spoilage

Ensure that the building structure is secure not only from people but also from animals. Screens should be placed over drains and windows to keep out disease-carrying rodents and flies.

Storage areas should be well-ventilated and large enough so that excessive heat and moisture do not cause spoilage in milk products.

2.6.4 Solid Waste

Re-use organic waste. Some organic waste such can be used as animal fodder.

Modify waste disposal to facilitate faster decomposition/breakdown of organic material. Add layers of dirt and dry organic material to waste pits, or spread waste over large areas of land. This type of composting and “land spreading” can speed up decomposition and quickly lowers waste volume. Ensure material does not attract disease-carrying vectors including birds, rodents, and insects.

Minimize wastes by improving production processes. Identify and change elements of production that may be inefficient or produce excess waste. For example, improved techniques for cutting food produce can reduce waste and yield more product.

2.6.5 Poorly Maintained Machinery

Schedule regular machine maintenance checks and repairs. Ensure up-to-date training in operation and maintenance. Do not wait until machinery is broken before checking it; leaks can occur long before serious equipment breakdown and may be costing you money. If possible and cost-effective, replace faulty

machinery with more efficient machinery. If machinery is difficult to access, then monitor wastes or emissions to detect leaks. For example, check for puddles underneath machinery or chemical/fuel smells.

Use wood shavings, drop cloths and/or oil/water separators to catch spills and leaks.

If you are disposing of organic and chemical wastes separately, ensure that chemical or fuel waste does not contaminate the organic waste.

If it is not cost-effective to replace or to repair machinery, make sure harmful effects are minimized. Increase ventilation around any machinery that has high gas or chemical emissions.

2.6.6 Water Use

Decrease water usage through “dry clean-up”. Dry clean-up involves an initial cleaning without water (sweeping, wiping down) before washing. This method reduces the amount of water required to dislodge solid wastes from floors or machinery.

Regulate water flow. Using high-pressure water hoses can ease cleaning and cut water use; usually this only involves adding a new nozzle to the end of a hose.

Reuse water. Some food processors use steam to purify or clean packaging materials; a closed-loop system can cycle hot water back into the system. This process saves money on both water and energy costs.

2.6.7 Liquid Waste

Practice water reduction strategies mentioned above, including “dry clean-up”, to minimize the amount of wastewater created and the amount of waste materials in the wastewater.

Separate fats, grease, and solids from wastewater. Oil separators or oil traps can be purchased or made at relatively low cost and can reduce the amount oil in wastewater dramatically. Drain stagnant pools of liquid or water away from holding pens and working areas.

Consider constructing waste treatment ponds. Both solid and liquid waste can be treated in these, which can aid decomposition and reduce disposal costs. Since they may attract mosquitoes and other insects, site such ponds away from animals and places of human activity.

2.6.8 Noises and Odours

- Locate waste disposal sites away from housing or town centres.
- Modify waste disposal or production practices to minimize odours. For example, if treating waste in lagoons or compost pits, make sure they are large enough to accommodate the volume of waste that is produced–if too small, the effectiveness of the treatment decreases and smell increases.
- Provide earplugs for workers.
- Repair and maintain machinery so that excessive grinding or squeaking is minimized. This may increase the machinery’s efficiency and make it last longer.

2.7 Conclusion

Cleaner Production is the most effective way to design and operate industrial processes and to develop and produce products and services. The costs of wastes and emissions, including negative environmental and health impacts, can be avoided or minimised by applying the Cleaner Production concept from the beginning and apply it continuously and throughout the entire life cycle. The costs of the traditional, reactive environmental strategy - the end-of-pipe strategy - are well known. These costs continue to grow, and as regulations become more aggressive and precise will increasingly add to the burden of business. In contrast, when Cleaner Production is applied, processes become more efficient because they require fewer raw materials and/or generate less waste. Cleaner Production approaches recognise that change has to come from within and sustainable change cannot be imposed from external sources against the needs or desires of the firm. Generally with a Cleaner Production approach, there are inevitably substantial economic benefits that can be directly related to the program.

Heating of milk has been rationalized to a great extent by introducing the chemical engineering approach. In this approach, the milk is described as a fluid with a number of key components and the equipment is described as a number of chemical model reactors. Processes for heating can be designed on the basis of the desired product specifications. After determination of the optimal temperature-time combination, the appropriate heating equipment can be selected and designed. Although heating is a well-developed and relatively robust preservation technology, there are still a number of challenges for improvement. For example, to improve the nutritional value of heated dairy products, there is a need for heating technologies that realizes hyper-short treatment at high temperature. Developments such as the ISI technology should

be encouraged. Also, for a number of products, the (bio) fouling of the heating equipment and its related negative consequences limits the application.

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3

ISO 14040 Life Cycle Assessment (LAS) as a Tool for Effective Environmentally Friendly Waste Management in the Food Industry

3.1 Introduction

The increased awareness of the importance of environmental protection, and the possible impacts associated with products both manufactured and consumed, has increased interest in the development of methods to better understand and address these impacts. One of the techniques being developed for this purpose is lifecycle assessment (LCA). LCA can assist in

- identifying opportunities to improve the environmental performance of products at various points in their life cycle,
- informing decision-makers in industry, government or non-government organizations (e.g. for the purpose of strategic planning, priority setting, product or process design or redesign),
- the selection of relevant indicators of environmental performance, including measurement techniques, and
- marketing (e.g. implementing an Eco labelling scheme, making an environmental claim, or producing an environmental product declaration).

For practitioners of LCA, ISO 14044 details the requirements for conducting an LCA. LCA addresses the environmental aspects and potential environmental impacts (e.g. use of resources and the environmental consequences of releases) throughout a product's life cycle from raw material acquisition through production, use, end-of-life treatment, recycling and final disposal (i.e. cradle-to-grave). There are four phases in an LCA study:

- The goal and scope definition phase,
- The inventory analysis phase,

- The impact assessment phase, and
- The interpretation phase.

The scope, including the system boundary and level of detail, of an LCA depends on the subject and the intended use of the study. The depth and the breadth of LCA can differ considerably depending on the goal of a particular LCA. The life cycle inventory analysis phase (LCI phase) is the second phase of LCA. It is an inventory of input/output data with regard to the system being studied. It involves collection of the data necessary to meet the goals of the defined study. The life cycle impact assessment phase (LCIA) is the third phase of the LCA. The purpose of LCIA is to provide additional information to help assess a product system's LCI results so as to better understand their environmental significance. Life cycle interpretation is the final phase of the LCA procedure, in which the results of an LCI or an LCIA, or both, are summarized and discussed as a basis for conclusions, recommendations, and decision-making in accordance with the goal and scope definition. There are cases where the goal of an LCA can be satisfied by performing only an inventory analysis and an interpretation. This is usually referred to as an LCI study. This International Standard covers two types of studies: life cycle assessment studies (LCA studies) and lifecycle inventory studies (LCI studies). LCI studies are similar to LCA studies but exclude the LCIA phase. LCI studies are not to be confused with the LCI phase of an LCA study. Generally, the information developed in an LCA or LCI study can be used as part of a much more comprehensive decision process. Comparing the results of different LCA or LCI studies is only possible if the assumptions and context of each study are equivalent. Therefore this International Standard contains several requirements and recommendations to ensure transparency on these issues. LCA is one of several environmental management techniques (e.g. risk assessment,

environmental performance evaluation, environmental auditing, and environmental impact assessment) and might not be the most appropriate technique to use in all situations. LCA typically does not address the economic or social aspects of a product, but the life cycle approach and methodologies described in this International Standard can be applied to these other aspects.

3.2 Description

Every day in the marketplace, people make choices that affect, directly or indirectly, the environment. Manufacturers choose from among different materials, suppliers, or production methods. Consumers, for their part, either choose among products or whether to use a product at all. Those who would like to make environment ally responsible choices need reliable information which is frequently related to life cycle assessment (LCA) (http://www.tc207.org/articles/). LCA considers the environmental aspects and the potential impacts of a product or a service system throughout its life - from raw material acquisition through production, use, and disposal (from cradle to grave). This information is very important and can be of great help in identifying ways to improve environmental aspects of a product at various stages in its life cycle, to support decision-making in industry, governmental or non-governmental organizations. It can substantially help the selection of various indicators of environmental performance (EP) and (with proper precautions) to promote the marketing of products or services (EPA, 1994). The Society for Environmental Toxicology and Chemistry (SETAC) defines LCA as the:

> *Process to evaluate the environmental burdens associated with a product, process, or activity by identifying and quantifying energy and materials used and wastes released to the environment; to assess the impact of those energy and material uses and releases to the environment; and to identify*

and evaluate opportunities to effect environmental improvements. The assessment includes the entire life cycle of the product, process or activity, encompassing extracting and processing raw materials; manufacturing; transportation and distribution; use, re-use, maintenance; recycling; and final disposal (EPA, 1994).

The main focus of LCA is to determine the environmental impacts of the system understudy in the areas of ecological well-being, human health, and resource depletion (Tansey and Worsley, 1995). ISO 14041 is entitled 'Life Cycle Assessment – Goalsand Definition/Scope and Inventory Analysis' and is intended to describe the special requirements and guidelines for the preparation, conduct and critical review of the lifecycle inventory analysis. The most widely accepted LCA structure is the one suggested by SETAC as described in Table 1.0. The term 'life cycle analysis' is often employed for the analysis stage of a life cycle assessment. Goal definition (ISO 14040) is perhaps the most important component of LCA. The inventory (ISO 14041) is an analysis, qualitative and/or quantitative, of the resources used and the emissions generated in the life cycle. The impact assessment (ISO 14042) can be divided into classification, characterization, and valuation (Andersson *et al*., 1994). The assessed impacts fall into three broad categories: human health, ecological health, and resource use (Tansey and Worsley, 1995). Characterization is the aggregation of inventory data within the impact categories by the use of equivalency factors (Andersson *et al*., 1994). It is a largely quantitative step that analyses the relative contribution of the multiple inputs or outputs by category (Tansey and Worsley, 1995). Valuation can be carried out either qualitatively or quantitatively by expert panels or by comparison of environmental loading profiles, respectively (Andersson *et al*., 1994; Boudouropoulos and Arvantioyannis, 1999). Interpretation (ISO 14043) stands for conclusions based on the assessment and suggestion of improvement actions.

Table 3.1 *Structure of LCA suggested by the Society of Environmental Toxicology and Chemistry (SETAC) Source: Andersson et al., 1994, Tibor and Feldman, 1997.*

Analysis	Goal definition and scooping Inventory analysis
Assessment	Impact assessment, which is divided into: Classification Characterization Valuation
	Improvement analysis

International Standards are playing a critically important role in all industries formational production, international terminologies, safety, and health protection, measurements, analysis, quality control, and environmental protection, particularly in the energy field, where standards for the interfaces in energy flows are indispensable, such as electric connectors, fuelling devices, calibration methods, and electrical safety. Besides the specific standards for petroleum, coal, nuclear and hydro-power, hydrogen and the vast field of electricity, the energy standards series ISO 13600 allows the characterization, analysis and comparison of all energy systems and soon will issue a global energy statistics and planning matrix for the transition to environmentally sound sustainable economics. These standards allow integrated resource planning, including all new renewable options, such as the increasingly important direct and indirect solar energy, co-generation, hybrid systems, small decentralized units, bio-energy, ambient temperature use by heat pumps and substitutions of muscle-powered systems or vice versa, besides the more efficient production and use of conventional finite and renewable energy-sources (Grob, 2003). The total cost of their emissions, their net gray, i.e. re-usable embedded energy, can be determined, total and relative efficiencies can be calculated and their life cycles and risks can be assessed with this new standard tool in conjunction with the many existing and emerging standards on specific energy systems or parts thereof (Figure 3.1).

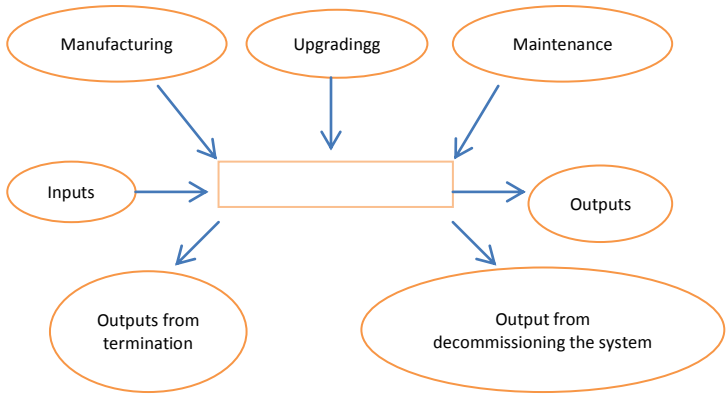

Figure 3.1 *Factors affecting the total cost of emissions (adapted from Grob, 2003).*

LCA is a process:

To evaluate environmental burdens related to products, processes or activities, to identify potential impacts on the environment coming from energy or material consumptions, to identify and to evaluate possible product improvements (SETAC, 1993).

3.3 General Analysis

The 'from cradle to grave' perspective, which LCA can take into account, makes it possible to judge and improve environmental performances over the entire life cycle, as well as appraise embodied improvements at particular levels. Nevertheless, depending on the specific requirements of a company, LCA may also be used in a limited perspective ('from process to process'), which can be of particular interest should the company wish to analyse carefully a limited part of the whole life cycle, the one under its own control (De Monte *et al*., 2005).

As regards the potential applications of LCA, Azapagic (1999) from Hospido *et al*. (2003) put forward the main uses as follows:

- Identification of environmental improvement opportunities.
- Strategic planning or environmental strategy development.
- Product and process optimization, design and innovation.
- Environmental reporting and marketing.

Life cycle assessment (LCA) is an analytical tool for the systematic evaluation of the environmental aspects of a product or service system through all stages of its lifecycle. A graphical description of LCA methodology based on the principles of ISO 14040 is shown in Figure 3.2.

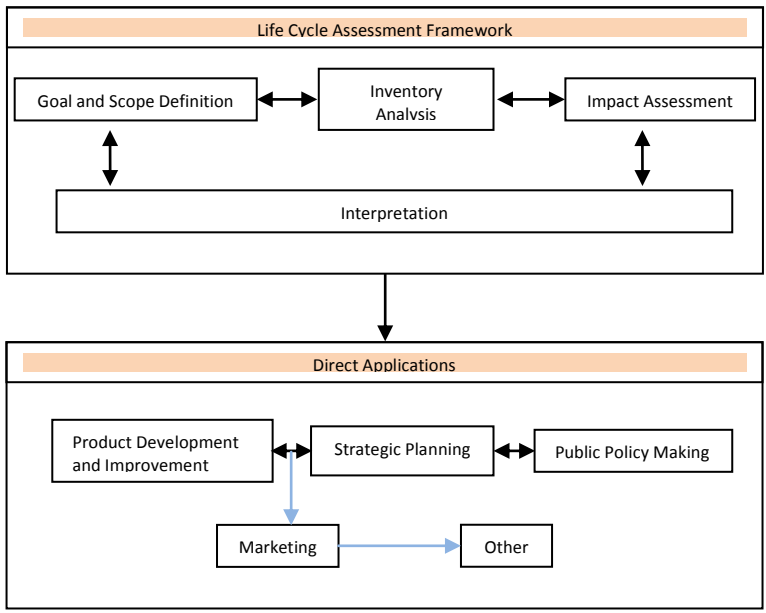

Figure 3.2 *Phases and applications of an LCA (ISO 14040, 1997).*

The main applications of LCA are in:

- Analysing the origin of problems related to a particular product,
- Comparing improvement variants of a given product,

- Designing new products,
- Choosing among a number of comparable products (LCA part 1).

Pennington *et al*. (2004) recently compiled a review on the life cycle impact assessment (LCIA) phase, focusing on the key attributes of the supporting models and methodologies.

These models and methodologies provide LCA practitioners with the factors they need for calculating and cross-comparing indicators of the potential impact contributions associated with the wastes, the emissions and the resources consumed that are attributable to the provision of the product in a study. ISO 14042, entitled 'Life Cycle Assessment – Impact Assessment (LCIA)', proposes to provide guidance on the impact assessment phase of LCA. This phase of LCA is aimed at evaluating the significance of potential environmental impacts using the results of the life cycle inventory analysis (Haklik, 1998). The environmental load unit taken from the natural resource or substance effect index multiplied by the amount of the substance used or released produces total environmental load value (ELV) for the particular product or process (Kuhre, 1995). Because of the inherent subjectivity in impact assessments, the most critical requirement for their conduct will be disclosure, so that decisions and assumptions can be clearly described and reported (Haklik, 1998). LCA consists of both mandatory and optional elements, as illustrated in Figure 3.2 (ISO 14042, 2000):

- Selection of the impact categories of interest, the indicators per impact category, and the underlying models (a procedure also considered in the initial goal and scope phase of an LCA).

- Assignment of the inventory data to respective impact category. Impact categories include climate change, stratospheric ozone depletion, photo-oxidant formation (smog), eutrophication, acidification, water use, and noise.

The food production industry requires large inputs of resources and causes several negative environmental effects. The food production systems are oriented and optimized to satisfy economic demands and the nutritional needs of a rapidly growing world population. Environmental issues, however, have not been given much attention. There are many difficulties in conducting life cycle studies of food products. Ideally, a complete study should include agricultural production, industrial refining, storage and distribution, packaging, consumption and waste management, all of which together comprise a large and complex system (Koroneos *et al*., 2005).

LCA Studies on Foods

Table 3.2 *LCA Studies on Foods; Source: I. S. Arvanitoyannis 2008.*

Product (Food)	Country	Effects	Reference
Peas, bread and milk product	The Netherlands	Environmental impacts from packaging in proportion to other parts of the supply system	Kooijman, 1993
Margarine	The Netherlands	Two margarines and two low-fat products were investigated, concentrating on the fat components and packaging	*Vis et al.*, 1992
Bread and Meat	Denmark	Qualitative study with a very broad definition of environmental parameters	Pederson, 1992
Canned cooked Products	Austria	Application of the Institute for Okologische Wirtschaftsforschung's eco-balance system. The study involved four canning industries and one producer of packaging	IOW Wien, 1992
Fruit Yogurt	Germany	Comparison of processed products from ordinary and ecological agriculture	Ott, 1992
Bread, beer and Cheese	Germany	Comparison of processed products from ordinary and ecological agriculture	Ott, 1992
Tomatoes and Cucumbers	Switzerland	Comparison of seven hydroponic systems and eight ordinary production systems (under glass, In tunnels, and outdoor production)	Hahn, 1992
Tomatoes	Switzerland	Comparison of seven different ways of producing Tomatoes under glass	Cysi and Reist, 1990
Potatoes	Switzerland	Investigation of seven combinations of thermal, mechanical and chemical potato topping	Jolliet, 1993
Milk Chain	Sweden	The total use of energy and packaging materials seems to be	Sonesson

Product (Food)	Country	Effects	Reference
Supply		crucial to the outcome. More knowledge of the amount of wastage in households is required, both in absolute numbers and as influenced by the type of packaging	and Berlin, 2003
Ketchup Production	Sweden	Six alternative sub-systems, including packaging, processing, and transportation, were modelled and simulated. The environmental impact categories included were energy use, global warming, acidification, eutrophication, photo-oxidant formation, and the generation of radioactive waste. It was concluded that the contribution to acidification can be reduced significantly and the environmental profile of the product can be improved for either the type of tomato paste currently used or a less concentrated tomato paste	Anderson and Ohlsson, 1999
Milk Production	Spain	Different sub-systems were identified and thoroughly studied – farms, fodder factories, and dairies – and even through the collection of their inventory data took place throughout one complete year, some values were found to vary considerably. Raw milk production, specifically the agricultural phase, and packaging manufacture have been identified as the crucial elements. Other aspects such as formulation of animal food at farms and emission from boilers at dairies are also decisive when improvement actions are to be set up	Hospido *et al.*, 2003
Beer	Greece	The impact categories most affected by the beer Production are the earth toxicity or heavy metals and the Category of smog formation. Bottle production, followed by packaging and beer production are found to be the sub-systems that account for most of the emissions	Koroneos et al., 2005
Wild caught And farmed Salmon	Norway	The fishing phase for the cod and the feeding phase for both salmon and chicken dominate for all Environmental impacts considered. Chicken is most energy effective followed by salmon and cod, which are almost on the trawling is around 100 times larger than the land area needed to produce the chicken feed for production of the 0.2kg fillet. There is potential for improvement of environmental performance, both for salmon farming and cod fishing, especially when it comes to energy use	Ellingsen and Aanondsen, 2006
Danish fish Products	Denmark	Energy consumption is a key factor contributing to the environmental burden for all investigated fish products. The (quantitative) LCA suggests that the environmental hotspot for flatfish is the fishing stage. The same applies to cod, Norway lobster, shrimp, and prawn. Generally, however, the use and retail stages are also important, while the processing stage only represents an important impact potential for certain types of fish products (pickled herring, canned mackerel and mussels)	Thrane, 2006
Fish	The Netherlands	Impacts such as climate change, stratospheric ozone depletion, photo-oxidant formation (smog), eutrophication, acidification, toxicological stress on human health and ecosystems, the depletion of resources and noise. The need exists to address these product-related contributions more holistically and in an integrated manner, providing complementary insights to those of regulatory/process-oriented methodologies	Pennington *et al.*, 2004
Canned Tuna	Spain	The system under study included landing at harbour, Transport to the factory, processing inside the factory, Final product distribution to markets and use in households. The results show that processing accounted for the greatest percentage in all the	Hospido *et al.*, 2006

Product (Food)	Country	Effects	Reference
		impact categories, except human toxicity potential. Inside the factory, the production and transportation of tinplate was identified as the most significant contributor and, consequently, improvement actions were proposed and evaluated, such as an increase in the percentage of the recycled tinplate	

3.4 Actualisation: LCA Case Studies Reported – Dairy Processing

The dairy sector has been extensively studied from the perspective of LCA in Norway: milk production (Hogaas, 2002); Sweden: milk production focused on the farm level (Cederberg and Mattson, 2000) and semi-hard cheese (Berlin, 2002); and Germany: milk production, with a special interest on impacts associated to agriculture (Cederberg and Mattson, 2000; Haas *et al*., 2001). Although all dairy products are essential for the everyday nutritional regime, milk production has been chosen in this work as the most representative due to its outstanding position as an important staple food. In fact, most citizens consider consumption of milk in infancy, childhood and throughout adult life, as a prescription for good health. Regarding the different types of milk, skimmed milk (0.5% fat), semi-skimmed milk (1.5–1.8% fat) and whole milk (3.5% fat) are the most important ones (Hospido *et al*., 2003). The life cycle of milk production included in the analysis by Hospido *et al*. is shown in Figure 3.4.

In this analysis, six categories have been considered (global warming, stratospheric ozone depletion, acidification, eutrophication, photo-oxidant formation, and depletion of abiotic resources) as well as a flow indicator, energy consumption; on the contrary, others such as eco- and human toxicity and land use were not analysed. In a qualitative way, they considered that potential damage over those categories should not be very significant if the nature of emissions (with the exception of pesticides at the agricultural phase) and the

Galician land characteristics (low population density and low industrial character) are borne in mind. Among these six categories, three have been reported as significant: eutrophication, acidification, and global warming. Several actions focused on these categories (specifically on eutrophication potential (EP) and acidification potential (AP)) have been proposed and the percentages of reduction have been measured. However, it is necessary to indicate that there is an action that, although it cannot be quantified, had to be pointed out due to its importance on global warming potential (GWP). Methane emitted by cows on farms is responsible for more than 30% of greenhouse gas emissions, so it has an outstanding weight. The elements involved at animal feed turned out to be accountable for an important percentage of all the impact categories at the farm level. In addition, the item 'impacts associated with milk production', which includes emissions to air as well as to water, were identified as responsible for certain categories (GWP and PCOP). The milk supply chain model consists of two main parts: a background and a foreground system (Figure 3.4). The actual handling of dairy products is the foreground system. However, to supply the foreground system with such necessary inflows as packaging material, water, and energy in various forms, and also to take care of its residues, background systems are necessary. The results from a simulation thus include emissions from both background and foreground systems as well as all use of primary energy carriers caused by the milk supply chain. All models are static, i.e. the emissions and use of energy change linearly with changes in the flow, no economies of scale are assumed.

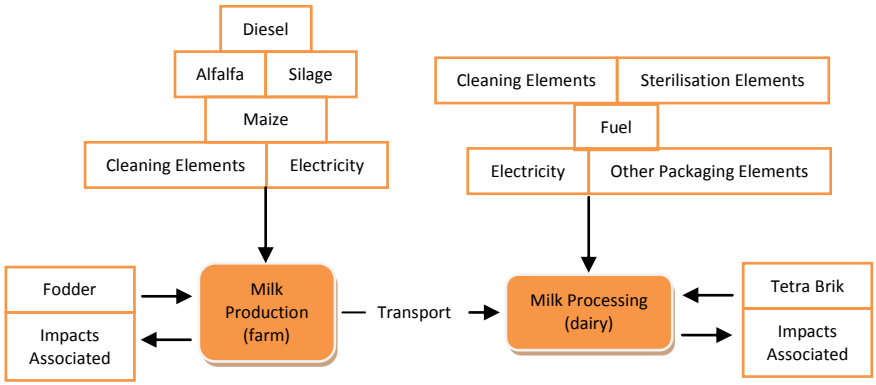

Figure 3.3 *Schematic flow chart of the life cycle of milk. (adapted from Hospido et al., 2003).*

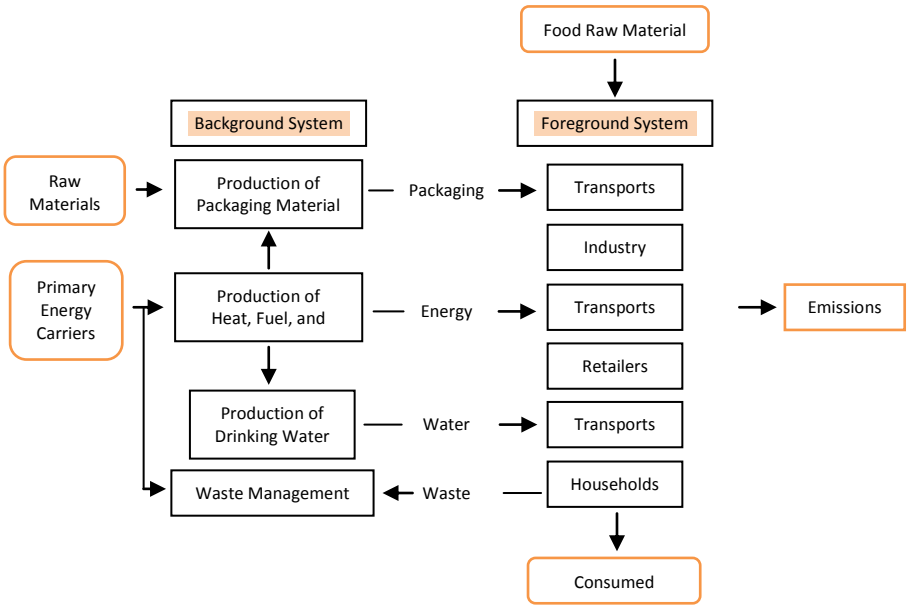

Figure 3.4 *LCA in future milk supply chains in Sweden (adapted from Sonesson and Berlin, 2003).*

The subsystems involved are as follows:

- Truck transport.
- Car and van transport.

- Dairies.
- Retail.
- Households.
- Energy system.
- Production of packaging material.
- Production of drinking water.
- Waste management (Sonesson and Berlin, 2003).

Within dairies, more products and more frequent deliveries to retailers probably result in less efficient dairies. The effects are also noticeable in distribution, retailing, home transport, and wastage within households. The model used in this study could still be improved to reflect fully the strong trends in this direction and provide reliable indications of their effects on environmental impacts. The total use of energy and packaging materials seems to be crucial to the outcome. More knowledge of the amount of wastage in households is needed, both in absolute numbers and as influenced by the type of packaging. In future studies, it might be interesting to include agriculture in the analysis since different future scenarios will probably also affect the structure of agriculture. In this study, agricultural patterns would probably differ in the large-scale scenario and in the green IT-wave scenario and thus would have different impacts on the environment.

3.5 Discussion

Nowadays, there is an increasing awareness that today's life style should aim at more sustainable production schemes in conjunction with limited use of renewable resources and minimal environmental impact on land, water and air

(Environment inhttp://www.tc207.org/articles/). All processes have to be envisaged as potential resources since their by-products provide the primary material for a subsequent process in a continuous regenerative loop (Tansey and Worsley, 1995). Life cycle assessment, though not a brand new tool any more, is still able to analyse and assess the environmental impacts associated with a product, process or service by multi attribute product evaluations. The importance of LCA as an environmental decision support tool continues to increase rapidly. A distinction between the objective and subjective elements of LCA is bound to take place in order to clarify the structure of the method and be of great help to the decision-making. Goal definition and scoping as well as interpretation of the inventory results would benefit most from decision analytic approach and methods. In these phases, subjectivity and the overall goal of the process have a major impact. The important dimensions of the decision problem could be presented in a value tree and this could be exposed to general discussion and modification before deciding the actual content and scope of the study. Pertinent answering of the prioritization questions at an early stage of the study is anticipated to help greatly the decision-makers in terms of identifying both the real decision alternatives and the concrete environmental problems closely linked with the product's environmental impacts thus providing the required inventory data. Valuation referring to values is another subjective issue and is closely linked to preference data (Miettinenand Hamalainen, 1997).

3.6 General Recommendations

3.6.1 Waste Management

What is being recommended is that multi-station sampling points from different unit operations gather composite samples of representative flows

discharged per shift in food processing areas for analytical purposes. These samples need to be tested for COD or BOD, plus those components specific to the foodstuff being processed. If we assume the raw material produced into a food contains carbohydrate, fat, proteins, and minerals besides water, then the different components have variable values depending on the ingredient source at the sampling station. Obviously, raw material components would be less valuable than components lost in products at other stages prior to the finished good value. For example, product X might be worth 20 cents/kilogram as raw product when the worth of components with carbohydrates is 5 cents/kilogram, 15 cents/kilogram for the protein fraction and 10 cents/kilogram for fat content. Further up-line in processing, when product is cooled and concentrated, the protein, and fat content are increased. In fact, the kilogram value might double to 30 cents for protein and 20 cents for fat losses with little change in carbohydrate value. When product loss occurs at this step more loss value needs to be applied to components sent to floor drains. This kind of an evaluation process quickly shows that affirm needs to begin paying strict attention at key steps to maximize product yield. All losses are vital, but we are perfectly safe in thinking more money will be lost in finished goods or partially finished products than for raw material in a food processing sequential step operation. Here are the things that need to be done:

- Establish a loss sampling station.
- Gather and test representative samples of loss materials sent to drains.
- Assign dollar values to different components.
- Provide unit operation supervisors loss information promptly so corrective actions might be taken to improve operations. On the other hand, the data

may well confirm to people that the operation is being carried on with success. This too is vital for people to know.

- Inventory wastage with the same vigour you would do for other items in your shift or day accounting system.

3.6.2 Life Cycle Assessment

Reporting the results of LCA must be impartial and complete. Objective reporting is made to third party; the requirements of the standard should cover at least the following aspects:

- general aspects;
- defining the purpose and scope;
- life cycle inventory analysis;
- life cycle impact assessment;
- life cycle interpretation;
- Critical analysis.

Critical analysis is essential and it can be made by an independent internal expert LCA study or by an external expert analysis that will give an analysis statement. SR EN ISO 14041 standard - Definition of the purpose, scope and inventory analysis – establishes requirements and procedures for setting up and training the purpose and scope definition for a Life Cycle Assessment (LCA) and for the development, interpretation and reporting cycle inventory analysis Life (ICV). The product-system is detailed here and the final product is indicated next to intermediates, auxiliary inputs, uncontrolled emissions, data quality, sensitivity analysis, uncertainty analysis, etc. For Life Cycle Inventory analysis, the “system product” which is composed of “units of product” must be defined more clearly.

It is essential that function, functional unit and reference flow to be clearly defined and to determine the boundaries of the original system. Inventory Analysis is made according to the logical scheme of Figure 3.5.

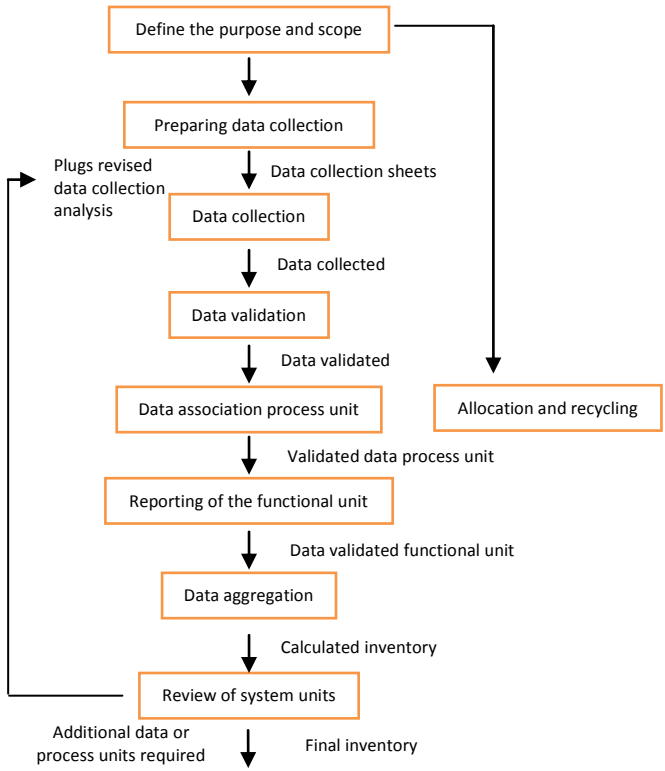

Figure 3.5 *Simplified procedures for the analysis of inventory.*

3.7 Conclusion

Integration of companies from various parts of the world in production and distribution of ordinary products (salad, fruit, etc..) is a globalized phenomenon. Of course, transportation is no longer a problem, technology on a bed of closed-circuit water, organic loading is widely controlled. Disputes of GM food technology have social implications in employment in overcrowded areas. To

gain access to these disputes the only way is to implement LCA management systems. So we have the technical data and even the possibility of participating in the dialogue provided by the policy in the field.

In recent years there has been accumulated large volume of communications on Custodies, and almost each of them claims fairness. To speak a common language we should relate strictly to the implementation of the reference standards SR EN ISO14040 series and procedures and records required by them. It is good to make implementation a consultant may, by Curriculum Vitae, evidence that leads directly to people and businesses. Vision is needed for overall management, linking the action system - product assembly and parts (sub-systems) components. LCA requires that analysis be made by independent experts, preferably external, to ensure objectivity. Objectivity ensures correct conclusions that will lead to finding that waiver decisions on economic and environmental grounds, a number of other systems - a product which actually represents the chain of companies and business, technical facilities, capital and workers with families and local communities. There are no acknowledged and accredited certification bodies of referential standards SR EN ISO 14040 series, still their application can solve the problem out – by developing a dialogue between stakeholders and consulting firms, creating regional accreditation bodies for our benefit.

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4

Novel Non Thermal Preservation Techniques in Meat Processing: High Hydrostatic Pressure as a Model Technology

4.1 Introduction

As a consequence of market globalization, the production and manufacture of meat products is at a stage of innovative dynamics. In order to keep or to reinforce their leading position, meat and food companies need to take into consideration the evolution of the purchasing and consumption habits of consumers, as well as the perception and definitively the trends of the consumers' demands. These consumers' demands are continuously changing, but some of the main parameters or axes are consolidating. Consumers demand high quality and convenient meat products, with natural flavour and taste, and very much appreciate the fresh appearance of minimally processed food. Besides, they require safe and natural products without additives such as preservatives and humectants.

To harmonize or to blend all these demands without compromising safety, it is necessary to implement new preservation technologies in the meat industry and in the food industry in general. High hydrostatic pressure (HHP) represents an attractive non-thermal process for meat products to avoid post-processing contamination. When combined with antimicrobials, like bacteriocins, the death rate may be increased because of sub lethal injuries to living cells. HPP is a powerful tool to control risks associated with *Salmonella spp*. and *Listeria monocytogenes* in raw or marinated meats. The HPP treatment could extend the shelf life of the marinated beef loin by controlling the growth of both spoilage and pathogenic bacteria.

Storage of chilled meats in air leads to rapid spoilage by psychotropic bacteria, predominantly *Pseudomonas* spp. and *Brochothrix thermosphacta*. Traditional packaging systems for meat products have been very successful in slowing the

rate of microbial spoilage and extending the shelf life of meats. These systems are designed to manipulate the gas environment surrounding the product. Such systems include oxygen - permeable overwrap for short – term retail display to maintain the bloom colour of red meats. For long-term storage, vacuum packaging (complete removal of headspace gases) or modified atmosphere packaging (MAP)/controlled atmosphere packaging (CAP) is employed. The success of these packaging systems is such that the majority of red meat produced in the United States is vacuum or MAP packaged (Siragusa et al. 1999).

The past decade has seen development of non - thermal technologies for the control of meat spoilage microorganisms and extension of shelf life. Information is now available on the types of microbes found on meats and conditions that lead to spoilage (Marshall and Bal'a 2001; Nychas et al. 2007). New information related to revolutionary packaging innovations such as gas scavenging and antimicrobial impregnation systems is now also available. So is information on recent developments in natural product biological interventions (phage, bacteriocins, chitosan, essential oils, and enzymes), chemical interventions (organic acid salts, acidified sodium chlorite, phosphates, ozone, and electrolyzed water), and physical interventions (ionizing irradiation, high pressure, hydrodynamic shockwave, pulsed electric fields, high intensity light, and cold plasma).

Many of the interventions remain at the theoretical stage and will require extensive validation and economic analysis before practical introduction to industry. Others, however, have found widespread use and will likely remain a mainstay in industry.

4.2 Description

4.2.1 Ionizing Irradiation

Irradiation is a safe and effective method to improve food safety and quality. Ionizing irradiation employs gamma rays (cobalt – 60 and caesium - 137 as radioactive sources), x - rays (machine - generated), and e beam (high - energy electrons, machine - generated) as treatments to successfully kill microbes in foods. Irradiation damages microbial DNA, resulting in cell death. According to Aymerichet al. (2008), viruses are most resistant to irradiation, followed by bacterial spores, yeasts, moulds, Gram - positive bacteria, and Gram - negative bacteria. This technology has excellent penetration power. For example, x - rays and gamma rays can penetrate 80 to100 cm while e beams have less penetrating power, ranging from 8 to 10 cm. None of these ionizing treatments make food radioactive, making questionable negative consumer fears about the technology. Irradiated foods should bear the internationally recognized radura symbol together with a "treated with irradiation" statement on the label to inform consumers.

4.2.2 Phage Technology

Bacteriophages (also known as phages), from "bacteria" and Greek *phagin*, "to eat" are viruses that infect bacteria. Phages consist of an outer protein shell with enclosed DNA or RNA. Phages infect, grow, and multiply only inside bacterial cells. Lytic phages cause bacterially is (cell death), which leads to the spread of more phage in the environment. Some phages lyse only a fraction of infected cells and keep other cells alive while continuously shedding new phages. Phages capable of lysogeny integrate phage DNA into the bacterial host DNA without causing cell death. Most reports on the use of phage technology focus on

applications to control meat -derived bacterial pathogens. For example, specific phages have been investigated against *Escherichia coli* O157: H7, *Listeriamonocytogenes*, *Campylobacter jejuni*, and *Salmonella enterica* Typhimurium (Bigwoodet al. 2008). In 2006, *L. monocytogenes* phage was approved by the FDA as a food antimicrobial (Stahl 2007). Several advantages of phage technology for meat spoilage control are described by others (Greer 2005; Hudson et al. 2005). For example, phages are self - reproducible and release more phage after bacterial lysis. Phage specificity may be an advantage if selective for spoilage micro flora only. On the other hand, specificity may diminish phage activity against broad - spectrum spoilage micro flora. Whitman and Marshall (1971a) noticed that phages from bacteriophage – host systems isolated from refrigerated food products usually attacked only those hosts upon which they were isolated. Phages are generally more stable than their hosts and can survive processing (Koo et al. 2000). Greer (1988) showed that phage concentration remained stable (5 to 6 log 10 PFU/cm 2) on the surface of refrigerated (4 ℃) beef rib – eye steaks during 14 days of storage in air. Whitman and Marshall (1971b) showed that some *Pseudomonas* phages isolated from beef may remain infectious after heating to 60 ℃, pH change to 4.0, and exposure to 4 MNaCl. Phages are naturally present entities and constitute part of the environment. Whitman and Marshall (1971a) isolated total of 38 host - phage pairings from ground beef, sausage, chicken, raw milk, and oysters. Phage concentration as high as 6.3 × 10 6 PFU/gas is found on chicken skin. Not surprisingly, most isolated were invaders of *Pseudomonas* spp., followed by Gram - positive cocci and members of the Enterobacteriacea family. Similarly, Atterbury et al. (2003) isolated 34 *Campylobacter* phages from retail chicken meat.

4.2.3 High Pressure Processing

High hydrostatic pressure (HHP) treatment involves placing packaged meat in a pressure vessel and applying isostatic water pressure of 100 to 900 MPa. HHP processing is considered non-thermal, since temperatures increase only 3 ℃ for every 100 MPa applied (Aymerich et al. 2008). Equipment for HHP is commercially available, including manufacturers Avure Technologies (United States) and Nicolas Correa Hyperbaric (Spain). HHP kills bacterial cells through a combination of actions, with the bacterial membrane the primary site of damage. Gram – negative bacteria are more susceptible, followed by Gram - positive bacteria and spores (Hugaset al. 2002). Linton et al. (2004) reported that the micro flora of chicken mince became less diverse and shifted to Gram - positive bacteria after HHP treatment. Regarding cell shape, rods (elongated) are more susceptible than cocci (round). It is generally believed that HHP does not significantly change the sensory quality of meats, although cooked colour (at 150 MPa), oxidation of ferrous myoglobin (at 400 MPa), and lipid oxidation has been reported in fresh and marinated meats (Hugas et al. 2002). Results of studies showing prevention of meat spoilage with HHP treatment are summarized in Table 4.1.

Table 4.1 *High hydrostatic pressure treatment of meat products.*

Product	Target bacteria	Results	Process	Reference
Minced beef muscle	Total micro flora	3 to 5 log 10 reduction	450 MPa, 20 min, 20 ℃	Carlez et al. 1994
Mechanically recovered poultry meat	Mesophilic bacteria	3.6 log 10 reduction	450 MPa, 15 min, 2 ℃	Yuste et al. 2001
Marinated beef loin Dry cured ham Cooked ham	Aerobic total count	> 4.5 log 10 reduction > 2.5 log 10 reduction > 6 log 10 reduction after 60 days at 4 ℃	600 MPa, 6 min, 31 ℃	Garriga et al. 2004
Minced chicken	Aerobic plate count	1 log 10 reduction	500 MPa, 15 min, 40 ℃	Linton et al. 2004

4.2.4 Hydrodynamic Shockwave Treatment

Hydrodynamic shockwaves (HDS) are generated either electrically (capacitor discharge system) or by using explosives in water. Besides tenderizing meat products by disrupting the myofibrillar structure (Schillinget al. 2003), HDS might influence bacterial counts as well, resulting in extended product shelf life (Raloff 1998). Explosively produced HDS are not commercially feasible because it is a batch - type process, has specific packaging requirements, and has potential worker safety concerns. In contrast, electrically generated HDS has been commercialized by Hydrodyne, Inc. (Claus et al. 2001). Mixed results are found in the literature on the effectiveness of HDS to inactivate microbes on meats. Williams - Campbell and Solomon (2002) showed that explosively generated shockwaves caused immediate reduction of aerobic plate counts by 1.5 to 2.0 log 10 CFU/g in fresh beef. After 14 days of storage, treated beef counts were 4.5 logs less than control samples. Schilling et al. (2003) showed that blade - tenderized beef treated with HDS had lower standard plate counts (0.5 log difference) compared to controls after 14 days of storage. On the other hand, Moeller et al. (1999) found no significant difference in aerobic plate counts and coliform counts between explosive HDS -treated pork muscle and control. Thus, aside from the obvious increase in tenderness, HDS treatment as a tool to decrease microbial loads and prolong the shelf life of meat products remains undetermined, and additional research is needed to support this concept.

4.2.5 Antimicrobials

(a) Bacteriocins

Bacteriocins are cationic and hydrophobic peptides produced by lactic acid bacteria, with antibacterial activity against related Gram-positive bacteria (Chen

and Hoover 2003). In addition to bacteriocins, lactic acid bacteria produce other antimicrobials, such as lactic acid, acetic acid, diacetyl, ethanol, and carbon dioxide among others (Davidson and Hoover 1993) Bacteriocins, usually named after the bacterium that produces it, can be classified into four major classes, with class I and class II being the most investigated (Hugas 1998). A brief summary of bacteriocins is presented in Table 4.2.

Table 4.2 *Summary of bacteriocins and their producing bacteria.*

Bacteriocin	**Producer**	**Bacteriocin**	**Producer**
Nisin, lacticin	*Lactococcus lactis*	Sakacin	*Lactobacillus sakei*
Lactocin	*Lactobacillus sakei*	Curvacin	*Lactobacillus curvatus*
Pediocin	*Pediococcus acidilactici*	Curvacitin	*Leuconostoc curvatus*
Enterocin	*Enterococcus faecium*	Bavaricin	*Lactobacillus bavaricus*
Brevicin	*Lactobacillus brevis*	Leucocin	*Leuconostoc gelidum*
Divergicin	*Carnobacterium divergens*	Carnobacteriocin/Piscicolin	*Carnobacterium piscicola*

Application of nisin in meat products is somewhat challenging due to its binding ability to meat components, low solubility (hydrophobic nature), and loss of efficacy at pH > 5 (Scannell et al. 1997; Murray and Richards 1998). For example, Rose et al. (1999) showed that glutathione, which is present in raw ground beef, can inactivate nisin. Scott and Taylor (1981) showed the need for greater nisin concentration to inactivate *Clostridium botulin* in cooked meat compared to microbiological medium. Finally, Chung et al. (1989) showed a 70% loss in nisin activity in raw meat during storage at 5 ℃ for 4 days. Hugas (1998) mentioned that pediocin might be more effective than nisin in meat applications, since it is derived from the meat-fermentation bacterium *Pediococcusacidilactici*. Another approach for bio preservation might be use of lactic acid – producing bacteria that also produce bacteriocins as direct protective cultures on meats (Hugas1998) due to the fact that lactic acid bacteria do not induce significant spoilage until large population numbers are reached (Nychas etal. 2007). Bloukas et al. (1997) extended shelf life of vacuum -

packaged frankfurters stored at 4 ℃ by one week using commercially available protective culture of *Lactobacillus alimentarius*.

(b) Lactic Acid, Sodium Lactate, Diacetate, and Acetate

Table 4.3 *Lactic acid - derived antimicrobials.*

Product	Antimicrobial	Result	Reference
Sliced poultry sausage	2% Na lactate	3 ×to 4 ×shelf – life extension, 5 to 7 ℃, air 7 ×shelf - life extension, 5 to 7 ℃, N2	Cegielska – Radziejewska and Pikul 2004
Pork chops	Na acetate Na lactate Na lactate/diacetate	Na lactate/diacetate treatment had lowest APC and least discoloration after 96 - h display	Jensen et al. 2003
Low - fat Chinese - style sausage	3% Na lactate	Lower microbial counts after 12 weeks storage at 4 ℃	Lin and Lin 2002
Retail beef cuts	1.2% acetic acid, 120 s 1.2% lactic acid, 120 s	Paler meat, but small sensory difference; 1 to 2 log 10 CFU/g reductions in Escherichia coli and APC count within 9 d storage	Kotula and Thelappurate 1994
Pork loin chop	2% Acetic acid 10% Na lactate dip	Pale soft exudates appearance, > 9 day shelf - life. Extended shelf - life by 3 days compared to control (9 vs. 6)	Lin and Chuang 2001
Vacuum packaged fresh pork sausage	1% Na lactate 2% Na lactate	1 to 2 weeks shelf – life Extension 2 week shelf - life extension	Brewer et al. 1993
Vacuum packaged cooked beef loins	4% Na lactate	Lower APC after 7 days at 10 ℃	Maca et al. 1999
Vacuum packaged beef bologna	3% Na lactate	Lower APC after 10 weeks storage at 4 ℃	Brewer et al. 1992
Vacuum packaged frankfurters	2% Na lactate	2 to 3 week shelf – life extension at 4 ℃	Bloukas et al. 1997

The U.S. government allows the use of lactic acid, sodium lactate (4.8%), sodium diacetate (0.25%), and sodium acetate (0.25%) on meat products as extensive research has shown their safety for human consumption (FDA 2000). Whether produced by lactic acid bacteria or chemically derived, the listed compounds are antagonists to food-borne pathogens and to general spoilage micro flora due to nonspecific mechanisms of action (Kim et al. 1995a, b; Marshall and Kim 1996; al' A and Marshall 1998; Kim and Marshall 2000). Numerous publications have documented the effectiveness of these compounds against *L. monocytogenes, E. coli O157:H7, Clostridium perfringens, and Salmonella spp*. (Glass et al. 2002; Porto et al. 2002; Juneja 2006; Michaelsen

et al. 2006; Paulsonet al. 2007). Lactate efficacy can be improved by combining with diacetate (Jensen et al. 2003; Serdengecti et al. 2006). The main drawback of using straight organic acids instead of their salts is lowered pH and the pale/watery appearance of fresh meats (Kotula and Thelappurate 1994; Lin and Chuang 2001). A summary of organic acid applications (with an emphasis on lactate) for meat product shelf life extension is presented in Table 4.3.

(c) Chitosan

Chitin is the second - most abundant natural biopolymer after cellulose and is a starting material for chitosan (deacetylated derivative of chitin) manufacturing. Since biodegradation of chitin is slow, its accumulation during crustacean processing (mainly shrimp and crab shell wastes) is a disposal challenge. The production of value - added chitin by - products, such as chitosan, could provide a solution to crustacean processing waste accumulation (Shahidi et al. 1999). Chitosan is reported to have antimicrobial properties. Factors that improve antimicrobial activity are a low degree of acetylation and a low pH, both of which increase solubility (Shahidi et al. 1999). Due to the highly reactive nature of polycationic chitosan, which readily interacts with proteins, fats, and other anionic compounds, chitosan antimicrobial activity is less in foods than in vitro (Rhoades and Roller 2000). Chitosan has achieved self - affirmed GRAS status (FDA - CFSAN 2004), removing regulatory restrictions on its use in some foods. Studies by Darmadji and Izumimoto (1994) showed that 1% chitosan addition to minced beef stored at 4 ℃ for 10 days inhibited growth of spoilage bacteria, reduced lipid oxidation and putrefaction, and resulted in better sensory quality. Specifically, an initial reduction of total bacterial count by 0.5 log 10 CFU/g was observed, with average count reductions after 10 days storage at 4 ℃ of 1.0, 2.6, 1.0, 1.4, > 2.0, and > 2.0 log 10 CFU/g for total bacterial, pseudomonad, staphylococci, coli form, Gram – negative bacteria, and micrococci counts,

respectively. Sagoo et al. (2002) showed that the addition of 0.3 and 0.6% chitosan to an unseasoned minced – pork mixture reduced total viable counts, yeasts and moulds, and lactic acid bacteria by up to3 log 10 CFU/g for 18 days at 4 ℃ compared with an untreated control. Juneja et al. (2006) found that addition of 3% chitosan to ground beef and ground turkey prevented growth of inoculated *C. perfringens* after cooking and inadequate cooling. Their results showed a 4 to 5 log 10 CFU/g reduction in *C. perfringens* spore germination and outgrowth over12 -, 15 -, and 18 - hour cooling cycles and a2 log 10 CFU/g reduction during a 21 – hour cooling cycle. Three treatments of fully cooked grilled pork (air packaged, vacuum packaged, or treated with chitosan and vacuum packaged) were investigated for the duration of shelf life (Yingyuad et al. 2006).

(d) Essential Oils

Plant - derived essential oil components may be active against bacteria but are difficult to apply in foods due to significant changes in sensory quality (Davidson 2001). Seydim and Sarikus (2006) compared the antimicrobial activity of oregano, rosemary, and garlic essential oils in whey protein isolate films (1.0 to 4.0% wt/vol) against *E. coli O157:H7, Staphylococcus aureus, almonella* Enteritidis, *L. monocytogenes*, and *Lactobacillus plantarum* on agar plates. Film with 2% oregano essential oil was the most effective compared to films with garlic effective at 3% and 4%) or rosemary extracts (no effect). Oussalah et al. (2004, 2006) also showed that alginate - based or protein – based edible films containing oregano essential oil were more effective than cinnamon orpimento in the extension of shelf life of whole beef muscle. They found that application of oregano oil edible film caused 0.9 and1.1 log 10 CFU/g reductions in *Pseudomonas* and *E. coli O157* counts, respectively after 7days of storage at 4 ℃ (Oussalah et al. 2004) ikewise, Skandamis and Nychas (2002) found that oregano essential oil extract extended shelf life of refrigerated MAP -stored fresh meat.

Allyl isothiocyanate is one of many volatile natural antimicrobials found in cruciferous plants, such as horseradish, black mustard, cabbage, and turnip. Nadarajah etal. (2005a) prepared paper disks containing1 ml of 65% allyl isothiocyanate mixed with corn oil. They then applied the paper disks to ground beef patties that were then vacuum packaged and stored for 15 days at 4 ℃. Results showed a delay in natural micro flora growth and significant population reduction in inoculated *E. coli O157: H7*. They argued that the antimicrobial might have use as vapour. When 5% to 20% mustard flour was used as a natural source of allyl isothiocyanatein ground beef, inoculated *E. coli O157: H7* population declined but no effect on spoilage micro flora was noted (Nadarajahet al. 2005b). Sensory evaluation results showed that panellists could detect mustard treatment, but considered mustard – treated meat to be acceptable.

(e) Enzymes

Lysozyme is a naturally occurring (human saliva, egg white), 14.6 kDa, single – peptide protein that has antimicrobial activity due to its enzymatic ability to hydrolyse β (1 – 4) glycosidic linkages in bacterial cell walls Proctor and Cunningham 1988). It is more active against Gram - positive bacteria, and activity against Gram - negatives can be increased by use of membrane disrupting agents (detergents and chelators), such as EDTA (Padgett et al. 1998). Because of this narrow activity range, most studies use lysozyme in combination with other antimicrobials. Gill and Holley (2000) showed that combined lysozyme, nisin, and EDTA treatment of ham and bologna sausages reduced populations of *B. thermosphacta* to no detectable levels for up to 4 weeks, while during storage at 8 ℃, growth of *Lactobacillus curvatus*, *Leuconostoc mesenteroides*, and *Listeriamonocytogenes* was slowed for up to 3, 2, and 2 weeks, respectively. Cannarsi et al. (2008) showed that the combination of 0.5% lysozyme and 2% EDTA extended the shelf life of chilled buffalo meat, with an antimicrobial effect

on all micro flora present, including *B. thermosphacta*. Nattressand Baker (2003) combined nisin and lysozyme as an antimicrobial treatment on pork loins, with successful inhibition of lactic acid bacteria and preferential growth of Enterobacteriacea. However, the authors noticed that aerobically displayed nisin - lysozyme treated meat spoiled sooner than untreated meat. They attributed this to inhibition of lactic acid bacteria and a resultant shift to putrefactive bacterial spoilers. In summary, a combined lysozyme/nisin/EDTA mixture may be a promising tool for extension of the shelf life of anaerobically packaged meats by inhibiting lactic acid bacteria, which is the predominant bacterial spoilage group capable of growth in such conditions.

4.3 General Analysis

4.3.1 Shelf Life Extension in Meat Products Treated with HPP

(a) Fresh Products

The application of HPP to fresh meat products results in a cooked-like aspect, and sometimes the products may develop a rubbery consistency. Murano, Murano, Brennan, Shenoy, and Moreira (1999) tested the usefulness of applying a mild heat treatment at 50 ℃ simultaneously with HPP in ground pork patties to lower the D values of Listeria monocytogenes obtained with only HPP. With a treatment of 414 MPa and 50 ℃ for 6min they obtained a 10-log^{-1} reduction in the most resistant strain of Listeria monocytogenes. Shelf life studies were also conducted, spoilage levels for control samples were reached after 5 days of storage at 4 ℃ and after 28 days for treated samples. Sensory evaluation of uninoculated grilled patties showed that panellists could not distinguish between those treated by heat and HPP and untreated controls. Thus, treatment by HPP in combination with mild heating can be used successfully to produce safer, long-

lasting fresh pork without affecting quality. Marinated beef loin, which is a raw uncooked meat product with high water activity, a low level of salt and without nitrite, harbours a mixed flora of spoilage and pathogenic microorganisms from the slaughterhouse cutting and trimming operations. Sliced, skin vacuum-packaged marinated beef loin was treated by HPP at 600 MPa for 6 min at 31 ℃. Aerobic, psychrophilic and lactic acid bacteria counts showed at least a 4 log 10 cycle reduction after treatment and remained below the detection limit ($<10^2$ cfu g^{-1}) during the chilling storage of 120 days, helping to prevent the sour taste and off-flavours while untreated samples reached 10^8 cfu g^{-1} after 30 days in the same conditions. Enterobacteriaceae were kept below 10 cfu g^{-1} during the whole storage period in HPP treated samples, while untreated samples reached 10^5 cfu g^{-1} after 30 days. HPP is a powerful tool to control risks associated with Salmonella spp. and Listeria monocytogenes in raw or marinated meats. Most of the untreated samples showed presence in 25 g from one or both of the pathogens, whereas all pressurized samples showed absence in 25 g (Garriga, Aymerich, & Hugas, 2002). The HPP treatment could extend the shelf life of the marinated beef loin by controlling the growth of both spoilage and pathogenic bacteria.

Main Technological Effects of HHP in Meat

About colour:

- In fresh or marinated meat, the iron in the myoglobin changes rom ferrous to ferric and globin is denatured: the red colour is lost.

About texture:

- Inhibition or stimulation of the proteolytic activity in muscles activity muscles (depending on processing conditions).

- Proteins are partially denaturized in products where proteins have not been previously modified by other process: heating, drying and fermentation.

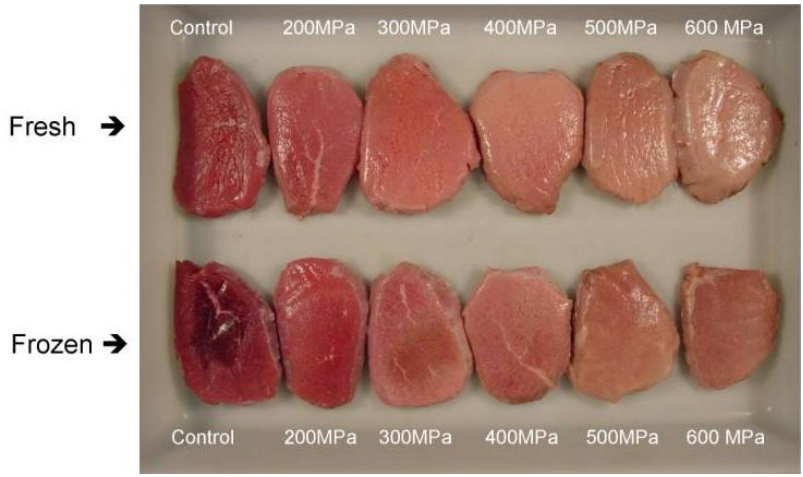

Figure 4.1 *Top view of the fresh and frozen beef samples treated by HHP Source: IRTA.*

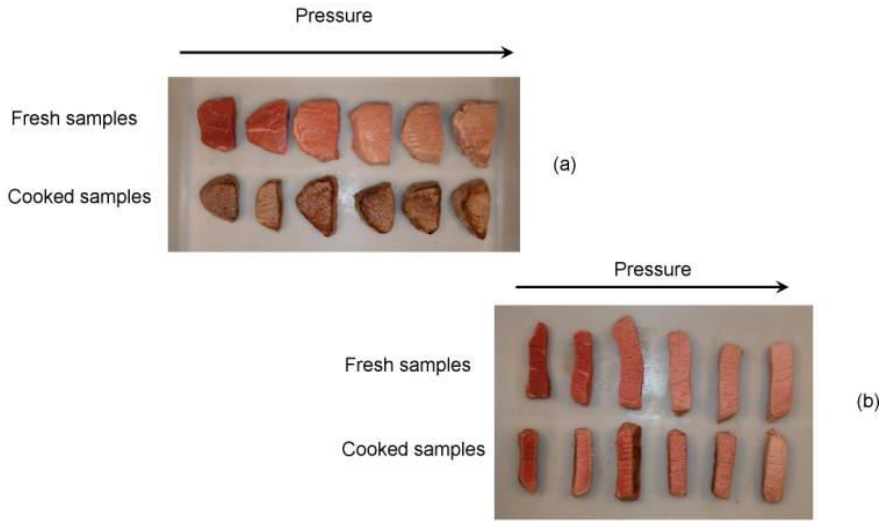

Figure 4.2 *Cooked samples: view from the top (a); view of the inside (b) Source: IRTA.*

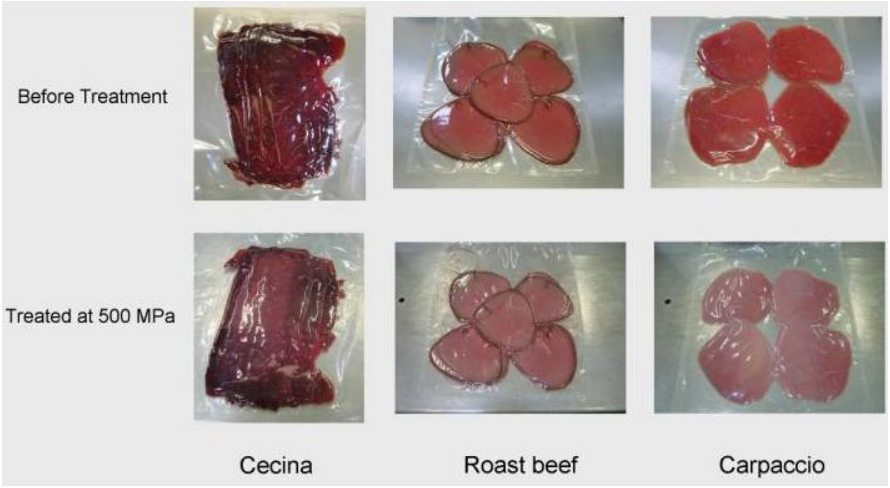

Figure 4.3 *Commercial beef products before and after HHP treatment, Source IRTA.*

(b) Cooked Ham

Sliced vacuum-packaged cooked ham is a highly perishable product due to its composition, pH and water activity and the lack of a background flora competing with spoilage or pathogenic microorganisms. The physico-chemical and microbiological characteristics of cooked ham do not represent any hurdles to bacterial growth. Its shelf life depends on the hygienic characteristics of the final product after post-processing as well as to the packaging methods where cross-contamination is more likely to occur. The techniques used to reduce cross-contamination include good manufacturing practices, post-pasteurization after packaging or even the use of ‘‘white rooms’’ at the slicing and packing stage. Sliced, skin vacuum-packed cooked ham treated by HPP at 600 MPa for 6 min showed a significant delay in the growth of spoilage associated microorganisms compared with untreated samples, thus contributing to the maintenance of organoleptic freshness for at least 60 days after treatment (Garriga, Aymerich, & Hugas, in press). The HPP process helped to prevent any sour taste, off-flavours, ropiness and colour changes. Thus, HPP processing on cooked ham in the

conditions mentioned earlier was an effective process to avoid the growth of yeasts and Enterobacteriaceae, with the potential to produce off-flavours and gas. Accordingly, it contributed to the shelf life extension in this highly perishable meat product. Dry cured ham is a dry, bone-in, salted and dried, non-fermented meat product. Because of the low water activity and high salt content of this type of product, spoilage microorganisms are mainly gram-positive cocci and yeasts. They may be present on the surface of whole hams and reach the sliced product during final boning, slicing and packaging operations. Sliced, skin vacuum-packed dry cured ham samples, treated by HPP at 600 MPa for 6min, showed a significant reduction of at least two log10 cycles for spoilage associated microorganisms after treatment. The surviving microbiota was kept at low levels during the storage period; contributing to the preservation of the organoleptic freshness during shelf life (120 days) and helping to prevent off-flavours, sour taste and gas formation. Enterobacteriaceae and Escherichia coli were below the detection limit, both in HPP and untreated samples. Listeria monocytogenes was present (in 25 g) in one untreated sample, but absent in all HPP treated samples during the whole storage period. (Garriga, Aymerich, & Hugas, 2002). Demonstration of the substantial equivalence of HPP meat products after evaluating the proximate composition of marinated beef loin, cooked ham and dry cured ham pressurized at 600 MPa for 10 min at 30 ℃ compared with control non-pressurized samples (Table 4), small differences have been observed which could be more related with the variability of samples and raw materials than with the technological procedures. A slight decrease in phosphate content was detected in samples of HPP-treated dry cured ham, indicating a possible enhancement of phosphatase activity. The differences in chloride and phosphate contents ($P<0.001$) fell within the typical variability between samples in whole muscle meat products. As a general conclusion HPP did not show any influence in the proximate composition of cooked ham, dry cured ham and marinated beef loin.

Non-significant differences were found in the non-protein nitrogen fraction in the three meat products studied when HPP treated and compared with controls. In the same sense, no differences were observed in their amino acid content (Garcı´a-Regueiro, Sa´rraga, Horto´s, Dı´az, Valero, & Rius, 2002). These results agree with a lack of protein breakdown due to HPP. For the fatty acid composition and the cholesterol content in the three products studied no significant differences between samples were found, with the exception of acid. With this fatty acid, an increased stability was observed in pressurized marinated beef loin ($P<0.05$). According to the levels obtained in cholesterol oxides, less cholesterol oxidation was obtained in pressurized products. 7 Ketocholesterol which was high in beef control samples was strongly reduced in beef subjected to HPP. However, it is necessary to study if HPP processing could have some influence on the recovery of cholesterol oxides by analytical methods. The vitamin content did not present any significant differences between HPP-treated and untreated samples, at least on the B group vitamins. In general, no significant differences were found in the mineral composition of pressurized samples compared with control. The decrease of calcium content in HPP cooked ham is difficult to explain and more experiments should be carried out to verify if the solubility of some ions is modified by HPP. An increase in the iron content of HPP beef loin can be explained according to the results of Ledward (2001), who reported a release of iron from non-heme complexes at pressures higher than 400 MPa as well as from the heme proteins denaturation above 300 MPa. Such changes do not apparently occur in cured meats. As a general conclusion it can be stated that from a physico-chemical point of view, cooked pork ham, dry cured pork ham and marinated beef loin, vacuum packed and high pressure treated at 600 MPa for 10 min at 30 ℃, are substantially equivalent to the same untreated products. The effect on the bioavailability of nutrients was also assessed. The solubility of proteins in cold 1% SDS was higher in marinated meat HPP than in untreated samples, whereas

no differences were found in dried cured ham or cooked ham. The proteins solubilised in this medium are representative of the cytoplasmic fraction, excluding most of the myofibrillar proteins. The solubility of the myofibrillar protein fraction in a selective solvent (1 M KCl) was markedly reduced by pressure treatment, but it is even more dramatically decreased by traditional cooking. Analysis by SDS– PAGE of different conditions of protein extraction, showed only minor differences confirming that pressure did not affect the primary structure of proteins. Nevertheless, precipitation by TCA after KCl extraction as well as solubilisation by 6M urea and SDS–PAGE confirmed the lower major proteins' solubility in the pressurized materials except in dry cured ham.

Table 4.4 *Proximate composition of pressurized meat products: marinated beef loin (A), cooked ham (B) and dry cured ham (C) pressurized at 600 MPa, 10 min 30 ℃; Garcı´a-Regueiro et al., 2002.*

		Control	SD[a]	HPP[b]	SD[a]
(A)	Marinated beef loin				
	Moisture (%)	74.11	0.60	73.78	0.65
	Fat (%)	4.54	0.76	3.68	0.46
	Protein (%)	20.64	0.83	21.43	0.50
	Hydroxyproline (ppm)	677.0	316.7	558.6	130.3
	NO_2 (ppm)	5.00	0.00	5.00	0.00
	NO_3 (ppm)	9.67	2.31	15.67	4.04
	Chloride (%)	0.74	0.03	0.83	0.09
	Ash (%)	1.68	0.13	1.96	0.08
	Carbohydrate (%)	0.71	0.04	0.65	0.06
	Phosphate (ppm)	4786	411	3795	320
	Ascorbate (ppm)	<10	0.00	<10	0.00
	pH	5.44	0.01	5.80	0.03
(B)	Cooked Ham				
	Moisture (%)	75.20	0.24	74.02	0.40
	Fat (%)	2.63	0.38	2.97	0.89
	Protein (%)	22.67	0.58	20.64	1.44
	Hydroxyproline (ppm)	993.7	136.3	1043.3	56.52
	NO_2 (ppm)	103.3	6.66	91.0	3.00

	Control	SD[a]	HPP[b]	SD[a]
NO_3 (ppm)	38.33	3.06	38.0	3.61
Chloride (%)	2.06	0.04	1.80	0.01
Ash (%)	3.16	0.05	3.18	0.09
Carbohydrate (%)	0.52	0.03	0.52	0.02
Phosphate (ppm)	4592	74	3051	269
Ascorbate (ppm)	234	16	219	14
pH	6.42	0.02	6.52	0.04
(C) Dry Cured Ham				
Moisture (%)	50.64	0.28	50.17	1.03
Fat (%)	12.9	1.46	14.6	1.36
Protein (%)	30.56	0.70	28.88	0.50
Hydroxyproline (ppm)	2035.3	144.3	1873.0	18.08
NO_2 (ppm)	5.00	0.00	7.67	0.58
NO_3 (ppm)	98.67	3.51	81.67	12.7
Chloride (%)	3.76	0.10	4.63	0.14
Ash (%)	6.24	0.09	6.41	0.11
Carbohydrate (%)	0.19	0.02	0.22	0.04
Phosphate (ppm)	4590	360	3663	980
Ascorbate (ppm)	58	1	74	6
pH	5.48	0.44	6.11	0.03

Key:

a. SD, standard deviation

b. HHP, high pressure processing

*. P<0.5

**. P<0.01

***. P<0.001

4.4 Actualisation

Study by J. Yuste, M. Mor-Mur, I M. Capellas, B. Guamis, and R. Pla- Mechanically Recovered Poultry Meat Sausages Manufactured with High Hydrostatic Pressure; 1999 Poultry Science 78:914–921.

The effect of high pressure processing at high temperature on texture and colour of frankfurter type sausages made with different contents of mechanically recovered poultry meat (MRPM) was evaluated and compared with that of a standard cooking process. Five types of sausages containing 100, 75, 50, 25, and 0% MRPM and 0, 25, 50, 75, and 100% of minced pork meat (MPM), respectively, were manufactured. They were pressurized at 500 MPa for 30 min at 50, 60, 70, and 75 C or cooked at 75 C for 30 min. Pressure treated sausages were less springy and firm, but more cohesive. Moreover, colour of pressurized sausages was lighter and more yellow than that of conventionally cooked sausages. Addition of MPM increased cohesiveness, hardness, and force at 80% compression. Minced pork meat also caused the appearance of sausages to be lighter, less red, and less yellow. Cooked sausages made with MRPM can have an attractive appearance and texture via high pressure processing.

Compared to a standard cooking process, high pressure processing at high temperature yielded less springy and firm but more cohesive sausages, which were also lighter and more yellow. The addition of MPM increased cohesiveness, hardness, and force at 80% compression. It also caused lighter, less red and less yellow sausages. In this study, formulation influenced textural parameters more than type of treatment; this effect was very clear, particularly in the case of absence of MRPM. Significant differences were caused by the three variables (formulation, temperature of treatment, and type of treatment) and also by the interactions among them. Thus, pressurization could be a good choice to achieve desirable characteristics in the case of meat products containing MRPM, because two of the main drawbacks of this meat as an ingredient are its appearance (too dark) and texture (too pasty and soft). Tartarisation of MRPM would possibly increase the range of products prepared from this raw material (Froning, 1976; Jones, 1988). Moreover, a certain

amount of MPM can help to solve the disadvantages and to improve the properties of these products, but this raw material should not be added excessively because it could lead to very firm products.

Dhillon and Maurer (1975), Froning (1976), Newman (1981), and Radomyski and Niewiarowicz (1987) stated that combinations of MRPM and hand deboned poultry meat gave desirable sensory and functional properties and economic advantages. From the results obtained, it can be stated, as reported by Cheftel and Culioli (1997), that pressure treatment with previous, simultaneous, or subsequent cooking is the most suitable way of processing fresh whole or minced meat, taking into account the modifications induced by pressurization.

Final cooked meat products would be obtained directly from this process. Cooked sausages containing MRPM with better appearance and texture than the traditional ones can be obtained by means of high pressure processing. Moreover, the ability of pressurization to inactivate microorganisms and, therefore, to enhance the safety and to extend the shelf-life of some food products must be emphasized (Hoover *et al*., 1989; Hayashi, 1991; Ludwig *etal*., 1992; Yuste *et al*., 1998). Thus, high pressure processing is a technique with a promising future in the processing of meat and meat products and, in general, in food technology.

4.5 Discussion

In the near future, the new non-thermal technologies will very likely replace current technologies. However this may cause confusion to the consumer. Does this mean that current technologies are not guaranteeing the safety of foods we are consuming every day? New technologies can tackle the problem of new emergent pathogens which concern the consumers but they could also be very

useful for the development of new products. A representative survey (Baron et al., 1999) of consumer attitudes concerning HPP of foods was carried out among 300 adults aged 14 years and over in France, Germany and the United Kingdom in face-to-face computer assisted personal interviews. The variable to be predicted using the model was the willingness to buy products preserved by HPP. The acceptability values were 71% for France, 74% for Germany and 55% for the UK. The average acceptability rate of 67% was clearly above the threshold value of 60% (a pragmatic market research threshold) which is extremely positive for such an emerging technology. The best predictor which optimizes the classification result of potential buyers and non-buyers in the three countries is mainly the hope for more personal advantage from this new technology. Before the total implementation of the new preservation technologies, several issues need to be addressed such as: the mechanisms of microbial resistance and adaptation to these new technologies, the mechanisms of microbial and enzyme inactivation, the identification of the most resistant and relevant microorganisms in every food habitat, the role of bacterial stress, the robustness of the technologies, the increased safety versus current technologies and last but not least, the legislation needed to implement them. In some years, there will be new technologies to be used: gamma irradiation, electron beams, microwave heating, ohmic heating, high pressure, pulsed electric field, submerged arcing, pulse lights on surfaces, etc. Some of them have a high likelihood of being used in combination with other technologies. The applications in the real world of the new technologies are new challenges to the food technologists and food researchers. The need to convince consumers and stakeholders about the improvement these new technologies represent is a must. To do so, it is very important to present convincing data, to identify stakeholders and to provide clear, objective and unbiased information including the potentially negative aspects and their limitations. It is very important to

demonstrate that the technology is available or that there is existing potential to develop a given technology. Pressure treatment is maybe, the most available emergent technology. However, it is still costly, mainly because of the initial capital expenditure, and this may limit its application. It is expected than these costs will go down as a consequence of further progress in technology, the acceptance of and resultant investment in the requisite equipment for HPP by an increasing number of manufacturers. As an example, the treatment cost of cooked ham is 0.1E per kg which is a cost quite affordable for the consumer.

4.6 General Recommendations

1. The use of HP as a possible alternative processing method to thermal treatment has brought about the need to study the pressure–temperature behaviour of macromolecular food ingredients since, for example, the mechanisms of protein denaturation under pressure are far from fully understood.

2. It is well known that HP can modify the activity of some enzymes and the structure of some proteins. Although covalent bonds are not affected, hydrogen bonds as well as hydrophobic and intermolecular interactions may be modified or destroyed. From this perspective, some concern about the potential risks of HP may arise. It is necessary to compile data in order to clarify the role of HP towards toxicity, allergenicity, loss of digestibility and the eating and nutritional quality of foods (Hugas et al., 2002).

3. There have been many studies of the use of HP as a pre-treatment method to improve the textural properties of food products. As a pre-treatment tool, HP processing appears effective in improving gelation properties of meat, egg or soy proteins, as well as improving the coagulating properties of

milk (Galazka et al., 2000). Further studies are also required to understand the potential of the technology for rheological control in food protein systems, as well as to optimize the operating conditions that should be used during actual processing.

4. Before any food product can be produced commercially using HP, optimization of processing conditions is essential to ensure product safety (McClements et al., 2001).

5. Food companies must be able to make a realistic cost-benefit analysis of the potential rewards in investment in HP processing. The value of HP in terms of increasing food safety assurance, in some cases, may alone be sufficient to justify such investment.

4.7 Conclusion

The application of any new technology presents significant challenges to food technologists and food researchers. HP processing offers the food industry a technology that can achieve the food safety of heat pasteurization while meeting consumer demand for fresher-tasting minimally-processed foods. In addition, other favourable organoleptic, nutritional and rheological properties of foods have been demonstrated following HP, in comparison to heat processing. The retention of colour and aroma and the preservation of nutritive components are enormous benefits to both the food processing industry and consumers. Also, from a food processing/engineering perspective, key advantages of high-pressure applications to food systems are the independence of size and geometry of the sample during processing, possibilities for low temperature treatment and the availability of a waste-free, environmentally-friendly technology. Application of HP can inactivate microorganisms and enzymes and

modify structures, while having little or no effects on nutritional and sensory quality aspects of foods. HP food processing is today being used on an ever-increasing commercial basis. Opportunities clearly exist for innovative applications and new food product development. HP can affect the functionality of protein and carbohydrate molecules in often unique ways, which may allow the optimization of food manufacturing processes and the production of novel foods. The range of commercially-available HP-processed products is relatively small at present but there are opportunities for further development and the production of a wide range of HP-treated products. The main drawbacks of pressure treatment of solid foods are the use of batch or semi continuous (the latter for liquids only) processing and the high cost of pressure vessels. HP is an environmentally-friendly, industrially-tested technology that offers a natural alternative for the processing of a wide range of different food products. This method prolongs product shelf-life while at the same time preserving organoleptic qualities, by inactivating microorganisms and enzymes while leaving small molecules such as flavours and vitamins intact. It is a technology with many obvious advantages, especially for food products with a high added value, targeted at a growing group of consumers that demand maximum safety and quality in the products they purchase.

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5

Application of Statistical Techniques in Food Science: Chemical Analysis Data

5.1 Introduction

There are many applications of statistics in the field of food studies. One of the earliest was in agriculture where R. A. Fisher used experimental design to partition variation and to enable more precise estimation of effects in crop plot experiments. There was even an early sensory experiment on tea tasting (Fisher 1966), and since then statistical applications have increased as food science emerged as a distinct applied science subject. Some examples of the form of statistical applications in food are given in Table 1. Preparation of data summaries is one general application of statistics that can be applied across the board. It is one of the simplest applications and can be done manually if necessary, depending on the requirements. A variety of simple graphs and table methods are possible, which allow rapid illustration of results. These summaries are taken further in statistical quality control where measures such as the mean value are plotted 'live', as a process is on-going. The graphs (control charts) used include limit lines which are set by using other statistical methods, which allow detection of out-of-limit material, e.g. food product packs which are below statutory minimum net weight. Statistical methods can also be applied to evaluate the trustworthiness of data obtained by any method of measurement. This application has been used extensively in evaluation of chemical data generated by analytical laboratories. The statistical analysis provides an evaluation of how dependable the analytical results are. This can range from within-laboratory to between-laboratory comparisons, globally. Enforcement agencies rely on such checks so that they can monitor adherence to legal requirements with confidence. Food research application brings in analysis of differences and relationships. Here, hypotheses are put forward on the basis of previous work or new ideas and then magnitudes of effects in sample statistics can be assessed for significance, for instance, examination of the change in

colour pigment content during frozen storage of vegetables. Examination of relationships requires that different measurement systems are applied and then compared. There are many examples of this in studies of food where data from instrumental, sensory and consumer sources are analysed for interrelationships. The process of sampling of items, including food material and consumer respondents, can be controlled using statistical methods and here a statistical appreciation of variability is important. Experimental design takes this further, where sources of such variation are partitioned to improve precision or controlled and minimised if extraneous. A common example is the unwanted effect of order of samples in the sensory assessment of foods – design procedures can minimise this. In fact, *all* the above examples rely on design procedures if the result is to be valid and adequately interpreted.

Table 5.1 *Some applications of statistics in the food field.*

Method	Application
Summaries of results	Tables, graphs and descriptive statistics of instrumental, sensory and consumer measures of food characteristics
Analysis of differences and relationships	Research applications on differences in food properties due to processing and storage; correlation studies of instrumental and sensory properties
Monitoring of results	Statistical control of food quality and parameters such as net filled weight
Measurement system integrity	Uncertainty of estimates for pesticides and additives levels in food
Experimental design	Development and applications of balanced order designs in sensory research

5.2 Description

5.2.1 The Approach

Progress in food science and all its associated disciplines is underpinned by research activity. New information is gathered by investigations and experiments, and in this way knowledge is advanced. The scientific approach to research and exploration follows an established paradigm called the *positivism method*. This

postulates that events and phenomena are objective and concrete, able to be measured and can be explained in terms of chemical and physical reactions. All scientists are familiar with this viewpoint, which is described as the *scientific deductive approach* (Collis and Hussey 2003). It is largely based on *empirical methods*, i.e. observations from experiments. The scientific style of approach can be used for any type of investigation in any subject. The procedure uses deduction from theory based on current knowledge. To advance knowledge, experiments can be designed to test advances on existing or new theory, using a *hypothesis process*. The findings can then be disseminated and knowledge increased. Results are generalised and can be used to establish new theories and to model processes and event reactions, which in turn allows prediction in the formation of new hypotheses. The term *quantitative research* is also used in reference to the scientific approach. This strictly refers to the nature of the data generated, but it implies the deductive positivistic viewpoint. In this process, the researcher is assumed to be objective and detached. Ultimately, the deductive method searches for an explanation on the basis of *cause–effect* relationships. Without such procedures, there would be no progress and they form the foundation of the scientific approach in many food disciplines. Amore recent approach is that of *phenomenology* where an *inductive approach* can be used to examine phenomena on the basis that they are socially constructed. Theories and explanations are generated and built up from data gathered by methods and techniques such as interviews (Blumberg *et al.* 2005). These methods are often described as *qualitative*, which again refers to the data which are in the form of words rather than numbers. The modern food practitioner needs to be aware of such data as there are several qualitative methods (e.g. interviews and focus groups) used in sensory and consumer work. Analysis of data from *qualitative methods* can be summarised by numerical techniques such as counting the incidence of certain words and phrases, but usually statistical analysis as such is not involved. Typical

use of the scientific approach in food studies entails identifying a topic for research or investigation then posing a *research question(s)*. Deductive reasoning from existing knowledge is examined to develop a *research hypothesis*. A plan can then be drawn up with an experimental design and specification of measurement system, etc. Data are gathered and then statistical analysis is used to test the hypothesis (quantitative). The scope of the procedure can be from a simple investigation of the 'fact-finding' type, e.g. determination of chemical content values, to a complex experimental design, e.g. a study on the effect of temperature, pressure and humidity levels on the drying properties of a food. In this latter case, the objective would be to identify any significant differences or relationships. Experimental control means that results can be verified and scrutinised for validity and other aspects. Simple experiments do not usually require stating of hypotheses, etc. In circumstances where differences or relationships are being examined, e.g. 'Does process temperature affect yield of product', a more formal procedure is used or, at least assumed (Fig. 1.). The conclusion of one investigation is not the end of the process as each piece of work leads to new ideas and further studies, etc.

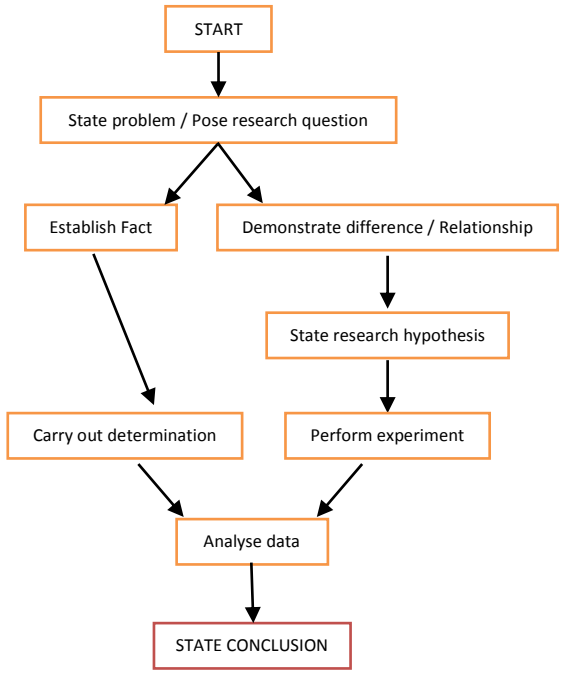

Figure 5.1 *The approach to investigation.*

5.2.2 Chemical Analysis

The chemical analyst is interested in the end result, but also in the *uncertainty* of the estimation; some researchers state that unless a measure of uncertainty is included the results themselves are useless (Mullins 2003). This view could well apply to all scientific measures, but there are still occurrences of it not being adopted for chemical data. Many investigations have taken place to examine error components and to quantify their contribution to uncertainty. Also, cost considerations are included in these studies as reducing uncertainty usually means additional analyses and hence cost in terms of time, resources and personnel. The interest here is in the balance between *gain in certainty*, against the *increased cost* to the laboratory (Lyn *et al.* 2002; FSA 2004a). Another unique aspect of studies in this topic is that the uncertainty is examined

not only for location measures estimate such as the mean, but also for that of the level of variability – thus the uncertainty of the standard deviation is also of interest. *Method proficiency testing* is one aspect of this protocol that has been developed for some common standard methods with measures all focused on uncertainty in analytical chemistry. In addition to analysis coming under this latter umbrella, where analyses such as pesticides are determined at very low level, there are many proximate analyses and 'crude content' methods used for food. These may exhibit higher levels of uncertainty, but their results and in fact, those from any instrumental measure can be subjected to some of the calculations detailed below. Food analysis methods have received special attention via The *Food Analysis Performance Assessment Scheme (FAPAS)*. Patey (1994) described the initial stages and progress of this initiative – there was some improvement, but not for all analyses and all laboratories. A relatively simple check on performance for proficiency testing schemes is based on calculation of a form of *z*-score

$$z = (\text{Test result} - \text{reference value})/\text{sigma}$$

Sigma is a designated 'target value' for the standard deviation of the method data, based on realistic estimates. The larger the discrepancy (error) between the test and the reference is, the larger the *z* value is. The calculation produces *z* in a standardised form – values *equal or less than 2* are required for the laboratories' result to be declared 'satisfactory'.

Accuracy and Bias in Chemical Analysis

As stated above, accuracy of a chemical method is a measure of how close it is to the 'true' value. Variation from the true can occur due to error in the form of bias (Kane 1997). This circumstance can apply to a number of stages in the analysis (Table 5.2)

Table 5.2 *Bias Sources.*

Source
Operator
Lab
Preparation
Run
Method

It is crucial that this source of error is quantified and removed, or at least accounted for in any analytical determination, although this is not always done (O'Donnell and Hibbert 2005). Bias can be calculated as the *error of the mean*, and by the location of the range specified by a *confidence interval*. The 'true value' is represented by *reference samples* or the nearest equivalent.

5.3 General Analysis

5.3.1 Errors and Measurement Uncertainty

The term, "experimental error" is used extensively in student lab books to account for all manner of unexpected results. While this may be appropriate the error can be allocated to a number of possible sources which can usually be identified as discussed below. *Gross* errors (e.g. a misread balance or grossly incorrect additions /omissions of reagents) are usually *accidental* in nature and with care they can be avoided. In the Kjeldahl analysis an obvious gross error would be seen if there was omission of the catalyst for one of the replicates. Rejection of that value could be considered and there are statistical tests for such "outlier" values. Thus these errors may not affect all measurements in a set and often can be easily detected. Other types of error occur even when the greatest care is taken. *Systematic* errors (e.g. a balance which requires servicing and calibration, unrecognized faulty technique by the analyst, or a method related

systematic error) usually affect all the analyses in a similar manner. The systematic error effect is also known as bias and affects accuracy. Note that even if a balance is calibrated (i.e. set to weigh accurately using certified weights) it may still give an inaccurate reading if the balance model is unable to read beyond ascertain level. Thus the lack of calibration is determinate error, and can be changed, but the other is constant. Calibration improves accuracy and reduces or removes any bias which instruments may have. A blank determination is another aid to detection of a systematic error. Another source of error is detected if the test sample was analysed more than once. Even if gross and systematic errors are absent, repeated measurements may show some variation. These are caused by *random* errors, e.g. small errors in weighing, use of volumetric devices and other analysis instrumentation. Even highly trained analysts using top of the range equipment might not avoid random error. The random error effect in a series of measurements causes the individual results to fall on either side of the mean. They may be accidental in nature but are indeterminate as they are difficult to remove entirely. Random errors affect the precision of the analysis method. These errors can occur at any stage of the analysis and accumulate to produce the overall error. Some errors augment one another whereas others may cancel one another out. The replicate values in Table I are all different and possible error sources could be deduced by examination of each stage of the Kjeldahl analysis. An estimate of error magnitude in the final results can now be calculated.

Table 5.3 *Quality control laboratory data for percentage of crude protein analysis on food product.*

Replicate Number	Percentage of protein Analysis A	(N2 x 6.25) Analysis B
1	7.3	8.4
2	8.5	9.1
3	-	8.7
4	-	8.2
Mean	7.9	8.6

Note: True/most probable value = 8.8 per cent.

5.3.2 Accuracy and Precision in Measurement

Accuracy is the extent of agreement between the determined value and the true or most probable value; and precision is the extent of agreement among a series of measurements of the same quantity. It is important to note that with these terms the presence of one does not automatically imply the other: a high degree of precision does not imply accuracy and vice versa.

(a) Measures of Accuracy

The degree of concordance with the true value can be calculated as the error of the mean which can also be expressed as the relative error of the mean (REM):

$$EM = M - T \quad \%\,REM = \frac{EM \times 100}{T}$$

Where:

EM = error of the mean

T = "true" or "actual" value

M = mean value.

The true value may not be available for unknown samples; unless an independent analysis has been performed giving a confident estimate. If an indication only is required then a rough estimate can be given by "typical" values from text books and/or food product labels. In the food production situation (Table 3), the true expected value can be calculated for quality control purposes from knowledge of the chemical composition of the specified ingredients. Alternatively a standard or control material of known composition can be analysed along with the unknown under the same analysis conditions, thus enabling the above calculation. A suitable crude test material can be made up by

the analyst, formulated from constituent chemicals or purified constituents. Another possibility used in some laboratories is use of a previously analysed material which is kept in stable storage and sampled along with the new samples. For more critical circumstances a CRM (certified reference material) would be required. While many RMs are available, not all common constituents such as nitrogen are found in the certified lists and the matrix (i.e. the physical and chemical "makeup") of the RM may be different from the unknown food sample. There have been some developments to answer these food specific requirements, e.g. the FAPAS (food analysis performance assessment scheme) initiative run by MAFF (Ministry of Agriculture, Fisheries, and Food) which has food product test materials for proximate analyses such as nitrogen protein. The use of a hierarchy of reference standards from secondary RMs to certified RMs and ultimately primary RMs forms part of the traceability chain for chemical composition instigated by VAM. The presence of errors will affect the magnitude of the percentage REM obtained. Assuming the absence of gross and systematic errors then a percentage REM of zero is possible but unlikely due to random errors. Usually negative or positive percentage REM values are obtained representing results which are below or above the true value respectively. These statistics can now be calculated for the data of Table 5.3. As could be easily deduced by inspection of the mean values, both analyses have underestimated percentage protein, and the magnitude of this is shown (Table 5.4) by the negative percentage REMs. Analysis B has a greater agreement with the most probable value.

(b) Measures of Variability (Precision)

The standard deviation (SD) and the mean absolute deviation (MD) introduced previously are measures of precision. These can be standardized as the percentage coefficient of variation (%CV; also known as the relative standard deviation) and the percentage relative mean deviation (% RMD) respectively:

$$\%\,\mathrm{CV} = \frac{\mathrm{SD}\times 100}{\mathrm{M}}$$

$$\%\,\mathrm{RMD} = \frac{\mathrm{MD}\times 100}{\mathrm{M}}$$

Where

SD = standard deviation

M = mean

MD = mean deviation.

These measures are related (MD is approximately 0.8 times SD). Both are included here as MD is perhaps easier to understand and calculate. In the form above, erroneous comparisons between data sets possessing different measurement scales are avoided, e.g. an MD often for a mean of 10,000 gives a very low percentage RMD (0.1 per cent), but with the same MD for a mean of 100 the RMD is very high (10 per cent). Two other related measures are important. Repeatability is the precision obtained when a method of analysis is repeated under the same conditions, i.e. by the same analyst using the same equipment, on the same sample material, etc. (also referred to as "within laboratory" or "within run" precision). The analyses in Table 3 can be assumed to have been done under repeatability conditions. Reproducibility is the precision obtained when the same method of analysis is repeated on the same test material but under different conditions, i.e. a different analyst, different setoff equipment or a different laboratory or even different method (also known as "between run" or "between laboratory" precision). Its usual to find that repeatability conditions result in greater precision than those of reproducibility. In fact the poor reproducibility shown by different laboratories when analysing the same samples was one of the reasons for instigating the VAM project. The magnitude of the

percentage CV (or percentage MD) will range from zero upwards. "Perfect" precision would produce a CV percentage of zero and although this can occur, more commonly small values are obtained, caused by random error. Large percentage Values may point to gross errors. Note that even if the method is perfectly precise, repeated values could still vary owing to inherent variation within the food material itself. Calculation of precision for the data of Table 5.3 shows that precision is relatively poor in seta (high %CV, %RMD values). Pertinent to these measures is the number of repeated measurements.

Table 5.4 *Accuracy measures for percentage of protein data.*

	Analysis A	**Analysis B**
Number of replicates	2	4
Mean (%)	7.9	8.6
% REM	-10.2	-2.3
Note: Most probable value = 8.8 per cent		

5.3.3 Acceptable Level of Replication

The level of replication is an important consideration as it affects the statistical measures and the cost of the analysis in terms of time and personnel. In practice the costs can limit the degree of replication. For routine analyses with established techniques, modern instruments and trained analysts, minimal replication maybe common, except where the technique is very rapid and low in cost, e.g. as with modern nitrogen analysers based on the Dumas method (2.5 minutes per sample). Thus duplicate determinations or even a single one done along with a reference or standard analysis for the run may be typical. If a single determination is made there is no reference point for error detection. Statistically, the greater the number of determinations is, the more reliable or accurate the result is. Whether or not a low level of replication is acceptable depends on several factors: the experience of the analyst and the laboratory itself; the method of analysis and its history with

respect to the food in question; and the importance of the decisions which are to be based on the results. Certainly, low levels of replication in isolation provide a weak basis for making confident decisions regarding the data obtained, e.g. standard deviation based on only two values is an extremely shaky foundation on which to base further inferences. The difference in magnitude between the SD values (Table 5.5) for two and four replicates, for data sets with similar ranges, illustrates this point. This does not, however, preclude the routine use of duplicates. The final consideration is how to use the calculated measures (Tables 5.4 and 5.5) to answer questions concerning the acceptability of the obtained levels of precision and accuracy

Table 5.5 *Accuracy measures for percentage of protein data.*

	Analysis A	**Analysis B**
Number of replicates	2.0	4
Mean (%)	7.9	8.6
Range (%)	1.2	0.9
MD	0.6	0.3
SD	0.85	0.39
%RMD	0.6	3.9
%CV	10.74	4.55

5.3.4 Acceptance Level for Precision

The deviation of a set of replicates around the mean depends on the precision of the measurement system and on the degree of variability of the population from which the samples originate. If both are of a completely unknown nature then whether or not to accept a set of replicates cannot be decided easily. Some measure of variability must be established. This can be done by carrying out an initial set of a larger number of replicates than is envisaged for routine use, e.g. at least ten, or if appropriate, by proceeding with duplicate analyses without considering variability until a “data bank” of typical values has been established

from which an estimate of deviation in the form of the standard deviation can be calculated, i.e. comment concerning the "expected variation" for a set of replicates cannot be made until some measure of variability has been established. Once this is available then an error estimate, known as a confidence interval (CI) can be calculated for the population mean of the measurement. It gives a region within which we are confident that the population mean will be located, with a specified probability or "certainty" level. This statistic can be used as an estimate of bias (accuracy) and the width of the interval gives another perspective on precision, as it emphasizes the effect of sample size. To understand a confidence interval we need to appreciate the nature of a population distribution. Put simply, if we know how a population is "mapped out" then it can be used to make estimations based on samples taken from that population. Imagine that the food product (Table 5.3) is analysed a very large number of times for crude protein content and grouped values are plotted on a histogram – then it is likely that a rough inverted cone shape would be obtained (see Figure 5.1). Increasing the number of points would have smoothing effect on the shape and with a very large number bell shaped curve would be obtained. Ultimately with an infinitely large number of values the curve would be smooth

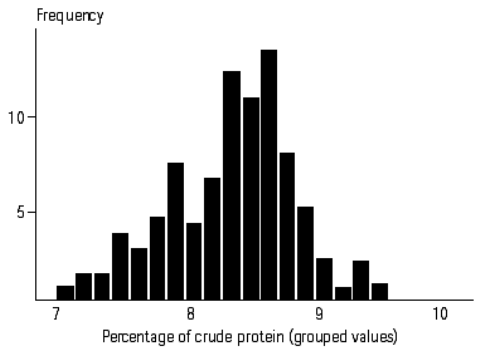

Figure 5.2 *Frequency Distribution of 100 per cent Crude Protein Content Determinations.*

and would represent the population distribution for the measurement. Note that these measurements are the entire same constituent on the same material, using the same technique, etc. The curve shape would be typical of a normal distribution (see Figure 5.2) and similar distributions, "normal" in this case meaning "standard". The curve has certain properties which allow powerful inferential statistics to be performed– the mean (μ), mode and median are centrally located; on either side of the centre the two "tails" are of such shape that more values are clustered towards the centre than at the edges; in terms of variation the proportion of the curve at one or more standard deviations(s) from the mean can be marked and measured. It can be seen that when selecting a random sample from such a population, there is a higher probability of obtaining a percentage protein value within ± 1 standard deviation of the population mean than further away, as there is more area under that region of the curve. In fact approximately 68 per cent of all the values lie in this region, and approximately 95 per cent lie within ± 2 standard deviations. Most chemical and physical measurements on food samples are likely to come from abnormal population. Even if the parent population deviates from normality, statistical theory proves that the distribution of the means of samples from such a population will approximate to normality. Thus this distribution will also possess the above properties and provides the basis for determining the confidence interval for the population mean based on the sample mean. Large sample sizes provide adequate estimates of the population parameters to allow calculation of the confidence interval using the proportions described above. For small samples of the order likely to be used in chemical analysis more appropriate distribution "standard" for making estimates is the *t*-distribution – it is similar in shape and characteristics to the normal distribution but is wider and flatter, having more "spread" (especially for small numbers of samples or replicates). Thus the interval will be wider, reflecting the increased uncertainty. A measure of the degree of confidence must

be specified and it is expressed on a probability scale of zero to 100 per cent, with 100 per cent representing absolute certainty. Unfortunately, choosing the 100 per cent level of confidence would result in an interval of very large width, unusable in practical situations. Usually the 95 or 99 per cent limit is selected, representing high degrees of confidence. The confidence interval limits are calculated using the *t*-value from the *t* distribution based on the number of replicates:

$$95\%\,\mathrm{CI} = \mathrm{M} \pm \mathrm{t} \times \mathrm{SD} / \sqrt{\mathrm{a}}$$

Where

n= number of replicates.

The value of *t* is obtained from statistical tables and its magnitude depends on the confidence level and on the number of samples analysed (more specifically on the degrees of freedom, which is equal to the number of samples minus1). Thus a high confidence level combined with low replication would maximize the *t*-value and the interval width and vice versa. These calculations can now be done for the data of Table 3 and are summarized in Table 5.6.

Thus, assuming no systematic error for Analysis B, the analyst would be confident that 95 per cent of the time, the population mean for percentage of crude protein content would lie between 8.0 and 9.2 per cent. The width of the interval can guide the acceptability of precision. Whether or not it is acceptable depends in turn on how confident the analyst is in the validity of the SD. In set A it is based on only two determinations and gives a confidence interval of 15.2 per cent owing to the large *t*-value and the large SD – in isolation such a wide interval would be unacceptable.

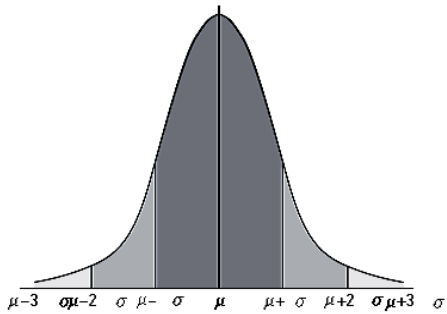

Figure 5.3 *A normal distribution frequency curve.*

Table 5.6 *Confidence intervals for percentage of protein data.*

	Analysis A	**Analysis B**
Number of replicates	2	4
Mean (%)	7.9	8.6
Range (%)	1.2	0.9
SD	0.85	0.39
CI (95%)	0.3-15.5	8.0-9.2
t-value (95% confidence)	12.71	3.18

Analysis B, using four replicates, cuts the interval to 1.2 per cent, obviously more acceptable. This fact seems to condemn low replication but if a confident SD is established initially by a larger number of replicates, or on series of at least six duplicate analyses, then this can be used to calculate a useful statistic, a form of repeatability for subsequent analysis with two replicates:

$$r = t \times \left(\sqrt{2}\right) \times SD$$

Where

r = estimated variability or repeatability which must not be exceeded;

t = value from table based on the larger original number of initial analyses;

SD = standard deviation of original number of repeat determinations under repeatability conditions.

Assuming such circumstances, an additional analysis based on ten crude protein determinations is given below (Table 5.6) along with the calculated *r* statistic. The *t*-value is smaller as it is based on the original ten determinations. Thus we would expect duplicate crude protein determinations to differ by less than 1.1 per cent, so although Analysis A looks more favourable now it could still be rejected on these grounds. Indeed, in the author's experience of the manual Kjeldahl technique on a range of food products, the precision of Analysis B (or better) is more typical and it is likely that a gross error has occurred in Analysis A. Following the above procedure now gives a more definite guide to accepting the level of precision.

5.3.5 Acceptable Level of Accuracy

The magnitude of the percentage of REM or the EM will decide this, but how large should it be before it is regarded as unacceptable? If it's based on comparison with typical values then these can vary by up to 10 per cent or more and this must be borne in mind when gauging accuracy via crude methods. Similarly "most probable" estimates are also approximations. Analysis B (Table 5.5) is within 10 present of the estimated true value whereas Analysis A exceeds this limit. The confidence interval detailed above as a precision check canals be used for accuracy, provided that confident measure of the SD was obtained – if the expected value lies within the interval then this is acceptable. In the example (Table 5.5), both analyses achieve this level of acceptance, but the Analysis A result is rejected because of the very large interval. Determination of crude protein is a proximate technique, and accuracy much beyond that of Analysis B may be an unrealistic target. For a certified RM, a similar procedure can be

applied – the determined interval should contain the certified analysed value. Additionally an interval will be quoted on the certificate – the mean value obtained by the analyst should lie within this interval. This is a more stringent test as the certificate interval is likely to be narrower. In either case, if the determined mean is out with the interval then the result can be viewed as inaccurate and this may indicate the presence of a systematic error. Depending on circumstances, there is some leeway in the decision-making process and individual analysts can decide on acceptable proximity to the precision and accuracy levels. Textbooks on analytical methods may not quote figures for acceptable accuracy and precision. Often it is left to the experience and knowledge of the analyst.

5.4 Actualisation

Study Case: Statistical Technique Application Example – Precision Calculations for Chemical Analysis Data; Source: J. A. Bower 2009.

Data gathered during routine chemical analysis of moisture content in foods were examined for the level of precision. Mean values were in the range 70–72 g/100 g and based on the data bank the population standard deviation was taken as 0.35 g/100 g. A duplicate measure was carried out under repeatability conditions.

Table 5.7 *Repeatability Calculation Data (Excel).*

Data	Moisture Content (g/100g)
Duplicate 1	71.5
Duplicate 2	70.9
Mean	71.2
sd(pop)	0.35
sd (unknown)	0.42
$t_{95\%,\ ldf}$	12.71
repeatability	0.97
repeatability	7.62

Table 5.7 shows the result of the duplicate moisture content determination. Assuming repeatability conditions as defined above, precision (repeatability) can be calculated in two ways.

As the population standard deviation (sigma (σ)) is known, then:

Repeatability 95%= z 95% × square root (2) × σ= 1.96 × square root (2) × σ

This is essentially the confidence interval for a duplicate determination, i.e. n = 2. The z value is the confidence level factor based on a normal distribution. In the example, repeatability has a value of 0.97%. Thus, duplicate determinations of moisture by the particular method in the same laboratory, same technician, reagents, etc., should differ by not more than 0.97%. The population sigma can be obtained from previous data as in the example, or by carrying out an initial larger set of determinations to give an improved estimate. If sigma is estimated as the sample *sd*, then:

$$\text{Repeatability } 95\% = t\ 95\%,\ 1\text{d}\ f \times \text{square root } (2) \times sd$$

The confidence level factor is based on the t distribution. This results in a much higher value (>7%), but it is also possible to use a t value based on a larger earlier set to give more representative measure of repeatability, and a narrower interval. In practice, duplicate moistures by oven drying give %*CV*s of <1%, thus the former estimate of repeatability is not usual. Some texts define repeatability as 'within laboratory' in a broader way in that it includes different operators, which is more realistic as it cannot be guaranteed that the same technician will analyse all incoming samples at one session, etc. Repeatability can also be obtained via the 'within variance' estimate in a two-way ANOVA.

5.5 Reproducibility

Repeatability is a critically important measure for a laboratory, but there are many analytical laboratories and there is concern that results can vary depending on which laboratory is used. There are many examples of such discrepancies in the literature (e.g. Thompson 1994), with z-scores (as above) attaining values well above the ±2 limit for some laboratories (±10 in some instances). This has given rise to a further definition of precision. The term is expanded to cover variation between different laboratories – *reproducibility*. This is the variability where all aspects other than the material being analysed are different, i.e. analysis in different laboratories, hence different technicians, reagents, times etc. The definition is calculated in a similar manner to that for repeatability, with the inclusion of the 'different laboratory effect': reproducibility is the magnitude of the interval for 2 determinations by any two laboratories. The calculation reflects the wider source of variation by incorporating the variance of both within- and between-laboratory sources:

Reproducibility 95% (population variance known):= z 95% × square root (2) × square root (variance within+ variance between)

And

Reproducibility 95% (variance estimated): = t 95%, 1 × square root (2) × square root (estimated variance within+ estimated variance between)

Table 5.8 *Estimation of within- and between-laboratory variance using ANOVA (Excel).*

Data	**Moisture content (g/100g)**					
	Lab A	**Lab B**				
	71.1	72.8				
	71.7	72.9				
Mean	71.4	72.85			Estimate	Known
sd(pop)	0.18	0.09	Within variance		0.093	0.065
sd(unk)	0.42	0.07	Between variance		2.10	1.6
$T_{95\%,1df}$	12.71	12.71				
$Reliability_z$	0.50	0.25	$T_{95\%,1df}$			
$Reliability_t$	7.62	1.27	$Reproducibility_z$			
			$Reproducibility_t$			
ANNOVA: single factor						
Summary						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Lab A	2	142.8	71.4	0.19		
Lab B	2	145.7	72.85	0.005		
ANNOVA						
Source of variation		*SS*	*df*	*MS*	*F*	*P - value*	F_{crit}
Between groups		2.103	1	2.10	22.73	0.041	18.51
Within groups		0.185	2	0.093			
Total		2.288	3				

The estimates in the second formula are conveniently obtained by ANOVA, which provides a 'pooled within variance' as well as the 'between variance'. This is shown for two laboratories – laboratory A compared with Laboratory B (Table 5.8). The variance of each laboratory is given and the average of this is the 'pooled within variance' – also shown as the 'within group mean square' (MS). The 'between variance' is the 'between groups MS'. These were used to calculate the reproducibility of these two laboratories. As seen, the reproducibility figures are higher than those of repeatability, especially when the variance estimates are based on two determinations (again, this can be improved using a known or an established more confident variance estimate). The above analysis is limited in that it cannot provide any indication of interaction of laboratories with different concentrations or levels of a particular analyte, e.g. a food with a higher or lower

level of moisture. Inclusion of a second set of replicates from another food type allows interaction to be assessed. Here, reproducibility is determined via estimates of *within*, *between* and *interaction*, which are adjusted to take into account the number of determinations, etc. Reproducibility is still high when more than one concentration of the moisture content is considered, but the differing concentration does not appear to cause a significant effect. Such work is done in inter-laboratory proficiency-testing schemes, and large precision studies can involve ten or more laboratories examining a particular method at several levels of concentration.

Table 5.9 *Reproducibility with interaction (Excel).*

Data	**Moisture content (g/100g)**						
	Lab A	**Lab B**					
s1	73.4	75.1					
	72.7	74.2					
s2	66.5	67.9					
	68.0	67.2					
Variance Estimates							
Within Lab	0.51		=*Within*				
Between Lab	0.24		=*columns – interaction / number of determinations per lab*				
Interaction	0.17		=*interact – within / number in each cell*				
$T_{95\%,4df}$	2.78		*df = within*				
Reproducibility	3.76						
Annova two – factor with replication							
ANNOVA							
Source of variation	*SS*	*df*	*MS*	*F*	*P - value*	F_{crit}	
Sample	83.21	1	83.21	164.76	0.0002	7.71	
Columns	1.81	1	1.81	3.57	0.13	7.71	
Interaction	0.84	1	0.84	1.67	0.26	7.71	
Within	2.02	4	0.51				
TOTAL	87.88	7					

5.6 Discussion

It is possible to evaluate scientific data without involving statistical analysis. This can be done by experienced practitioners who develop a ‘feel’ for what the data are ‘telling them’, or when dealing with small amounts of data. Once data accumulate and time is limited, such judgement can suffer from errors. In these cases, simple statistical summaries can reduce large data blocks to a single value. Now, both the enlightened novice and the experienced analyst can judge what the statistics reveal. Consequent decisions and actions will now proceed with improved confidence and commitment. Additionally, considerable savings in terms of time and finance are possible. In some instances, decision-making based on the results of a statistical analysis may have serious consequences. Quantification of toxins in food and nutrient content determination rely on dependable methods of chemical analysis. Statistical techniques play a part in monitoring and reporting of such results. This gives confidence that results are valid and consumers benefit in the knowledge that certain foods are safe and that diet regimes can be planned with surety. Other instrumental and sensory measures on food also receive statistical scrutiny with regard to their trustworthiness. These aspects are also important for food manufacturers who require assurance that product characteristics lie within the required limits for legal chemical content, microbiological levels and consumer acceptability. Similarly, statistical quality control methods monitor online production of food to ensure that manufacturing conditions are maintained and that consumer rights are protected in terms of net weights, etc. Food research uses statistical experimental design to improve the precision of experiments on food. Thus, manufactures and consumers both benefit from the application of these statistical methods. Generally, statistics provides higher levels of confidence

and uncertainty is reduced. Food practitioners apply statistical methods, but ultimately, the consumer benefits.

5.7 General Recommendations

1. Food manufacturers and producers should adhere and comply with national standard bodies through established analytical procedures, regulations and standards.

2. Research scientists should use statistical techniques in experimental and research work and present findings or results that are empirical, accurate and precise. Update information on new statistical software and packages should be adopted and included in educational curriculums for study programmes.

3. Application of statistical techniques in the following areas should be promoted:

   Instrumental measures - covering any measurement system from chemical and physical analysis to specific food instrumentation methods and process measures, e.g. protein content, Lovebird colour measures, air speed setting, etc.

   Sensory measures – to include all sensory tests used by trained assessors such as discrimination tests and descriptive analysis methods. *Consumer tests*– to include some sensory methods, which are affective or hedonic in nature, e.g. preference ranking. Generally systems should cover mostly laboratory measurements.

Consumer measures- should refer to questionnaire measures in surveys, such as consumers' views and opinions on irradiated foods. This should cover consumer applications which are usually non-laboratory in nature.

5.8 Conclusion

Food issues are becoming increasingly important to consumers, most of who depend on the food industry and other food workers to provide safe, nutritious and palatable products. These people are the modern-day scientists and other practitioners who work in a wide variety of food-related situations. Many will have a background of science and are engaged in laboratory, production and research activities. Others may work in more integrated areas such as marketing, consumer science and managerial positions in food companies. These food practitioners encounter data interpretation and dissemination tasks on a daily basis. Data come not only from laboratory experiments, but also via surveys on consumers, as the users and receivers of the end products. Understanding such diverse information demands an ability to be, at least, aware of the process of analysing data and interpreting results. In this way, communicating information is valid. This knowledge and ability gives undeniable advantages in the increasingly numerate world of food science, but it requires that the practitioner have some experience with statistical methods. Unfortunately, statistics is a subject that intimidates many. One need only consider some of the terminology used in statistic text titles (e.g. 'fear' and 'hate'; Sal kind 2004) to realise this. Even the classical sciences can have problems. Professional food scientists may have received statistical instruction, but application maybe limited because of 'hang-ups' over emphasis on the mathematical side. Most undergraduate science students and final-year school pupils may also find it difficult to be motivated with this subject; others with a non-mathematical background may

have limited numeracy skills presenting another hurdle in the task. These issues have been identified in general teaching of statistics, but like other disciplines, application of statistical methods in food science is continually progressing and developing. Statistical analysis was identified, two decades ago, as one subject in a set of 'minimum standards' for training of food scientists at undergraduate level (Iwaoka *et al.* 1996). Hartel and Adem (2004) identified the lack of preparedness for the mathematical side of food degrees and they describe the use of a quantitative skills exercise for food engineering, a route that merits attention for other undergraduate food science courses. Unfortunately, for the novice, the subject is becoming more sophisticated and complex. Recent years have seen this expansion in the world of food science, in particular in sensory science, with new journals dealing almost exclusively with statistical applications. Research scientists in the food field may be cognizant with such publications and be able to keep abreast of developments. The food scientist in industry may have a problem in this respect and would want to look for an easier route, with a clear guide on the procedures and interpretation, etc. Students and pupils studying food-related science would also be in this situation. Kravchuk *et al.* (2005) stress the importance of application of statistical knowledge in the teaching of food science disciplines, so as to ensure an on-going familiarity by continual use. Some advantages of being conversant with statistics are obvious. An appreciation of the basis of statistical methods will aid making of conclusions and decisions on future work. Other benefits include the increased efficiency achieved by taking statistical approach to experimentation.

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6

Application of Rheology in Food Engineering; Concentrated Food Emulsions and Dispersions

6.1 Introduction

Traditionally, foods have been classified as hard solids, soft solids and liquids. Some examples of these general physical categories are shown in Table 6.1.

Table 6.1 *General categories of foods.*

General Category	Food Examples
Hard solids	Chocolate, biscuits, hard cheese
Soft solids	Butter, ice-cream, tomato paste
Liquids	Water, honey, ketchup, mayonnaise

Many foods of commercial importance, such as baby foods, mayonnaise, salad dressings and plant food concentrates (orange, tomato, apple, etc.) are concentrated dispersions of solid (suspensions) or fluid (emulsions) matter in fluid media, which may behave as soft solids or highly non-Newtonian liquids. Rheology plays an important role in food manufacture and marketing [Barnes H; 2001], i.e. Design of handling systems, quality control and evaluation of sensory stimuli associated with oral and non-oral evaluation of viscosity [Rao M A; 1992]. Starting with processing, it is notorious that process variables determine the microstructure formed for a given formulation, and hence its rheology. Often, processing is itself affected by the rheology of the product, especially if it is very viscoelastic or very shear-thinning. When the final product is formed its rheology should be measured in a precise manner, accounting for any wall effects and selecting, for instance, the appropriate range of deformation rate for the particular application. A suitable constitutive equation should be selected, then, to fit the rheometry results. This step is still quite challenging for semisolids food products, taking into account their dramatic shear-thinning response and the probable appearance of instabilities in a significant range of shear rates [Bertola V, Bertrand F, Tabuteau H, Bonn D and Coussot P, 2003]. This suitable constitutive

equation can be used to calculate flows in other geometries, i.e. process equipment's, using Computational Fluids Dynamics. Finally, the measured product rheology should also be compared with the subjective consumer perception.

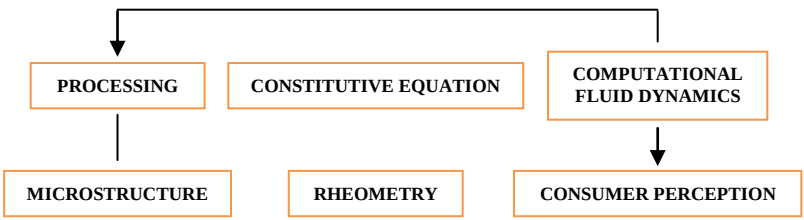

Figure 6.1 *Important areas in the study of Food Rheology (reproduced from Barnes H; 2001).*

6.2 Description

Although food scientists have some control over the final properties of a product, they must work within the physical constraints set by nature (i.e., the characteristics of the individual molecules and the type of interactions that occur between them). There is an increasing awareness within the food industry that the efficient production of foods with improved quality depends on a better understanding of the molecular basis of their bulk physicochemical and organoleptic properties (Baianu 1992, Kokini et al. 1993, Eads 1994). The individual molecules within a food emulsion can interact with each other to form a variety of different structural entities. A molecule may be part of a bulk phase where it is surrounded by molecules of the same type, it may be part of a mixture where it is surrounded by molecules of a different type, it may be part of an electrolyte solution where it is surrounded by counter ions and solvent molecules, it may accumulate at an interface between two phases, it may be part of a molecular aggregate dispersed in a bulk phase, it may be part of a three dimensional network that extends throughout the system, or it may form part of

a complex biological structure (Israelachvili 1992). The bulk physicochemical properties of food emulsions depend on the nature, properties, and interactions of the structures formed by the molecules. The structural organization of a particular set of molecules is largely determined by the forces that act between them and the prevailing environmental conditions (e.g., temperature and pressure). Nevertheless, foods are rarely in their most thermodynamically stable state, and therefore the structural organization of the molecules is often governed by various kinetic factors which prevent them from reaching the arrangement with the lowest free energy. For this reason, the structural organization of the molecules in foods is largely dependent on their previous history (i.e. the temperatures, pressures, gravity, and applied mechanical forces experienced during their lifetime). To understand, predict, and control the behaviour of food emulsions, it is important to be aware of the origin and nature of the forces responsible for holding the molecules together and how these forces lead to the various types of structures found in food emulsions. Only then will it be possible to create and stabilize foods that have internal structures that are known to be beneficial to food quality.

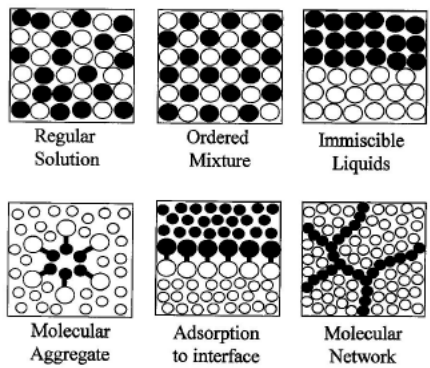

Figure 6.2 *The molecules in food emulsions may adopt a variety of different structural arrangements depending on the nature of their interactions with their neighbours.*

6.3 General Analysis

Emulsification is a complex unit operation in which many variables influence the processing and the final rheological characteristics of the product. The manufacture of emulsions usually requires the application of considerable mechanical energy. The two critical steps are the consecutive disruption of droplets and their coalescence, both of which are favoured by an intense agitation. Consequently, the improvement of the emulsification process requires the measurement of the droplet size of the dispersed phase and its polydispersity. Moreover, the emulsification process may be greatly affected by the viscous and viscoelastic properties of the continuous phase at which the disperse phase is added. One of the first studies on the influence of mechanical variables during processing was reported by Franco et al., [Franco J M, Guerrero A and Gallegos C; 1995] for emulsions stabilized by a mixture of macromolecular and low-molecular-weight emulsifier. Figure 6.3 shows the linear relaxation spectra of emulsions prepared with a rotor-stator turbine as a function of rotational speed and residence time. As can be observed the slope of the plateau region increases with the processing mechanical variables, because of the development of a three-dimensional network. This is also enhanced by a decrease in mean droplet size and polydispersity of the emulsion, yielding stronger inter-droplet interactions. As a result, stability against creaming was improved. Different results were obtained later on for emulsions stabilized by a sucrose ester non-ionic surfactant [Gallegos C, Sanchez M C, Guerrero A, Franco J M; 1996], which forms a gel-like structure in the continuous phase for a wide range of concentrations and temperatures [Madiedo. J. M; 1996]. As was previously mentioned, an increase in agitation speed or emulsification time also produces a decrease in droplet size and polydispersity. However, in this case, an increase in the agitation speed produced a decrease in the values of the dynamic viscoelasticity functions. The opposite

effect was found by increasing the emulsification time. Therefore, the viscoelastic properties of these emulsions depend on the balance between the formation of a larger interfacial surface and the breakdown of the gel-like structure of the continuous phase during processing. The influence of mechanical processing variables on vegetable protein stabilised emulsions is mainly affected by droplet size [Franco J M, Raymundo A, Sousa I and Gallegos C, J.; 1998]. An increase in emulsification time and, specially, agitation speed produces a decrease in the Sauté diameter and favours the development of an entanglement network, noticed by an enhanced development of the plateau region in the mechanical spectrum and a significant increase in the values of the dynamic functions (i.e. see figure 6.4). These authors also found an increase in emulsion viscosity and other textural parameters like firmness and adhesiveness.

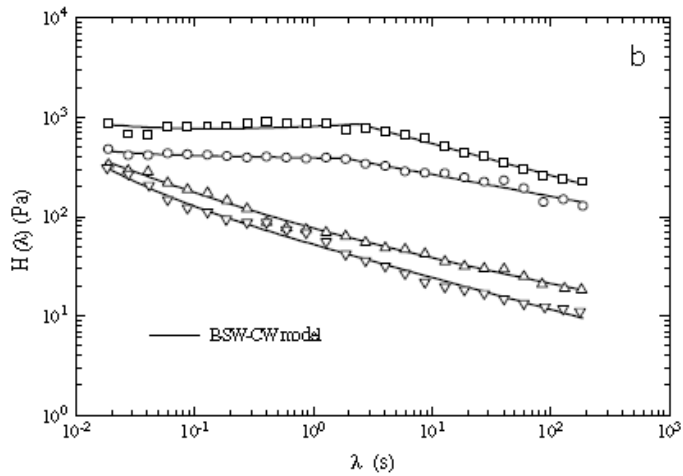

Figure 6.3 *Influence of emulsification time and agitation speed on the relaxation time spectra of salad dressing emulsions: 8000 rpm – 5 min, ○8000 rpm - 3min, Δ 5000 rpm – 5 min, ▼5000 rpm – 3 min. Adapted from Franco J M, Guerrero A and Gallegos C; 1995.*

Temperature is a crucial variable to be controlled during the processing of protein-stabilised emulsions. For instance, an increase in the temperature during

the emulsification, induced by the application of a severe mechanical energy, must affect the protein hydrophobicity [Raymundo A, Franco J M, Gallegos C, Empis J and Sousa I, Nahrung; 1998] and, consequently, favours the inter-droplet

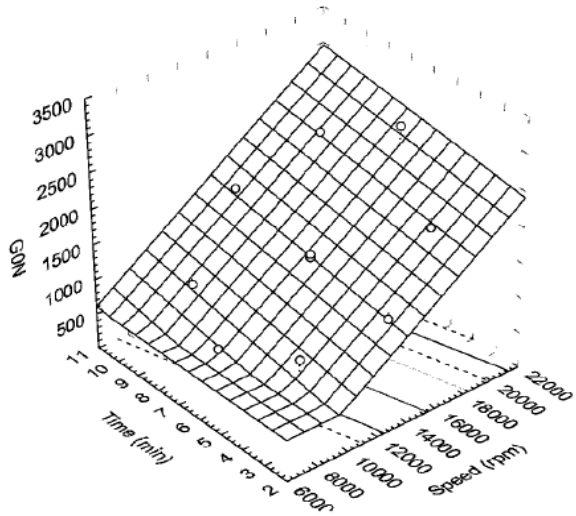

Figure 6.4 *Evolution of the plateau modulus for lupin protein-stabilized emulsions prepared with different emulsification times and homogenization speeds (reproduced from Franco J M, Raymundo A, and Sousa I and Gallegos C, J.).*

interactions. On the other hand, an increase in temperature or heating time favours the coalescence process, leading to larger droplet sizes and broader distributions, as found for pea-protein stabilised emulsions [Franco. J. M, Partal. P, Ruiz-Marquez D, Conde. B and Gallegos. C, J.; 2000]. However, a severe thermal treatment previous to emulsification leads to more viscous emulsions. Thus, for instance, the viscosity and the plateau modulus increases with temperature, specially up to temperature around 75 ℃ and then remain almost constant, in spite of higher droplet sizes, a fact that is related to the achievement of the protein extensive denaturation. The application of high temperatures during the emulsification process produces similar effects [Franco J M, Guerrero A and Gallegos C; 1995]. An even more severe previous thermal treatment on native egg yolk is the spray-drying process, usually required to

microbiologically preserve the final product, or as the first step previous to a cholesterol extraction process [Bringe N A and Cheng. J; 1995]. The rheology of spray-dried yolk stabilized emulsions is dramatically different to that found with native egg yolk-stabilised emulsions due to a significant denaturation of egg yolk lipoproteins, which confers a marked gel-like behaviour and significantly higher values of the linear viscoelastic functions to these egg product-stabilized emulsions [Guerrero A and Ball H R, J.; 1994; Moros J E, Franco J M and Gallegos C,; 2002]. The application of thermal treatments after emulsification may also have significant influence on the rheological behaviour of protein-stabilised emulsions. Dickinson and co-workers [Dickinson E and Yamamoto Y; 1996] have extensively studied, during the last few years, the rheological properties of heat-set whey protein-stabilised emulsion gels and the *in situ* gelation process through small-deformation oscillatory measurements. In all cases, the fresh emulsion was a very low viscous liquid-like system and became gel network by increasing temperature. Thus, a crossover between $G¢$ and $G¢¢$ was noticed at a relatively high temperature. Chen and Dickinson [Chen. J. and Dickinson E, J.; 1998] investigated the effects of protein concentration and the volume fraction of oil phase on the viscoelastic properties of heat-set whey protein emulsion gels. They conclude that protein concentration is the main factor affecting gel strength. The dispersed oil droplets act as space fillers but also help to build up the gel matrix structure through interactions between protein molecules at the droplet surface and those in the gel matrix. The filler effects of oil droplets on the rheology of heat-set egg yolk, soy and milk protein stabilised emulsion were respectively studied with some detail by Dickinson and Chen, Anton et al., and Kim et al. Although a simplified van der Poel ś equation [Smith. J. C, J.; 1975] to estimate the shear modulus of a particulate composite has been used by the authors, the fitting fails due to both oil droplet flocculation and droplet deformability. This thermo

rheological behaviour was shown by emulsions with relatively low volume fractions of oil phase. The improvement in the strength of highly concentrated gel-like emulsions, by means of thermo rheological treatments, was also studied by Moros et al [Moros. J. E, Cordobes. F, Franco. J. M and Gallegos. C; 2003]. They show how highly flocculated egg yolk-stabilised emulsions, in principle with a soft gel-like behaviour achieved during the emulsification process, are susceptible to improve their gel-like behaviour by applying different thermal treatments on fresh emulsions. Thus, for instance, the application of upward/downward temperature cycles, setting the maximum temperature at 67 ℃, avoids emulsion breakdown and yield significantly higher values of the rheological functions in comparison to those found with fresh emulsions, in spite of the thermal induced droplet coalescence observed. As may be observed in figure 6.5, in the first region, which corresponds to temperature range comprised between 25 ℃ and 67 ℃, the evolution of *G*¢ is typical of that found during an upward temperature ramp, an initial decrease of the dynamic functions up to around 45 ℃ and a subsequent increase up to around 70 ℃, related to a head-induced rearrangement of the egg yolk lipoproteins located at the interface of oil droplets. In the second region, where temperature is kept constant at 67 ℃ for different elapsed times, G¢ shows a rapid increase and then levels off. If temperature is kept constant at 67 ℃ for 500 s (cycles B_3 and B_4) the values of G¢ are significantly higher than those obtained by cooling the sample immediately after the maximum temperature was reached (cycle B_1). On the contrary, when the sample was maintained at 67 ℃ for a much longer period of time, i.e. 1500 s (cycle B_2), a slight decrease in G¢ is shown. These results were explained taking into account the aggregation of denatured lipoproteins, mainly live tins and LDL. However, if the sample is maintained at high temperature during an extended period (cycle B_2) a subsequent significant coalescence of oil droplets may be observed (i.e. *d43* = 25.2 matter the

application of cycle B2 versus $d\ 43 = 16.4\ \mu m$ after the application of cycle B_3). Finally, a further sample cooling produces a new increase in $G¢$, mainly due to hydrogen bonding. On the contrary, the cooling rate seems not to affect the final value of the linear viscoelastic functions, as may be deduced from the comparison of the final values of $G¢$ after the application of cycles B_3 and B_4 respectively. Afterwards, the values of $G¢$ do not change by keeping constant temperature at 25 ℃, which indicates an irreversibility of the gel reinforcement process. However, absolute values of G' after the application of these thermal treatments were significantly lower as compared to those found by others [Chen J and Dickinson E, J.; 1998; Chen J and Dickinson E,; 1999; Chen. J. and Dickinson. E; 2000], results that were obtained with milk protein-stabilized emulsions subjected to the same thermal treatments and containing similar protein concentrations, even with much lower fraction of dispersed phase, which indicates that egg yolk-stabilized emulsions are much less susceptible to enhance gel strength than milk protein systems.

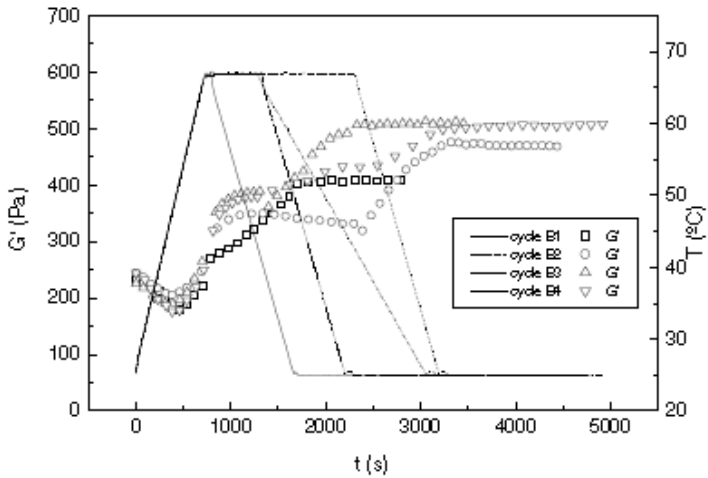

Figure 6.5 *Evolution of $G¢$ with time, during the application of different cycles of temperatures, for an emulsion containing 70% (w/w) oil and5% (w/w) egg yolk (reproduced from Moros J E, Cordobes F, Franco J M and Gallegos C; 2003).*

The reversibility of heat-induced gelation was studied by Dickinson and co-workers, for casein ate-stabilised emulsions, through viscosimetry measurements [Dickinson. E. and Casanova. H; 1999; Dickinson. E. and Eliot. C; 2003]. They showed that a thermo-reversible gelation in the temperature range of 30-45 ℃ occurs mainly depending on pH and calcium ion content. They divided the emulsions, attending to the reversibility, in three categories (see figure 6.5): i) liquid like emulsions which remains liquid when heated, ii) liquid-like emulsions which become gels when heated, exhibiting an increase in viscosity of, at least, one order of magnitude and decreasing viscosity again when it was cooling back down, and iii) emulsions that show already a gel-like behaviour when emerging from the homogeniser, which present an increase in viscosity of several orders of magnitude on heating above 30 ℃, without returning to low values on cooling back to ambient temperature. They presented a sort of phase diagram showing the different behaviours as a function of acid and calcium content. In addition to this, Chen and Dickinson [Chen J and Dickinson E; 2000] studied the effect of different programs of temperature on the reversibility of emulsions gels, attending to the evolution of the linear viscoelastic functions. As an example, they concluded that the application of a program 45 ℃, 5 ℃, and 45 ℃ on the emulsions leads to a temperature-reversible behaviour. G¢ and G¢¢ increase on cooling to 5 ℃ and recover their original values on reheating to 45 ℃. On the contrary, the program 5 ℃, 45 ℃, 5 ℃ produces a non-reversible rheological behaviour. Thus, they found G¢ values around 10 times higher than values before the application of the thermal treatment. An alternative to heat treatments is the high-pressure technology. The emulsifying and stabilising ability of some high-pressure treated vegetable and milk proteins were found inferior to those shown by the native proteins, which was attributed to an enhanced dissociation and/or aggregation through disulphide bridging [Galazka V B, Dickinson E and Ledward D A; 1999]. However, the viscoelastic parameters, i.e. the complex modulus, of

emulsions prepared with severely pressure-treated (up to 800 *MPa*) b-lacto globulin are higher in a wide range of pH than those found with untreated systems, even more when a thickener agent was included in the formulation, probably due to the development of high-pressure-induced inter droplet macromolecular linkages [Dickinson E and James J D,; 2000]. In any case, high-pressure processing seems to be a gentler processing operation in terms of changes in droplet size and instability [Dickinson E and James J D, J.; 1998] On the contrary, high-pressure treatments prior to emulsification (600 and 400 *MPa*, respectively) on ovalbumin [Galazka V B, Dickinson E and Ledward D A, J.; 2000] or lupin protein [Chapleau N and de Lamballerie-Anton, M; 2003] clearly improve the emulsifying efficiency and stabilizing properties of emulsions. Concerning the rheological behaviour, Chapleau and de Lamballerie-Anton [Chapleau N and de Lamballerie-Anton, M; 2003] found that viscosity and $G¢$ and $G¢¢$ values slightly increase with pressure, although the viscoelastic behaviour, typical of flocculated emulsions, was essentially the same.

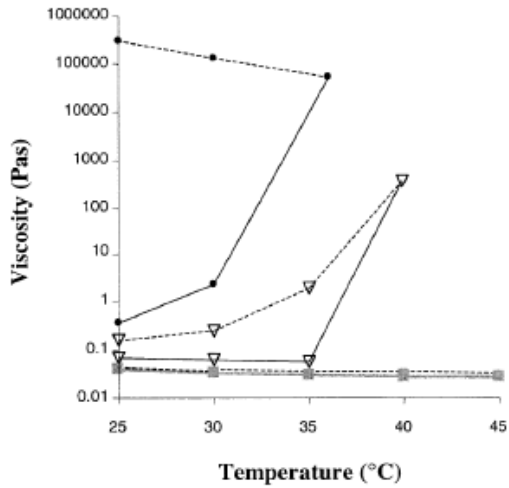

Figure 6.6 *Examples of the three categories of emulsions attending to the thermo reversibility of the rheological behaviour. Solid lines: heating; dashed lines: back-cooling. ■: category i), ▼: category ii), ●: category iii). Reproduced from Dickinson E and Eliot C; 2003.*

The influence of processing variables may be also studied by means of an *inside* rheological monitoring of the emulsification process [Edgar Chavez-Montes B, Choplin L and Schaer E; 2003]. The *in situ* rheological measurements were carried out in a small-scale semi-batch reactor, so called rheo reactor, equipped with a helical ribbon impeller adapted to a rheometer. An analytical method based on the Couette analogy allows to quantify correctly the torque-rotational speed to be transformed in the absolute rheological response, either under steady-state flow or in oscillatory regime [Ait-Kadi A, Marchal P, Choplin L, Chrissemant A S and Bousmina M, Can. J.; 2002]. The rheo-reactor not only provided real-time information during processing, but also allowed a complete rheological characterization of the final product without any sampling. Edgar Chavez-Montes etal. [Edgar Chavez-Montes B, Choplin L and Schaer E,; 2003] followed-up *in situ* the processing of one of the most complex food systems such as ice cream, consisting of an emulsion of milk-fat globules in a highly viscous continuous phase containing sugars, proteins and stabilizers together with ice crystals and air bubbles. Processing was divided in two crucial steps, foaming and subsequent freezing of mixes. They found that rheology of the incipient product was mainly influenced by the type and concentration of stabilisers. Figure 6.7 shows the evolution of specific viscosity with time during the foaming step for different blends of stabilisers and emulsifiers. In addition to this, a combination of two emulsifiers (i.e. Tween 20 and mono-and diglycerides) was necessary to produce a desired partial coalescence of fat globules, which is related to the melting resistance of ice creams.

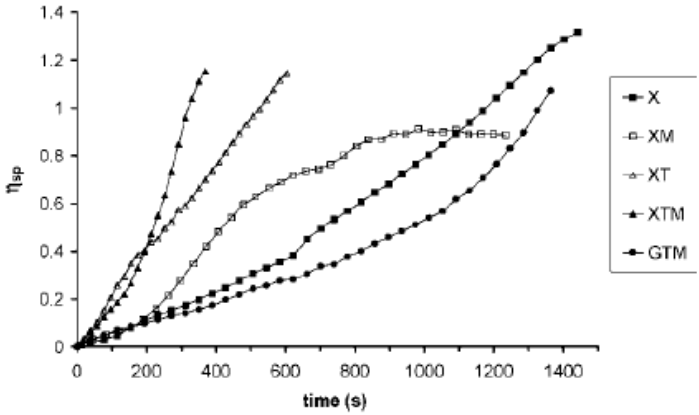

Figure 6.7 *Evolution of specific viscosity during foaming of mixes stabilized with different thickeners and emulsifiers (reproduced from Edgar Chavez-Montes B, Choplin L and Schaer E; 2003).*

6.4 Actualisation

The issue of wall slip requires special attention in relation to the understanding of the behaviour of disperse systems under shear conditions. At present, it is accepted that true slip at the wall does not occur during the flow of colloidal systems and, as Barnes [Barnes H A, J.; 1995] has pointed out, the term "wall depletion" is the most accurate way to describe this phenomenon in which the non-slip boundary conditions are not violated. Emulsions often show these effects due to the displacement of the dispersed phase away from solid boundaries, as for instance the walls of the sensor systems in aerometer or the walls of pipes or tubes, giving a depleted layer of liquid, which then acts as a lubricant. This leads to an apparent decrease in the measured bulk viscosity. In the case of emulsions, the deformability of the droplets and creaming enhance this effect. As has been detected in several food emulsions [Barnes H A, J.; 1995; Franco J M, Gallegos C and Barnes H A,; 1998] wall slip phenomenon is generally confined in a certain range of shear rates associated to constant values

of shear stress or, in other words, around a critical stress at which a sudden drop in viscosity takes place. The use of roughened plates or the vane geometry has been proposed as effective techniques for eliminating slip effects in emulsions [Pal R,; 2000]. However, in complex systems, such as food emulsions, this phenomenon is until now not fully understood from a micro-structural point of view. Franco et al. demonstrated that wall slip effects are strongly dependent on the composition and type of emulsion. Thus, for instance, a highly structured gel-like continuous phase dampens these effects, in contrast to oil-in-water emulsions with relatively low disperse phase volume fraction, in which creaming appears as a mechanism of instability. Sánchez etal. [Sanchez M C, Valencia C, Franco J M and Gallegos. C; 2001] proposed an empirical method to quantify the extension of wall slip as function of several structural parameters such as disperse phase fraction, emulsifier concentration and droplet size (influenced also by processing), according to the differences found in the flow curves obtained with both serrated and smooth geometries along the experimental range of shear rate or shear stress studied. More recently, Bertola et al. [Bertola V, Bertrand F, Tabuteau H, Bonn D and Coussot P, J.; 2003] have related the slip phenomena to the yielding behaviour and flow instabilities of emulsions. In addition to this, they demonstrated that slip does not occur when using rough surfaces by withdrawing the calculated shear rate due to slip from the data obtained with smooth surfaces. Then, flow curves almost exactly correspond to those obtained using rough surfaces. Although wall slip under the flow of food emulsions is a generally accepted phenomenon, some controversy appears in relation to the evidence of apparent wall slip in SAOS experiments. Ma and Barbosa-Cánovas and Plucinski et al. support the idea that no evidence of apparent wall slip in oscillatory shear is found for mayonnaises. On the contrary, Goshawk et al., which performed several tests with different plate-plate separations, found that the values of the linear viscoelastic functions, at a

given frequency, decrease as the plate-plate separation decreases, indicating that mayonnaise tested exhibits wall slip under SAOS, fact that is even surprising for the authors. Pal also detected that the oscillatory response of emulsions is strongly influenced by slip effects not only quantitatively but also from qualitative point of view since, in some cases, a solid like behaviour was found using the serrated plate geometry whereas a fluid-like response was obtained with smooth cone-plate geometries for the same emulsion. In addition to this, the linear viscoelastic range was clearly influenced by wall slip.

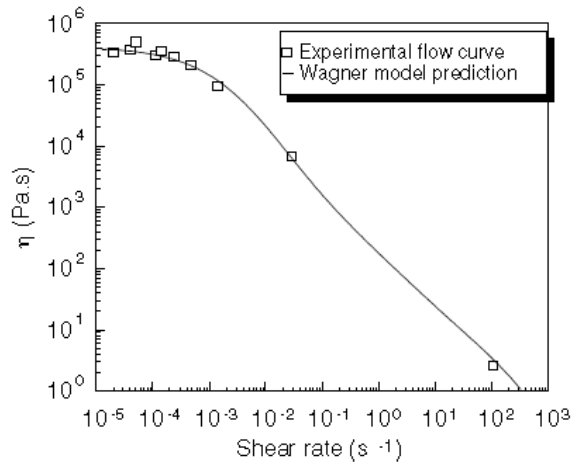

Figure 6.8 *Prediction of steady viscous flow for an oil-in-water emulsion containing 65% oil and 3% lupin protein.*

6.5 Discussion

Many natural and processed foods consist either partly or wholly as emulsions or have been in an emulsified state at some time during their production; such foods include milk, cream, butter, margarine, fruit beverages, soups, cake batters, mayonnaise, cream liqueurs, sauces, desserts, salad cream, ice cream, and coffee whitener (Friberg and Larsson 1997, Krog et al. 1983, Jaynes 1983, Dickinson

and Stainsby 1982, Dickinson 1992, Swaisgood 1996). Emulsion-based food products exhibit a wide variety of different physicochemical and organoleptic characteristics, such as appearance, aroma, texture, taste, and shelf life. For example, milk is a low-viscosity white fluid, strawberry yogurt is a pink viscoelastic gel, and margarine is a yellow semisolid. This diversity is the result of the different sorts of ingredients and processing conditions used to create each type of product. The manufacture of an emulsion based food product with specific quality attributes depends on the selection of the most appropriate raw materials (e.g., water, oil, emulsifiers, thickening agents, minerals, acids, bases, vitamins, flavours, colorants, etc.) and processing conditions (e.g., mixing, homogenization, pasteurization, sterilization, etc.). Traditionally, the food industry largely relied on craft and tradition for the formulation of food products and the establishment of processing and storage conditions. This approach is unsuitable for the modern food industry, which must rapidly respond to changes in consumer preferences for a greater variety of cheaper, healthier, and more convenient foods (Sloan1994, 1996; Katz 1997). In addition, the modern food industry relies increasingly on large-scale production operations to produce vast quantities of foods at relatively low cost. The development of new foods, the improvement of existing foods, and the efficient running of food-processing operations require a more systematic and rigorous approach than was used previously (Hollingsworth 1995). Two areas which have been identified as being of particular importance to the improvement of food products are:

- Enhanced scientific understanding of food properties.

  An improved understanding of the factors that determine the bulk physicochemical and organoleptic properties of emulsions will enable manufacturers to create low-cost high-quality food products in a more systematic and reliable fashion (Kokini et al. 1993, Rizvi et al. 1993).

- Development of new analytical techniques to characterize food properties.

  The development and application of new analytical techniques to characterize the properties of emulsions are leading to considerable advances in research, development, and quality control (Dickinson 1995a, b; Gaonkar 1995). These techniques are used in the laboratory to enhance our understanding of the factors which determine the properties of foods and in the factory to monitor the properties of foods during processing in order to ensure that they meet the required quality specifications.

Emulsion science is a multidisciplinary subject that combines chemistry, physics, and engineering (Sherman 1968a; Becher 1957, 1983; Hiemenz 1986; Hunter 1986, 1989, 1993; Evans and Wennerstrom 1994). The aim of the emulsion scientist working in the food industry is to utilize the principles and techniques of emulsion science to enhance the quality of the food supply and the efficiency of food production.

6.6 General Recommendations

- Food researchers and manufacturers should strive to have an improved understanding of the factors that determine the bulk physicochemical and organoleptic properties of emulsions that will enable manufacturers to create low-cost high-quality food products in a more systematic and reliable fashion.

- Producers should adhere and comply with national standard bodies through established analytical procedures, regulations and standards.

- There should be concerted effort to promote the development and application of new analytical techniques to characterize the properties of

emulsions leading to considerable advances in research, development, and quality control.

- A more systematic and rigorous approach is needed in the development of new foods, the improvement of existing foods, and the efficient running of food-processing operations.

6.7 Conclusion

Most food emulsions are much more complex than the simple three-component (oil, water, and emulsifier) systems. The aqueous phase may contain a variety of water-soluble ingredients, including sugars, salts, acids, bases, surfactants, proteins, and carbohydrates. The oil phase usually contains a complex mixture of lipid-soluble components, such as triacylglycerol's, diacylglycerols, monoacylglycerols, free fatty acids, sterols, and vitamins. The interfacial region may contain a mixture of various surface-active components, including proteins, phospholipids, surfactants, alcohols, and solid particles. In addition, these components may form various types of structural entities in the oil, water, or interfacial regions, such as fat crystals, ice crystals, protein aggregates, air bubbles, liquid crystals, and surfactant micelles. A further complicating factor is that foods are subjected to variations in their temperature, pressure, and mechanical agitation during their production, storage, and handling, which can cause significant alterations in their overall properties. It is clear from the above discussion that food emulsions are compositionally, structurally, and dynamically complex materials and that many factors contribute to their overall properties. Much of our knowledge about these complex systems has come from studies of simple model systems. Nevertheless, there is an increasing awareness of the need to elucidate the factors that determine the properties of actual emulsion-based food products. For this reason,

many researchers are now focusing on the complex issues that need to be addressed, such as ingredient interactions, effects of processing conditions, and phase transitions (Dickinson 1992, 1995b; Dickinson and McClements 1995; Dalgleish 1996a; Hunt and Dalgleish 1994, 1995; Demetriades et al. 1997a, b).

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7

Active, Intelligent and Modified Atmosphere Packaging: A Model Technology for the Food Industry

7.1 Introduction

Packaging may be termed active when it performs some role other than providing an inert barrier to external conditions. Hotchkiss (1994) includes the term 'desired' when describing the role and this is important in that it differentiates clearly between unwanted interactions and desired effects. This definition reflects the element of choice in how active packaging performs and the fact that it may play some single intended role and otherwise be similar to other packaging in the remainder of its properties.

These latter two aspects also reflect that active packaging is something that is designed to correct deficiencies which exist in passive packaging. Simple example of this situation is when a plastics package has adequate moisture barrier but an inadequate oxygen barrier. Active packaging solutions could be the inclusion of an oxygen scavenger, or an antimicrobial agent if microbial growth is the quality-limiting variable. Active packaging has developed as a series of responses to unrelated problems in maintenance of the quality and safety of foods. Accordingly arrange of types of active packaging has been developed. Each of these has arrange of descriptive terms. Horticultural produce has for some years now been packaged in 'smart films', and oxygen has been removed from package headspaces by oxygen scavengers, free-oxygen absorbers (FOAs) and deoxidisers. Carbon dioxide can be released by emitters or can be absorbed by getters, and microwaves can be controlled in packages by subsectors or shields (Sacharow and Schiffman, 1992). Regional differences in terminology are also seen. The terms 'freshness preservative' and 'functional' and ‘Avant garde’ are also used to describe active packaging materials (Katsura, 1989; Louis and de Leiris, 1991). There has been a range of trade names for those packages where a generic form has not been coined, with the result that we have Smart Cap (Zapata - Advanced

Oxygen Technologies) foreclosures for beer bottles and Oxyguard for Toyo Seikan Kaisha Ltd. for caps for similar use. Smart packages have been defined by Wagner (1989) as 'doing more than just offer protection. They interact with the product, and in some cases, actually respond to changes'. In this sense, most packaging media are active to some degree. However, there are forms of packaging which are clearly distinct subclasses. The term equilibrium modified atmosphere (EMA) packaging is used to distinguish the situation where the choice of the permeability ratio of oxygen/carbon dioxide determines whether respiring horticultural produce generates a viable gas atmosphere or not (Gill, 1990). Thus where modified atmosphere packaging (MAP) is used with processed foods and involves merely flushing with an initial gas mixture the packaging is not active. EMA packaging is one of the borders between active and passive packaging. If the physical interactions of a package with the food are removed we are left only with the chemical (and increasingly, biochemical) effects. Such restriction is probably unduly strict and in time we should expect to see further subdivision of active packaging to take account of whether “activity” is a property of the packaging material itself or of inserts within the package. We are beginning to see reference to the benefits of active packaging in the popular press with reference to 'packaging that is niftier and cooks your food' and 'Hi-Tech' packaging (Sprout, 1994). The active packaging includes subsectors and reflectors in microwaveable packs as well as horticultural smart films that absorb ethylene. These are described together with temperature sensitive labels that help determine when food is cooked, i.e. ‘doneness indicators’. There are other areas of packaging developing concurrently and there are areas of overlap with active packaging as noted with MAP above. Probably the closest area is Intelligent Packaging, a grouping of technologies defined in the CEST publication by Summers (1992) as 'an integral component or inherent property of a pack, product or pack/product configuration which confers intelligence appropriate to the

function and use of the product itself. This grouping covers the area of product identity, authenticity, traceability, tamper evidence, theft protection, and quality as indicated by time temperature indicators. The latter was originally included by Labuza (1987) in his seminal review of active packaging. Time-temperature indicators also fit the definition of active packaging given above; they play a role in defining the steps that need to be taken to ensure the quality and safety of the packaged food. A somewhat related field of packaging which so far has fallen between the two definitions is that of gas composition indicators. To date they have been used in the form of tablets to indicate when oxygen scavenging sachets have achieved their purpose (Anon, undated). There have been steady efforts made for several years to produce oxygen-indicating printing inks but thus far, like the pellets, these indicators largely change colour at oxygen levels below 0.1%. The description of this field as interactive packaging is also seen. There is some benefit in such a description as it links desired and undesired interactions of foods and their packaging, such as flavour scalping (Hirose *et al*, 1989).

7.2 Description

Active packaging, sometimes referred to as interactive or "smart" packaging, is intended to sense internal or external environmental change and to respond by changing its own properties or attributes and hence the internal package environment. Active packaging has been considered a component of the packaging discipline for several decades or since the first inclusion of desiccants in dry product packages. In their own moisture-permeable sachets, desiccants absorb water vapour from the contained product and from the package headspace, and absorb any water vapour that enters by permeation or transmission through the package structure. As separate entities within packages, active packaging sachets, pouches, patches, coupons, labels, etc., are not often integral to the

package-a semantic differentiation. Desiccant pouches are widely used in the packaging of hardware and metal goods. The best-known and most widely used active packaging technologies for foods today are those engineered to remove oxygen from the interior package environment. Oxygen scavengers reduce oxidative effects in the contained product. Most oxygen scavengers in commercial use today are gas-permeable, flexible sachets containing reduced iron (i.e., iron not in the fully oxidized state) particles inserted into food and other packages from which air is initially removed by vacuum or by flushing with inert gas. During the last two decades of the twentieth century, commercial incorporation of oxygen-removal materials directly into a package structure occurred with varying results. Several applications for beer and juice bottles became commercial in 2000. The goal of active packaging, in conjunction with other food processing and packaging is to enhance preservation of contained food and beverage products. For example, to optimize the effects of oxygen scavenging, oxygen should first be removed from the product during processing and packaging operations. The oxygen must also be thoroughly removed from the package interior and the package materials, and the package structure, including materials and closure, must be barriers to further oxygen entry. In other words, oxygen scavenging complements good oxygen-control practices. In addition, oxygen is certainly not the only vector that can influence the quality of the contained food. For example, moisture gain or loss, light, non-oxidative reactions, microbiological growth, and enzymatic activity may all, individually or collectively, be involved in food-product deterioration. Worldwide development efforts devoted to oxygen removal have indicated that analogous efforts by the same and parallel research teams continue to be applied to oxygen scavenging and are being studied for other active packaging forms. Michael Rooney's book, *Active Food Packaging* (Rooney, 1995), coalesced some of the many known active packaging concepts for foods into single volume. This book did not,

however, probe deeply into some of the more promising technologies that have been proposed and have entered the marketplace, including antimicrobial films, carbon dioxide emitters, aroma emitters, and odour absorbers. Each of these is discussed or referred to in this text. On review of the commercial situation in the United States, and especially the application of oxygen-scavenging compounds into the walls of beer bottles and processed-meat packages, the reasons for the notable paucities in the Rooney book become apparent. Although reviewed and referenced in commercial contexts, definitive scientific documentation and publications are lacking, unsubstantiated, or unclear. Descriptions of plastic bottle wall oxygen scavenging appear only in the patent literature, which does not, of course, detail specifics. The 1999 and 2000 George O. Schroeder conferences, "Oxygen Absorbers: 2000 and Beyond" and "Oxygen Absorbers: 2001 and Beyond" (Anonymous, 1999, 2000) set out to probe the expanding realm of oxygen-removal mechanisms. The presentations offered excellent reviews of historical and contemporary technologies and scientific studies but did not elucidate on the intriguing, but still largely proprietary, industrial world of oxygen scavenging. High-gas-permeability films, including some that increase their oxygen permeability with increasing temperature, are used for packaging fresh-cut produce. Use of these temperature-sensitive package materials is expected to increase because the technology developer has acquired a fresh produce packager who, of course, uses the technology in its package materials. Carbon dioxide and ethylene scavengers for modified-atmosphere (MA) or, more precisely, controlled-atmosphere (CA) food preservation are common enlarge bulk shipments. Carbon dioxide emitters to suppress microbiological growth have experienced limited success in modified-atmosphere packaging (MAP). Ethylene scavengers are among the more successful commercial active packaging technologies in the fresh-fruit bulk-shipment category. Odours generated or captured within closed food packages are undesirable, and their obviation has

been a research topic for years. Door removers incorporated into packaging are increasingly important in some classes of food packaging. Antioxidants and oxygen interceptors incorporated into package materials, such as tocopherols (vitamin E), have emerged in recent years and are increasingly employed to combat odours generated in plastic processing. Tocopherols, which are non-volatile, have not replaced volatile mutilated hydroxyanisole/butylated hydroxytoluene (BHA/BHT) which migrate into foods in product antioxidant applications, but they appear to be new antioxidants of choice for mitigating the effects of oxygen. Entities such as oxygen scavengers/interceptors react with oxygen to form new compounds. Oxygen absorbers may remove oxygen by any means, including physical. Antioxidants react with free radicals and peroxides to retard or block the actual oxidation reactions. Sequestering agents tie up inorganic catalysts that might otherwise accelerate adverse oxidative reactions. Members of the food technology and packaging communities have long regarded package materials as an ideal reservoir and delivery vehicle for antimicrobial compounds. For many years, sorbet acid has been incorporated sparingly on the interior of package structures as an antimitotic in a limited number of dry food packages. The obvious benefits of sorbet acid as a midland yeast inhibitor have been one foundation by which numerous other antimicrobial agents have found their way into food package materials. Unfortunately, most antimicrobial agents also exhibit toxicity when they enter the food from the package and would be consumed as part of the food. Thus, actual commercialization has been proceeding slowly, except in Japan where several compounds have been reported to function effectively as antimicrobials in commercial packages. As with oxygen scavengers, the major technological and commercial successes for antimicrobials have been achieved by Japanese organizations for packaging Japanese products in Japan. Nevertheless, the concept of integrating microbistatic and microbicidal materials and plastic packaging has been very attractive. Numerous attempts have been and

are being made to translate favourable laboratory results into safe and effective commercial food packaging. The growing list of successes in active packaging beyond oxygen scavenging has been noted by the food-packaging community.

7.3 General Analysis

Active packaging has developed as a series of topics which are related only because they involve the package influencing the environment of the food. The literature in this field consists very largely of patent applications, technical leaflets and reviews. The latter have often been presented at conferences where specialised audiences have been able to take up the ideas presented. Reports of academic scientific investigation have been limited largely to occasional assessments of the appropriateness of some of these technologies. The literature in this field is therefore discussed in terms of the reviews. Sneller (1986) reported on the impact of smart films on controlled atmosphere packaging although the first broadly based reviews were presented in Iceland and Australia in 1987 (Labuza, 1987; Rooney, 1987). The first use of the term active packaging was proposed at that time by Labuza, who defined active packaging as a range of technologies, some of which now represent the borderlines between active, 'intelligent', and modified-atmosphere packaging (Labuza and Breene, 1989). The essential features of these 'freshness enhancers' have been summarised in a short review by Sacharow (1988). Katsura (1989) reviewed the range of functional packaging materials which had been commercialised with particular reference to Japan. He demonstrated the attention that had been paid to freshness preservative packaging. Wagner (1989) summarised the range of smart packages and emphasised the role of microwaveable-food packaging. Rooney (1989a, b; 1990) concentrated on chemical effects, particularly oxygen scavenging. The role of oxygen scavengers in maintaining the benefits of MAP for processed foods was

reviewed by Smith *et al.* (1990) following their own research into suppression of microbial growth The International Conference on Modified Atmosphere Packaging at Stratford-upon-Avon (UK) in 1990 organised by the Campden Food and Drink Research Association included several reviews relating to active packaging. Louis described several innovative active packages which generated modified atmospheres. Abe gave the first comprehensive quantitative assessment of the impact of active packaging. He estimated the market size for each of the broad classes of such packaging systems. His review reveals that around 6.7 billion oxygen-scavenging sachets and 70 million ethanol-generating sachets were manufactured in Japan in both 1989 and1990. The estimated market for films containing mineral powders was only1000 tonnes in 1989 with 40% of consumption as home use. The review by Robertson (1991) emphasised the application of active packaging to processed foods. The emphasis was placed on crown seals for bottled beer, oxygen-scavenging plastics films and microwave susceptors. The use of the term active packaging rather than smart films was noted by that reviewer and by Sacharow (1991) who also noted the use of sachets of potassium permanganate in silica gel for ethylene removal in produce packs. By this time the claimed benefits of freshness preservation technologies for horticultural products were being examined critically, especially in Japan. Ishitani (1993a, b) surveyed the number of patent applications for this purpose from 1984 to 1989. Over the first two years the annual rate was around 35 applications. This increased to a peak rate of 220 per annum in the second half of 1987 before dropping to around 60 per annum in 1989. It was noted that initial developments were directed at the needs for low temperature maintenance and moisture control. The boom in 1987 was the consequence of the attention being paid to gas composition control and ethylene removal. By 1989 gas composition was the main object of developments but moisture control and coating methods were also important. Ishitani (1993a) observed two factors that led to much rethinking.

These were the lack of data on the requirements of produce and doubts about the capacity of powder-filled plastics to remove enough ethylene. More recent developments have been focused on ethylene removal at high humidities and on matching gas composition and temperature to the requirements of enzyme systems of plants. Several recent books on MAP have included discussion of the gas-packaging requirements for horticultural produce as well those for some processed foods (Ooraikul, 1993; Parry, 1993). The environmental aspects of active packaging have not been considered to any great extent in reviews to date. Rooney (1991) addressed some issues drawing attention to the need to consider the nature of the packaging which can be replaced by these new technologies. The current state of development and commercial application of active packaging has been reviewed in three papers at the symposium Interaction: Foods - Food Packaging Material held in Sweden in June 1994. Miltz *et al* (1994) reviewed the field in general, Ishitani (1994) concentrated on Japanese developments, especially antimicrobial films, Day (1994) concentrated on fresh produce and Guilbert and Gontard (1994) focused on edible and biodegradable packaging. Several posters described original research and that of Paik described photo processing of a film surface to generate antimicrobial properties. Perdue (1993) has briefly reviewed antimicrobial packaging from the viewpoint of the Cryovac Division of W. R. Grace Company and presents a somewhat pessimistic picture.

7.4 Actualisation

Reviewing the Current Status of the Technology

The range of active packaging is so broad that, with further development, many of these technologies will be able to aid in the preservation and quality retention of commercially processed and packaged food (Tables 1, 2, and 3). The challenge is that the numbers of different active packaging proposals and

commercialisations from around the world are very large. Further, many of these claims are often difficult to comprehend. Perhaps in the future, some of these active packaging concepts may be further developed into systems that are or would be applicable in the United States and Europe.

Table 7.1 *Types of Active Packaging Systems with Mode of Action and Representative Manufacturers (Excluding Oxygen Scavengers). Adapted from Floros et al., 1997.*

System / Action	Substance	Organisational Source
Ethylene absorbing	• Activated carbon / potassium permanganate	• Kuraray / Nippon (Japan) • Greener (Japan)
Ethanol emitting	• Micro – encapsulated ethanol	• Freund (Japan)
Moisture absorbing	• Polyvinyl alcohol encapsulation • Silica gel • Clay based	• Garace Chemical (Davison) • Capitol Speciality Plastics • Multisorb Technologies • Sud Chemie Performance Packaging
Anti- microbial releasing	• Sorbates • Benzoates • Propionates • Silver salts • Sulphur and mercurial compounds • Bacteriocins • Sub- micrometer cell wall penetrants • Zeolites • Chlorine dioxide	• Mitsubishi Gas Chemical (Japan) • Microban Products • Various from Japan • Mitsubishi Gas Chemical (Japan) • Shinagawa Fuel (Japan) • Techyon Energy (Japan) • Bernard Technologies • Englehard Corp
Antioxidant releasing	• BHA /BHT • TBHQ • Vitamin C or E	• Roche
Flavour / Odour absorbing	• Activated Carbon • Sodium Bicarbonate	• Arm & Hammer • Carbot
Chemical Stabilisers	• Tocopherol or Vitamin E	• Roche

Table 7.2 *Selected Patents on Various Active Packaging Technologies: CO_2 Absorbers/Emitters, Ethylene Absorbers, and Ethanol Generators (Excluding Oxygen Scavengers).*

Company	Function and Substance(s)	Patent Year	Patent Number
Freund Industrial Co. Ltd (Japan)	Ethanol – vapour generator: several different substances mentioned	1989	US 4820442
J. Velasco Perez	Ethylene Absorber / CO_2 generator: sepiolite and $KMnO_4$	1990	US 4906309
K. K. Nasa (Japan)	Ethylene Absorber far – IR radiating ceramic granules	1990	US 4927651
Kyoei Co. Ltd (Japan)	Ethylene Absorber: zeolite (for apples)	1988	USW 4759935
Mitsubishi Gas Chemical Co. (Japan)	Ethanol emitter: for example, activated carbon, SiO_2, clay, celite, zeolite, paper cotton1 acetaldehyde remover ($1O_2$ Absorber)	1992	EP 0505726A1
Mitsubishi Gas Chemical Co. (Japan)	CO_2 absorber / O_2 scavenger;	1988	US 4762722
Mitsubishi Gas Chemical Co. (Japan)	CO2 absorber / O2 scavenger; $Ca(OH)_2$	1982	US 4366179
Toppan Printing Co. Ltd (Japan)	CO2 absorber / O2 scavenger: Mn- Salt 1 Metal 1Alkali 1 Sulphite	1983	US 4384972
Toppan Printing Co. Ltd (Japan)	Ethylene absorber: zeolite 1 bentonite 1 active carbon	1982	US 4337276

Table 7.3 *Current and Potential Future Applications of Active Packaging Technologies.*

	Food Groups					
Applications	**Dry**	**Minimally Processed**	**Meat and Dairy**	**Frozen Foods**	**Bakery**	**Beverages**
Ethylene Emitter	All dry foods	Fruit / Vegetables				
Ethylene scavenger	All dry foods	Fruit / Vegetables				
Moisture absorber	All dry foods					
Moisture regulator	All dry foods	Fruit / Vegetables; meats etc.				
Ethanol emitter	Semi dry fish; meat	Prepared foods	Cheese		Sweet baked goods; bread	
Antimicrobial releasing film		Fruit	Cheese; meat		Bread; Cakes	
Antimicrobial none releasing film	Breakfast cereal				Hard baked goods	Bag in box wine
Flavour containing and emitting film	Cereals	Prepared foods		Ice cream		Orange juice
Colour containing film			Surimi			
Anti stick film			Cheese slices	Frosting; candy		
Enzyme inhibitor		Fruit / Vegetables				
CO_2 regulator		Fruit / Vegetables				
Light control	Snacks; lipids	Fruit / Vegetables; meats				

7.5 Discussion

Active packaging is still developing as a collection of niche markets so it is not surprising that a diverse range of packages active in the physical and chemical sense are either proposed or commercially available. Early among these was the use of the reaction of lime with water to generate heat for self-heating cans of sake (Katsura, 1989). The Verifrais process for meat packaging uses the reaction of organic acid with bicarbonate to produce carbon dioxide in response to meat drip in foam trays. The carbon dioxide released helps to suppress microbial growth. Some properties of foods which can be addressed by active packaging are summarised in Figure 7.1. These properties are grouped depending upon whether they are designed to sustain living foods, suppress insect or microbial life in any foods, prevent oxidative attack on food constituents, retain flavour, or facilitate serving of the food for consumption. Active packaging can be seen in one sense as a means of maintaining the optimum conditions to which a food was exposed at the immediately preceding step in its handling or processing. Passive packaging has been used in an effort to minimise the deleterious effects of a limited number of external variables such as oxygen, water, light, dust microorganisms, rodents and to some extent, heat. Hence, active packaging has the potential to continue some aspects of the processing operation or to maintain chosen variables at particular levels. This aspect of active packaging is a unifying theme and crosses the border between foods such as plant produce, and processed foods, including those thermally processed. A second aspect of active packaging is that it can be involved in the preparation of the food for consumption. This includes aspects of temperature modification either for organoleptic or food safety purposes. These properties therefore include heating, cooling, and foaming.

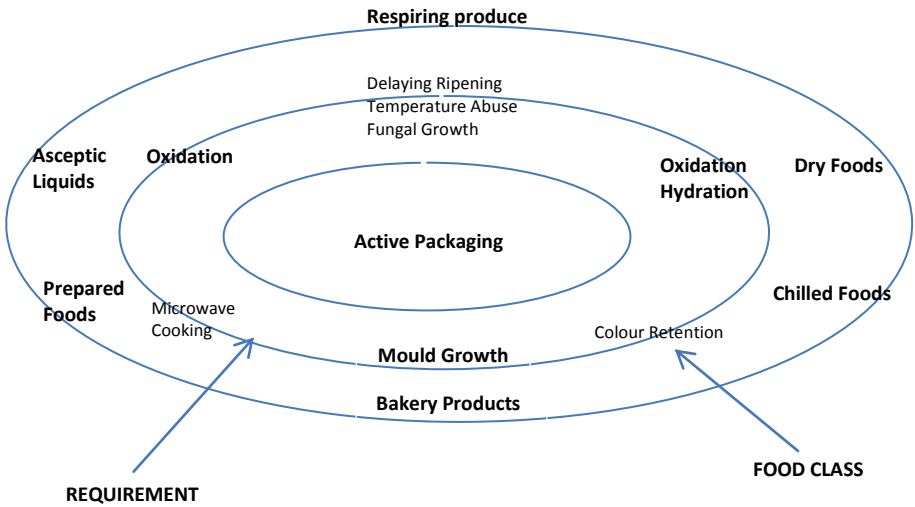

Figure 7.1 *Properties of foods amenable to active packaging.*

Non-processed, respiring food such as agricultural and horticultural produce, fish, crustaceans, and other seafood can be stored and/or shipped over long distances provided the respiration requirements are satisfied under controlled temperature conditions. Thus if the packaging can regulate the supply of oxygen to the animal or produce such that a minimum respiration rate can be sustained, an enhanced period of prime-quality life can often be achieved. In plant products the optimum oxygen concentration of the environment varies with the species, and levels down to 1% may be possible without inducing anaerobic respiration (Labuza and Breene, 1989). The generation of elevated levels of carbon dioxide to suppress ethylene synthesis and to suppress microbial action can be achieved by selection of plastics films of appropriate permeability's. However, achievement of the optimum balance of oxygen and carbon dioxide concentrations by use of plastics films alone is frequently impossible, particularly as allowance must be made for temperature abuse. It is possible to predict the potential packaging requirements for horticultural produce by modelling the properties of the food and the packaging film. There have been

several reports published on approaches to modelling such systems, and they have been compared by Solomos (1994), who has tabulated the characteristics provided for in each of the models. This form of packaging is commonly termed modified atmosphere packaging (MAP) or more appropriately equilibrium modified atmosphere (EMA) packaging. MA packaging involving selection of polymer films is, as mentioned previously, the borderline between active and passive packaging. Several approaches to overcoming the limitations of these films have been reported. One which is still in its infancy is the use of liquid crystal polymers which undergo a phase change at a characteristic temperature. The permeability of the polymer to oxygen sharply increases as this temperature is exceeded, thus providing the oxygen necessary to prevent packaged horticultural produce from switching from aerobic to anaerobic respiration. The present state of the art is not sufficiently advanced to cover EMA films which match produce over a wide temperature range, but research has opened up this possibility. An alternative involves pores in portions of a package which open when the temperature exceeds a precisely set value. This has been achieved by filling pores in a patch on a package with a wax which melts appropriately (Cameron and Patterson, 1992). This wax, when liquid, is drawn away by a wick such as a micro porous film to leave the pore open to gas exchange. This type of high-temperature emergency valve is applicable to packages over a wide range of sizes. Micro porous patches are already used commercially on retail trays of some fruit. The use of pores in packaging materials to actually balance the atmosphere in packages of respiring fruit has been the subject of some research and a large amount of marketing. Several forms of crushed rock, coral, and synthetic zeolites have been incorporated into extruded film but there has been very little disinterested scientific evaluation done. Such films extend the range of gas permeability values of the commodity films in current use. Some results for P-Plus, a porous film currently manufactured by Sidlaw Packaging,

Bristol (UK), have been presented (Gill, 1990). Predictive research and some experimental verification of the effects of single pores in produce packages have been reported (Mannapperuma and Singh, 1994). The effects of changes in temperature on gas composition need to be evaluated. Extension of the post-harvest life of fruits and vegetables requires more than EMA packaging. The water relations between the horticultural foodstuff and its atmosphere need to be balanced both to prevent dehydration and to avoid condensation induced by temperature abuse. Since the RH of such packages exceeds 95%, a temperature drop from 12 ℃ to 110 ℃ at the pack surface can cause condensation. Micro porous pads containing inorganic salts have been shown to buffer the water vapour pressure (Shirazi and Cameron, 1992). Some of these are used commercially in the USA and Japan but others are close to commercial development. There have been some patents directed towards use of combination effects in active packaging for horticultural produce. Thus there have been patents of combination CO_2 emitter/water vapour absorbers and otherwise similar compositions but including an oxygen scavenger as well. This would bring the advantages of reducing the time the packaged horticultural product is subjected to high oxygen levels and inhibiting the onset of ripening, particularly with climacteric fruit. The rapid oxygen scavenger films of Rooney (1982) and Maloba (1994) could be suitable for this purpose if they met with regulatory requirements. Other approaches to enhancing the storage life of horticultural produce have been directed towards removing ethylene produced by ripening fruits and vegetables. Since ethylene is both produced by ripening fruits and triggers their ripening it is essential to prevent those fruits which are further along the ripening process from triggering ripening of others in the same enclosed space. Injured fruits are a particular problem in this regard and this emphasises the need for strict quality control in EMA packaging. The isolation of packages containing fruit rapidly generating ethylene may be the appropriate

target of technologies for ethylene removal. The challenge appears to be to provide independently verifiable chemical processes which function satisfactorily to remove ethylene at physiologically significant concentrations in packages under conditions of high humidity and possibly in the presence of condensation. Since the quantities of ethylene are tiny, the cost should not be the major obstacle to commercial development. Produce packages normally have large headspaces so both sachet and packaging film scavengers should prove acceptable. Several other 'freshness enhancing' properties have been claimed for some commercial films but the processes occurring therein have not been clarified. Besides horticultural produce and living seafood which are meant to be kept alive during transportation, there is the very important field of chilled meats which retain muscular respiration for some hours or days post slaughter. While beef, for instance, is capable of oxygen scavenging by muscle respiration for a few days at meat works chiller temperatures of -1 ℃ to 1 ℃, it is no longer capable of doing so for the remainder of the desired storage period, usually 4-8 weeks. Lactic acid bacteria lower the pH and suppress the growth of *Bronchothrix thermosphacta* and *Pseudomonas* spp. and other species. There is scope for oxygen scavenging films in bag construction to prevent oxygen permeation and for lactic-acid-releasing films to enhance this effect in some cases. The removal of residual oxygen from MAP meat packs by oxygen scavengers would increase security and decrease the need for slow, sophisticated packaging processes in this case. The carbon dioxide levels are normally very high (> 99%), as in the Captech process (Gill, 1989), so oxygen scavengers would need to operate wet in this environment. An additional definition of active packaging specific to horticultural produce distinguishes between passive and active modified atmosphere packaging (Zagory and Kader, 1988). The passive form which we are considering as EMA involves choice of the packaging material for its ratio of permeability's to O2 and CO_2 as well as

for their absolute values. Active MAP has been defined as gas atmosphere replacement by flushing or evacuation-back flushing, although the option of adding other active agents has also been considered (Kader, Zagory and Kerbel, 1989). Modified atmosphere packaging of non-living foods is now a mature area of research and has resulted in filling significant niche markets, particularly in the bakery, cheese and fresh pasta areas. Fresh pasta, which has been a recent success internationally, is dependent on MAP (Castelvetri, 1990). The growth of moulds, while suppressed by elevated carbon dioxide concentrations, is not uniformly affected across the range of species. Low levels of oxygen can in some cases support some species of mould, particularly as carbon dioxide is lost by permeation of packaging films. There is a need to remove most residual oxygen which may reach more than1% when flushing is used without prior evacuation. Oxygen concentrations below 0.1% are desirable especially when cut surfaces are exposed, as in pizza-type cheeses and some MAP meats. Besides mould growth, chemical effects such as oxidative attack on colours in preserved meats (Andersen and Rasmussen, 1992), nutrient degradation such as vitamin C loss which can result in browning products (Waletzko and Labuza, 1976), and rancidity generation in fats and oils (Nakamura and Hoshino, 1983) can be prevented or minimised by use of oxygen scavengers. One benefit to researchers of oxygen absorbers is allowing ultimate effects of near-zero oxygen content atmospheres to be evaluated so that prediction of shelf-lives under other less perfect conditions can be more firmly based. Although initial development, and current commercial practice, is based on sachets of scavengers inserted into packs, much recent research and development has been directed towards scavenging polymers which can address problems with ox disable liquids such as beer, wines, fruit juices and other beverages. Polymers, because of their ease of melt formation, can take the scavenging capacity to

localised areas such as closures and to areas of close contact of product and packages found with meats, cheeses and wet foods generally.

The ability of polymers to act where there is close contact opens the way to provide a variety of food additives via a diffusive mechanism. This includes antimicrobial action (Halek and Garg, 1988) or antioxidant (Han *etal,* 1987) effects. To date, the use of such packaging has been restricted to controlled release of antioxidant into cereal products (Miltz *et al,* 1989). The benefit of slow release of antimicrobial agents and antioxidants is the potential for maintenance of the requisite high concentration at wet food surfaces. This applies especially to high-water-content foods in which diffusion from the surface into the bulk can deplete surface concentrations (Torres *et al,* 1985). This effect has been noted by Smith *et al* (1990) who investigated the effectiveness of ethanol-emitting sachets on the growth of *Saccharomyces cerevisiae* on apple turnovers. For active packaging to fulfil useful role in this field it will be necessary for it to provide controllable, slow release matched to the needs of the food. Water-triggered sachets of silica containing ethanol are very much a first generation approach to this form of packaging. Besides antimicrobials and antioxidants there is a wide variety of other agents that can be added to foods or which can act on them. Thus flavours can be added to offset degradation on storage, enzymes can remove oxygen or other undesirable food components, and insecticides can repel insects or kill them with permitted fumigants. There is a potential ethical dilemma which may arise from the application of such approaches to food packaging. There is also the potential for foods to be self-promoting via the aroma of their packaging. Thus desirable flavour might be generated by an outer layer of a package to attract customers rather than being released from an inner layer to offset scalping or processing losses. In an extreme case, supermarkets might become a confusing garden of unbalanced aromas competing for the organoleptic senses of the customer in

much the same way as package print attracts the customer visually. At this point the packaging ceases to be active in the sense of the present definition and can be described as intelligent in the definition of the CEST report (summers, 1992). Introduction of many of these forms of active or related packaging technologies will necessitate serious consideration of explanatory labelling. In some cases this may require regulation, as with oxygen-scavenging sachets in Japan, the USA and Australia where the "Do not eat label" is required.

7.6 General Recommendations

- If the potential of active packaging technologies is to be realised there will need to be a recognition that changes in packaging can open up new methods of presenting foods. The use of oxygen-scavenging plastics as chemical barriers to permeation should allow retortable plastics to provide product shelf-lives closer to those found using metal cans. Horticultural produce, such as flowers, should be transportable internationally with reduced losses.

- Any need for food-contact approval should be established before any form of active packaging is used.

- There may be a need for labelling in cases where active packaging can give rise to consumer confusion. Food-contact approval will often be required because active packaging may affect foods in two ways. A substance may migrate into the food or maybe removed from it. Migrants may be intended or unintended. The intended migrants include food additives which would require regulatory approval in terms of their identity and concentration. Unintended additives include active substances which achieve their purpose inside the packaging material and do not need

to enter the food. Food additive regulations require identification and quantitation of any such migration.

- The effect of active packaging materials on recycling may need to be determined on a case-by-case basis. Active packaging is often used currently to allow foods to be packaged with simpler materials than would otherwise be possible. The environmental impact of the food-package combination should be considered.

- Some form of external labelling may be required when various forms of indicator come into use. Such indicators would show gas composition, thermal history, or 'done-ness' in the case of microwaved foods. Some active packages may be expected to look different from their passive counterparts. It may be advisable to use labelling to explain this even in the absence of regulation.

7.7 Conclusion

The future of any innovation in packaging depends upon the extent to which it can satisfy the requirements of the product packaged. Commercial development therefore will be driven by needs as perceived in the food industry or in other industries with related problems. It is clear from patent searches that inventors of active packaging frequently see potential applications for their concepts in several industries. This is evident in claims for use of oxygen scavengers in the packaging of clothes, pharmaceuticals, fine chemicals such as amines, printing inks, electronic components, metals and many more areas. Some iron-based oxygen scavengers have been suggested for use in hand-warmers for skiers. If the potential of active packaging technologies is to be realised there will need to be a recognition that changes in packaging can open

up new methods of presenting foods. The use of oxygen-scavenging plastics as chemical barriers to permeation should allow retortable plastics to provide product shelf-lives closer to those found using metal cans. Horticultural produce, such as flowers, should be transportable internationally with reduced losses. Acceptance of active packaging solutions to food industry problems will continue to depend upon evidence of effectiveness demonstrated by independent investigators. The lack of hard evidence supporting many claimed benefits of some early horticultural produce packages has inhibited commercial usage. If the majority of patent claims already made prove useful and economically viable, active packaging has a bright future. Fermentation of traditional foods, as a hurdle technology, is profitable in terms of food quality, preservation, and decontamination of toxins, often found in food. Together with food safety, the nutritional and flavour profile of the products need to meet the expectations of modern consumers. Education of communities about benefits of consuming fermented foods needs to be part of health education. This technology needs to be further developed to enhance safety and ease of application in a rural poor-resource setting. Development of convenient starter cultures and processing methods will ensure that many people in Africa will reap the benefits of indulging in fermented foods and beverages both during cultural ceremonies and during their normal daily activities.

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8

Investigating the Effect of Cabbage Variety on the Characteristics of Sauerkraut Produced Using Local Cabbage Varieties

Abstract

Sauerkraut is encumbered with a lot of nutrients and phytochemicals and yet it is relatively cheap to make (www.tonytantillo.com/health). Sub Saharan Africa still faces the challenge of prevalence of undernourishment and if fully exploited sauerkraut can bring considerable changes.

The research undertaken sought to establish the influence of cabbage variety on sauerkraut quality. Sauerkraut was produced using cabbage varieties grown under Zimbabwean climatic and growing conditions. The prominent aspects of sauerkraut ranging from lactic acid development and pH reduction were evaluated. Sensory evaluation techniques including the paired comparison test and quantitative descriptive analysis were used to determine and compare the organoleptic properties of the sauerkraut that was produced. The nine point hedonic scale was used to assess the consumer preference.

It was found out that different tests produced different results. The quantitative descriptive analysis showed that cabbage variety has no significant influence on the quality of sauerkraut produced from cabbages grown under the Zimbabwean soils. The paired comparison test and the hedonic scale showed that differences and consumer preferences can be perceived respectively. Regardless of the perceived characteristics from the array of analysis carried out it was concluded that all the vast nutritional benefits in cabbage can be obtained by increased consumption through value addition to cabbage in the sauerkraut produced from any variety grown in the country. The differences in the results from different methods employed could only be due to the differences in the purpose and characteristics of the method which may include its sensitivity and specificity.

8.1 Introduction

Sauerkraut is finely shredded cabbage that has been fermented by various lactic acid bacteria, including *Leuconostoc*, *Lactobacillus*, and *Pediococcus*. Fermentation of sugars in the cabbage will lead to a sour taste and a longer shelf life. Lactic acid is the major contributing factor to the taste and shelf life of sauerkraut (Axelsson, 1998).

Lactic acid fermentation of cabbage and other vegetables is a common way of preserving fresh vegetables in the western world, China, and Korea (where *kimchi* is a staple in the diet). It is a simple way of preserving food: the raw vegetable is sliced or shredded, and approximately 2 present salts is added. The salt extracts liquid from the vegetable, serving as a substrate for the growth of lactic acid bacteria (Wood and Hodge, 1985) (Matususaki et al, 1997) (Adams and Nicolaides, 1997), (Gourama and Bullerman, 1995), (Nout, 1995). Anaerobic conditions should be maintained, insofar as possible, to prevent the growth of microorganisms that might cause spoilage. The sequence of organisms that develop in typical sauerkraut fermentation is as follows: *Leuconostoc mesenteroides* initiates the growth in the shredded cabbage over a wide range of temperatures and salt concentrations. It produces carbon dioxide and lactic and acetic acids, which quickly lower the pH, thereby inhibiting development of undesirable microorganisms that might destroy crispness. The carbon dioxide produced replaces the air and facilitates the anaerobiosis required for the fermentation. The fermentation is completed in sequence by *Lactobacillus brevis* and *Lb. plantarum. Lb. plantarum* is responsible for the high acidity. If the fermentation temperature or salt concentration is high, *Pecicoccus cerevisiae* develops and contributes to acid production. As would be expected, the rate of completion of the fermentation depends on the temperature and salt concentration. At 7.5 ℃ fermentation is very slow: under these

circumstances, *L. mesenteroides* grows slowly, attaining an acidity of 0.4 percent in about 10 days and an acidity of 0.8 to 0.9 percent in a month. Lactobacilli and pediococci cannot grow well at this temperature, and the fermentation may not be completed for 6 months. At 18 ℃ a total acidity (as lactic acid) of 1.7 to 2.3 percent will be reached, with an acetic to lactic acid ratio of 1:4, in about 20 days. At 32 ℃ a similar activity will be reached in 8 to 10 days, with most of the acid being lactic acid produced by the homofermentative bacteria *Lb. plantarum* and *P. cerevesiae*. Increasing the salt concentration to 3.5 percent results in 90 percent inhibition of growth and acid production for both *L. mesenteroides* and *Lb. brevis*. The ratio of non-volatile to volatile acid produced has a marked effect on flavour, *Lb. brevis* producing a harsh, vinegar-like flavour and *L. mesenteroides* a mild, pleasantly aromatic flavour. The homofermenters *Lb. plantarum* and *P. cerevesiae* yield unacceptable products (Vaughn, 1985).

Fermentation is a relatively efficient, low energy preservation process which increases the shelf life and decreases the need for refrigeration or other form of food preservation technology. It is therefore a highly appropriate technique for use in developing countries and remote areas where access to sophisticated equipment is limited. Fermented foods are popular throughout the world and in some regions make a significant contribution to the diet of millions of individuals (Saloheimo, 2005).

Fermenting fruits and vegetables can bring many benefits to people in developing countries. Fermented foods play an important role in providing food security, enhancing livelihoods, and improving the nutrition and social well-being of millions of people around the world, particularly the marginalized and vulnerable (Anon, 1995), (Anon, 1996).

Consumption of Sauerkraut in Southern Africa and in particular Zimbabwe is low though various varieties of cabbage are grown and consumed. A hypothesis is cited that notes that different cabbage varieties produce different qualities of Sauerkraut and lack of knowledge is the major cause of the apparent marked under consumption of Sauerkraut in Zimbabwe and the Southern African region (Collins et al, 2000). There is an apparent need to develop understanding of the processes that take place in the production of Sauerkraut and their adaptation for commercialisation.

8.2 Cabbage Varieties Mainly Grown in Zimbabwe

According to agronomists in the Zimbabwe Ministry of Agriculture, Cabbage varieties (cultivars) are generally classified according to season of maturity, leaf surface (smooth, savaged, or wrinkled), head shape (flattened, round, or pointed), and colour (green or red). Round, smooth-leaved, green heads are commonest. Varieties differ in their resistance to disease and in the tendency for heads to crack or split in the field. The commonly grown cabbage type in Zimbabwe is the green cabbage and the varieties grown are as follows:

8.2.1 Copenhagen Market

The cabbage heads are of fine quality with a solid construction that excels when used fresh or cooked. The interiors of the head are white and are ideal for making sauerkraut. The leaves are tightly wrapped, so this variety will stand for a long time without splitting and stores extremely well.

8.2.2 Drum Head

Late maturing variety, the heads are large, flat, and somewhat loose and drum shaped. Each head weighs 3-5 kg. Outer leaves are light green with prominent mid-rib.

8.2.3 Green Coronet F1 Hybrid

Green Coronet has a very firm flat round shaped head with an average head size (without frame leaves) of between 3-4 kg with an excellent flavor. An adaptable variety suited for the loose head and bagging market. Green Coronet has an excellent dark green colour, has a good holding ability and excellent bolting tolerance and is well adapted for different climates with exceptional cold tolerance.

8.2.4 Star 3311 F1 Hybrid

A large headed hybrid cabbage variety for the fresh market. A medium to large frame that has an upright leaf habit. STAR 3311 has very firm flat-round shaped heads with an average head size (without frame leaves) of between 2, 5 - 3, 0 kg with an excellent flavour. The colour of STAR 3311 is a typical grey-green which is highly sought after by market agents and hawkers.

8.2.5 Star 3316 F1 Hybrid

STAR 3316 has very firm round to semi-globe shaped heads with an average head size (without frame leaves) of between 3 and 5 kg with an excellent flavour.

8.2.6 Marcanta F1 Hybrid

Marcanta has a medium to large upright frame. Marcanta produces firm round uniform heads with excellent internal quality.

8.2.7 Klabish F1 Hybrid

A small to medium sized hybrid savoy cabbage variety for the fresh and prepacked market. Klabish produces firm round heads. Heads are uniform and internal quality of the head is excellent.

8.2.8 Golden Cultivar

This variety resembles Copenhagen Market in type; but the heads though not quite so large are more uniformly round than Copenhagen. There is an entire absence of coarse veins and leaves and it has the quality of hardening the head before it has attained its mature size.

8.2.9 Rotan F1 Hybrid

A small headed hybrid baby cabbage variety for the fresh and pre-pack market. Heads are uniform and internal quality of the head is excellent.

8.2.10 Adelita F1 Hybrid

Adelita produces firm semi-globe shaped heads. Adelita has good uniformity, allows for a uniform cut in the field.

8.2.11 Cape Spitz

Cape Spitz forms a pointed head of exceptional good quality and is certainly one of the most palatable cabbage cultivars. The leaves are dark green, fairly strongly crisped, so that the head sometimes resembles that of Savoy cabbage.

8.3 Spoilage and Defects in the Sauerkraut Process.

The majority of spoilage in sauerkraut is due to aerobic soil micro-organisms which break down the protein and produce undesirable flavour and texture changes. The growth of these aerobes can easily be inhibited by a normal fermentation. Soft kraut can result from many conditions such as large amounts of air, poor salting procedure, and varying temperatures. Whenever the normal sequence of bacterial growth is altered or disturbed, it usually results in a soft product. It is the lactobacilli, which seem to have a greater ability than the cocci to break down cabbage tissues, which are responsible for the softening. High temperatures and a reduced salt content favour the growth of lactobacilli, which are sensitive to higher concentrations of salt. The usual concentration of salt used in sauerkraut production slightly inhibits the lactobacilli, but has no effect on the cocci. If the salt content is too low initially, the lactobacilli grow too rapidly at the beginning and upset the normal sequence of fermentation (Farnworth, 2003). Another problem encountered is the production of dark coloured sauerkraut. This is caused by spoilage organisms during the fermentation process. Several conditions favour the growth of spoilage organisms. For example, an uneven distribution of salt tends to inhibit the desirable organisms while at the same time allowing the undesirable salt tolerant organisms to flourish. An insufficient level of juice to cover the kraut during the fermentation allows undesirable aerobic bacteria and yeasts to grow

on the surface of the kraut, causing off flavours and discoloration. If the fermentation temperature is too high, this also encourages the growth of undesirable micro flora, which results in a darkened colour. Pink kraut is a spoilage problem. It is caused by a group of yeasts which produce an intense red pigment in the juice and on the surface of the cabbage. It is caused by an uneven distribution of or an excessive concentration of salt, both of which allow the yeast to multiply. If conditions are optimal for normal fermentation, these spoilage yeasts are suppressed (Hammes and Tichaczek, 1994).

8.4 Methodology

8.4.1 Sauerkraut Production

Sauerkraut was produced using local varieties of cabbage as follows:

- Cabbage heads were trimmed, outer leaves removed including all bruised and soiled tissue.
- Cabbage heads were trimmed and washed thoroughly with tap water.
- Cabbage heads were cut in half and the hard, central core removed.
- Cabbage was shred using a knife.
- Shredded cabbage was weighed and sprinkled with salt such that a final concentration of 3% was achieved.
- Salt and cabbage were completely and thoroughly mixed.
- Shredded cabbage was packed into the beakers, filling to approximately 75-80% of total volume. The mixture was compressed moderately while

avoiding crushing or bruising the cabbage tissue until the cabbage was covered by juice.

- Beaker was placed in the anaerobic jars.
- The jars were incubated at 21 ℃ for 17 days.

8.4.2 The Experiments

The Table 8.1 below shows the experiments carried out:

Experiment	Cabbage variety
A(792)	Copenhagen market
B(620)	Green coronet
C(985)	Star 3315
D(781)	Golden cultivar

8.4.3 Lactic Acid and pH Determination

Using pH meter, the pH of the undiluted juice sample was determined. 10 ml undiluted juice sample was added to the Erlenmeyer flask followed by 10 ml of distilled water. The contents of the flask were boiled for 1 min to drive off the dissolved carbon dioxide. It was then cooled and 5 drops of phenolphthalein was added. Titration was done with 0.1 M NaOH until a light pink color persisted. The following formula was used to calculate the percent lactic acid (the predominant nonvolatile acid expected in the sauerkraut fermentation):

$$\% \text{ lactic acid} = [(\text{ml of } 0.1 \text{ M NaOH}) \text{ X } (0.9)] / [\text{sample volume}]$$

8.4.4 Sensory Evaluation Tests

Statistical tools were employed to determine if there was a significance difference in the quality of the sauerkraut produced using the different cabbage

varieties. The quantitative descriptive analysis and paired comparison tests were performed.

8.4.5 Quantitative Descriptive Analysis Tests

The analysis of variance technique in one way analysis of variance (ANOVA) was used and took a set of grouped data and determined whether the mean of a variable differed significantly between groups. The data set had measurements of saltiness, sourness, bitterness, consistency, texture, appearance, and aroma on four cabbage varieties. The analysis sought to evaluate if the sensory attributes changed with samples.

Graphical analysis: MATLAB (mathematical software) was used to analyse the data.

A grouped plot matrix of these variables was created using the gplotmatrix function

>>x= [s1 s2 s3 s4 s5 s6 s7];

>>gplotmatrix(x, [], s8, [])

MANOVA was then used to test statistically if the varieties were significantly different from one another with respect to the sensory quality.

Manova1 function

>> [d, p, stats] =manova1(x, s8)

This gives the results

d=0

p=0.2948

=0.8221

=0.9565

8.4.6 Paired Comparison Test

The paired-comparison test was used to determine whether two paired sauerkraut products from two different cabbage varieties differed in a specified attribute.

Two differently coded samples were presented to each panellist simultaneously and the panellist's task was to choose the one that is perceived as higher or more intense in the specified sensory attribute (www.jstor.org).

8.5 Results

8.5.1 pH and Lactic Acid

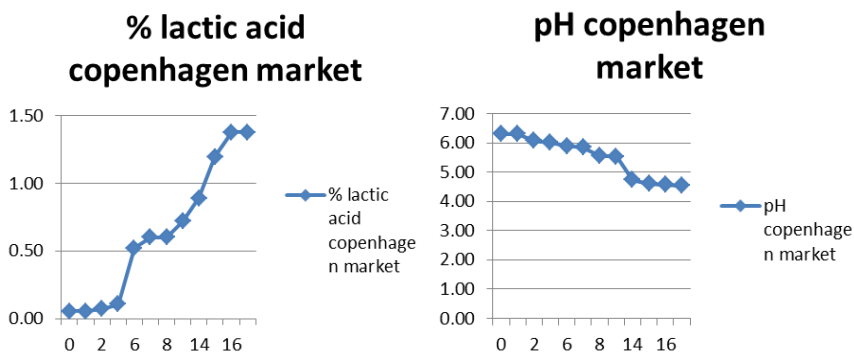

Figure 8.1 *Lactic Acid and pH results for Copenhagen cabbage variety.*

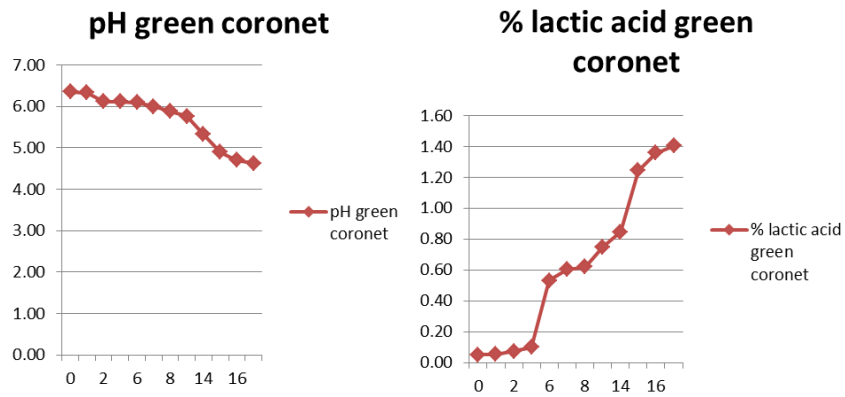

Figure 8.2 *Lactic Acid and pH results for Green coronet cabbage variety.*

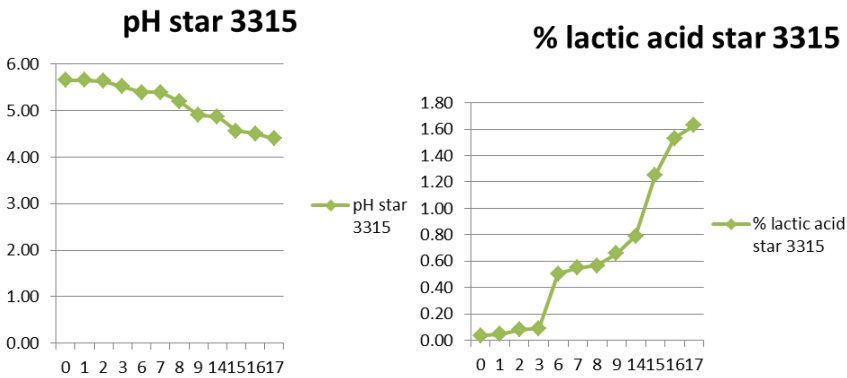

Figure 8.3 *Lactic Acid and pH results for Star 3315 cabbage variety.*

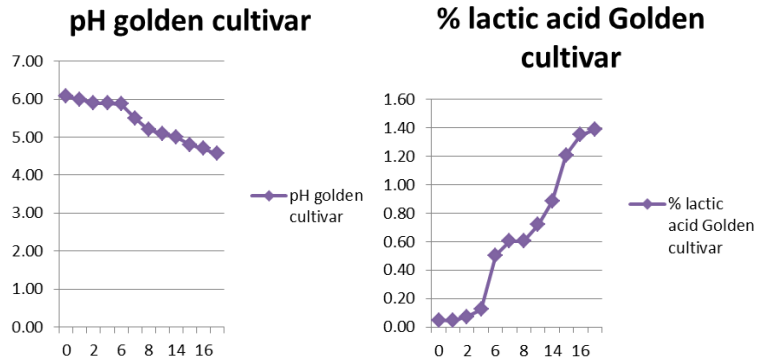

Figure 8.4 *Lactic acid and pH results for golden cultivar cabbage variety.*

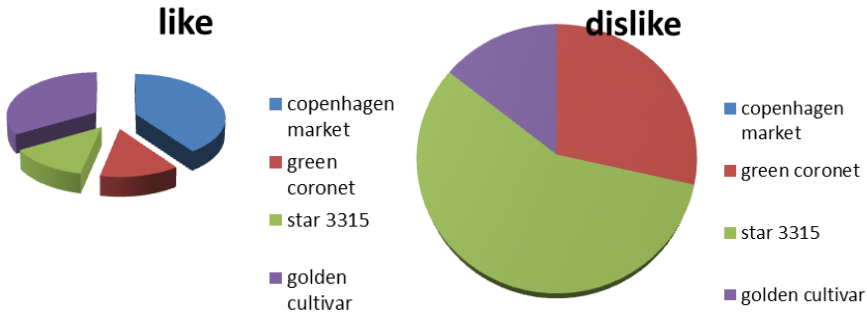

Figure 8.5 *Charts for the like or dislike from consumer preference test using the nine point hedonic scale.*

Table 8.1 *Results for the paired comparison test.*

Sample combinations	**Discussion**
Copenhagen market and Green coronet	Differences were detected by 100% of the panellists and Copenhagen market was preferred to Green coronet
Green Coronet and Star 3315	Difference detected and Star 3315 described to be more salty than Green coronet by 83.3% of the panellists and 16.7% differentiated using colour. Star3315 has a deeper colour than Green coronet
Star 3315 and Golden cultivar	100% of the panellists detected difference and described in terms of saltiness and sourness.
Copenhagen market and Golden cultivar	Differences detected by all the panellists and described in terms of bitterness, colour, odour and saltiness
Copenhagen market and Star3315	Differences detected by all panellists and described in terms of taste, colour, smell and sourness
Green coronet and Golden cultivar	66.6% detected difference and described the difference in terms of bitterness, texture, saltiness, colour and smell and 33.4% did not detect any difference

8.5.2 Qualitative Descriptive Tests Results

Table 8.2 *Qualitative descriptive analysis (QDA) results for the four cabbage varieties investigated.*

Varieties	Saltiness	sourness	Bitterness	Consistency	texture	appearance	Aroma
	6.50	7.30	7.40	5.70	5.40	3.90	7.10
	8.20	7.70	8.00	7.00	7.00	8.40	7.40
A(Copenhagen market)	2.80	6.00	3.10	8.00	8.50	4.00	3.40
	5.90	4.80	2.10	6.30	6.90	7.10	7.00
	9.20	1.20	0.90	5.10	6.30	5.30	2.00
	4.80	5.50	3.80	7.50	6.50	8.00	8.90
	5.50	3.80	3.90	5.50	5.60	3.90	5.30
	4.50	4.70	4.80	5.20	4.10	7.00	6.30
	2.20	8.10	3.10	1.30	8.50	1.50	4.10
B(Green coronet)	3.90	3.30	2.40	2.90	3.60	6.00	5.90
	8.60	1.30	2.20	2.20	7.10	3.70	3.10
	3.50	5.00	4.70	4.40	4.60	4.90	4.10
	9.20	8.20	8.60	6.70	7.00	5.00	3.30
	8.50	8.10	1.20	4.20	3.40	2.40	1.50
C(Star 3315)	9.10	7.50	7.80	1.80	9.10	9.20	2.60
	3.80	2.60	2.30	2.40	2.20	2.10	3.30
	9.00	5.90	5.10	4.90	6.60	6.10	4.40
	6.30	6.20	6.20	7.20	5.00	5.00	3.60
	9.80	7.50	8.00	5.30	3.50	3.60	5.30
	5.00	6.50	4.60	4.50	3.30	2.70	6.20
	1.50	2.80	1.50	1.40	7.40	1.60	3.90
D(Golden cultivar)	5.40	3.10	2.60	6.60	6.00	6.50	5.20
	9.00	7.00	5.00	6.20	6.70	7.20	2.50
	2.70	3.20	3.30	6.90	5.60	5.60	3.80

8.6 Graphical Analysis Results

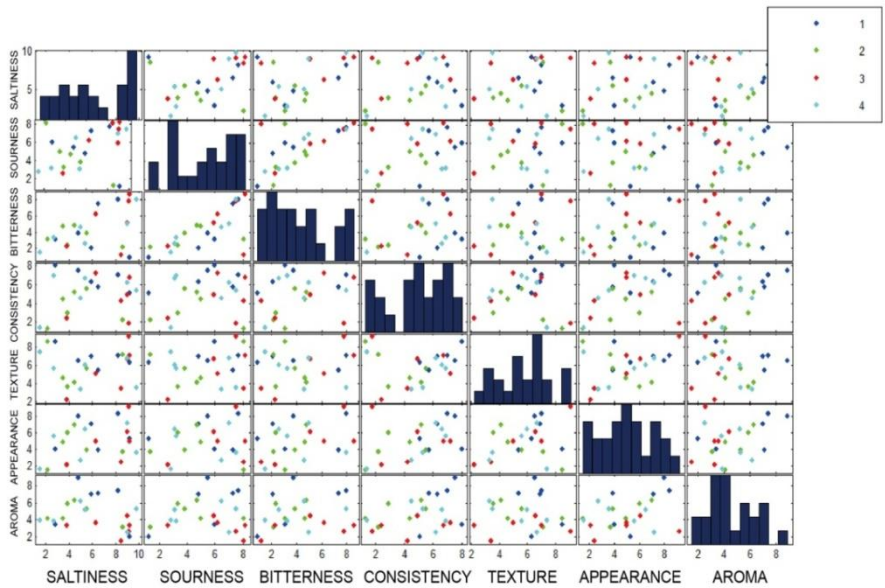

Figure 8.6 *Graphical analysis of the QDA results for the four cabbage varieties investigated.*

8.7 Discussion

Star 3315 had the greatest drop in pH, the final pH was 4.40.pH of Copenhagen market dropped from 6.31 to 4.54, green coronet's pH dropped from 6.35 to 4.63 and golden cultivar had a pH drop from 6.08 to 4.57. The drop in pH in the cabbage varieties is due to the production of lactic acid. Lactic acid was high in Star 3315 variety followed by Green coronet, Golden cultivar and lastly by Copenhagen market. From the hedonic scale results Copenhagen market variety was well liked scoring 40%. Golden cultivar scored 30% and Green coronet and Star 3315 had 13% each. In the range of dislike Star 3315 variety had 57%; Green coronet had 29% and golden cultivar scored 14%.

Copenhagen market was not disliked by any member of the sensory evaluation panel. It can therefore be concluded that Copenhagen market produced sauerkraut that was well liked by panellists.

The manova1 function produced three outputs:

- The first output, d, was an estimate of the dimension of the group means. If the means were all the same, the dimension would be 0, indicating that the means are at the same point. If the means differed but fell along a line, the dimension would be 1. If the dimension is 2, the group means fall in a plane but not along a line.
- The second output, p, was a vector of p-values for a sequence of tests. The first p-value tests whether the dimension, d, is 0, the next whether the dimension is 1 and whether dimension is 2.
- The third output was the stats but in this case it is of no particular importance.

Considering the results obtained the first p-value was small therefore the dimension was 0 and it can be concluded that statistically there was insignificant difference in the quality and sensory characteristics of sauerkraut produced using different cabbage varieties.

8.8 Conclusion

The results for the hedonic test and paired comparison showed that there are differences which can be perceived by consumers. On further analysis with the quantitative descriptive analysis it was found out the differences on the attributes which can be perceived organoleptically are not significant hence

consumption of sauerkraut can be from any cabbage variety. From the research results it was concluded that all the nutritional benefits from sauerkraut can be obtained by increased consumption of sauerkraut made using any cabbage variety that is grown in the country. The revelation of the knowledge of sauerkraut production will make it possible for Zimbabweans to make sauerkraut and draw the nutritional benefits contained therein.

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9

Food Irradiation as a Model Preservation Technique for the Food Industry: The Pros

9.1 Introduction

There's an old Chinese proverb that says "May you live interesting times". With respect to food irradiation (Borsa 2000), today's proponents and other observers of this technology have good reason to feel that indeed these are interesting times in this unfolding story. Studied intensively for more than half a century, and approved in some 50 countries around the globe for a wide variety of food products (ICGFI 2005), irradiation has been widely used for spices and other food ingredients for many years, but perishables (meat and produce) it is just now emerging into a significant commercial reality. In United States from basically a standing start at the beginning of this recent period, but powered by a high level of entrepreneurial energy and zeal, commercialization of irradiation technology in the food industry accelerated rapidly to reach heights far beyond anything previously achieved. Almost overnight, irradiated products appeared in literally thousands of retail and foodservice outlets (Sure Beam; 2001). Investors took notice (Titan Corp; 2001) and millions of dollars were raised for ventures targeting the opportunity presented by the very real needs recognised in food safety (Osterholm and Norgan; 2004) and quarantine security (IAEA 2004). The fact that those needs are evident all over the world added to the investment appeal. In these positive circumstances, interest in food irradiation rapidly escalated, giving rise to an exciting play in the investment world. Unfortunately, in 2004 a major business miscalculation intervened and this nascent industry suffered a significant setback just as it appeared to be getting over the hurdles associated with its launch. Not surprisingly, and to the great satisfaction of the sceptics and antitechnology activists, unreasonable expectations had exceeded the actual pace of adoption, especially by the major food processors, and the simple but inexorable math of the business world led

Sure Beam ™, the most prominent player in the field, to declare bankruptcy (Egerstrom 2004). This failure caused considerable consternation and uncertainty in the fledgling industry, raising concerns as to whether it would survive the setback. Now, more than 5 years later and with the dust largely settled, it appears that emerging from this uncertainty is a restructured food irradiation industry that is gradually regaining momentum. The fundamental benefits offered by the technology remain the same (Olson 2004) and the new path forward, although lacking the brash boldness and dash of the Sure Beam ™ approach, offers prospects for a more sustainable long – term future.

9.2 Description

Three types of ionizing radiation are used in commercial radiation to process products such as foods and medical and pharmaceutical devices (International Atomic Energy Agency [IAEA], 1982): radiation from high-energy gamma rays, x-rays and accelerated electrons. In accordance with the CODEX General Standard for Irradiation Foods (Codex Alimentarius Commission, 2003), only these ionizing rays are authorised to be used in food irradiation applications. These types of radiation are called "ionizing" because their energy is high enough to dislodge electrons from atoms and molecules and to convert them to electrically charged particles called "ions." Ionizing radiation may originate from different sources:

- Gamma rays which are produced by radioactive substances (called radioisotopes). The approved sources of gamma rays for food irradiation are the radionuclides cobalt- 60 (60Co; the most common) and cesium - 137 (137Cs). They contain energy levels of 1.17 and 1.33 MeV (60Co) and 0.662 MeV (137Cs).

- Electron beams, which are produced in accelerators, such as in a linear accelerator (linac) or a Van de Graaff generator at nearly the speed of light. Maximum quantum energy is not to exceed 10MeV.

- X-rays or decelerating rays, which can be likewise produced in accelerators. Maximum quantum energy of the electrons is not to exceed 5MeV.

Gamma rays and X-rays form part of the electromagnetic spectrum, like radio waves, microwaves, ultraviolet, and visible light rays. Gamma rays and X-rays are in the short wavelength, high –energy region of the spectrum. Both gamma and X-rays can penetrate foods to a depth of several decade centimetres. Energies from the previously mentioned radiation sources are too low to induce radioactivity in any material, including food. There is wide expertise in the design, building, and operation of both radionuclide and electrical machine irradiation facilities (Leemhorst and Miller, 1990). Radionuclide facilities are currently used for the treatment of food and for non-food applications, such as sterilization of medical supplies and for pharmaceutical, cosmetics, and veterinary products. Electron accelerators are used in the manufacture of certain packaging materials (e.g., cling film) and in the treatment of plastic wire insulation to improve its properties. Commercial irradiation facilities for food are available in approximately 50 countries. Food irradiation plants may be operated in batch or continuous mode. Batch facilities are considered to be more flexible and able to accommodate a wide range of doses (World Health Organisation, 1988). Continuous facilities are better able to accommodate large volumes of food products, especially when treating a single food, at a specific dose. Mobile irradiations have been used in research for the treatment of seasonal food, such as fruits and vegetables, and for fish irradiation on board ships. A comparison of radionuclide irradiators and electron accelerators is shown in Table 9.1. A food irradiation facility is composed of the following elements:

- A source of radiation (i.e., radionuclide or electron beam).
- Biological shielding to protect personnel operating the facility from radiation exposure.
- A carrier or conveyor system to take food product into the vicinity of the source for processing.
- An air evacuation system.

Table 9.1 *Comparison of Radionuclide Irradiators and Electron Accelerators; From Fink and Rehmann (1994).*

Radionuclide Irradiators	Electron Accelerators
Good penetration power of gamma rays; can be used to treat food in large packaging units	Relatively limited penetration power (5 8 cm).
Low dose rate	High, variable dose rate; allows high throughput (e.g., grain).
High reliability	More sensitive to breakdown; need for specialized personnel for regular maintenance.
Need to replenish radionuclide source	High requirements for power and cooling Machine can be switched off.
	Small units of equipment could be integrated into a production line.

- A safety interlock/control console system, which ensures that conveyor movement occurs when the source is exposed or the machine is switched on (no conveyor movement when the source is in a ‘‘safe’’ position or the machine is turned off) (Farkas, 1988).

The food irradiation facilities do not become radioactive and do not create radioactive waste. 60Co is manufactured in a commercial nuclear reactor by exposing nonradioactive cobalt to intense radiation in the reactor core. The cobalt sources used in irradiation facilities decay by 50% in 5 years and therefore require periodic replacement. The source are removed from the irradiation when the radioactivity falls to a low level, usually between 6 and 12% of the initial level (this takes 16-21 years for 60Co). The small radioactive cobalt “pencils” are

shipped back to the original nuclear reactor. The shipment occurs in special hardened steel canisters that have been designed and tested to survive crashes without breaking. Cobalt is a solid metal and even if something should break, it will not spread through the environment. 60Co may also be disposed of as a radioactive waste. Given its relatively short half – life (5 years) and its stable metallic from, the material is not considered to be a problematic waste. Electron beam and X-ray facilities do not use or create radioactive substances (IAEA, 1992).

9.2.1 Application

Applications of food irradiation are usually organized into three categories according to the range of delivered dose.

Low-dose (<1 kGy)

(a) Sprouting Inhabitation

In order to provide consumers a year –round supply of various sprouting foods, such as potatoes, yams, garlic and onions, storage durations of up to several months are often necessary (Ahari and Safaie2008; Ahari and Zafarani; 2008; Bibi et., 2006). Sprouting can be inhibited by refrigeration and the application of various chemicals such as hydrazide (preharvest) and isopropyl chlorocarbamate (postharvest). But, refrigeration is expensive and particularly so in the tropical and subtropical zones of the world. Whereas, the chemical treatments are relatively cheap and efficient, they do leave residue and many countries have banned their usage for health reasons. In such instances, irradiation can be recommended as a reasonable alternative. Sprouting prevention and reduced rotting and weight loss have been observed in potatoes, garlic, onions, and yams in the range of 50-150 Gy (IAEA, 1996 Lagoda, 2008; Marcotte, 2005).

(b) Insect Disinfestations

The best control for insects in grain and grain products can be achieved by using fumigants such as ethylene dibromide or ethylene oxide (IAEA, 1996 Landgraf et al, 2006). Until 1984, fruits and vegetables from infested areas were fumigated with chemicals to meet the quarantine regulations. However, the use of these chemicals has been banned or strictly restricted in most countries for health and environmental reasons. Whereas heat and cold treatments are capable of insect disinfestations, they can also acutely degrade the taste and appearance of the produce (Marcotte, 2005; Stewart, 2004b). Radiation processing has therefore been suggested as an alternative to fumigation. Disinfestations is intended at preventing losses caused by insects in stored grains, pulses, flour, cereals, coffee beans, dried fruits, nuts and dried fish (Farkas, 2004; Landgraf et al., 2006). Practical experience shows that the required radiation dose is in the range 150-700 Gy. A dose level of 250 Gy can be effective on quarantine treatment of fruits flies, whereas a dose of 500 Gy can control all stages of most pests (Farkas, 2004; Miller, 2005).

Meduim – Dose (1-10 kGy)

(c) Food Borne Pathogens

Beef, Pork, Poultry, Seafood, eggs and dairy products are all recognised as major source of food borne illness. The most serious contaminants are E. Coli, listeria and tapeworm for beef. For poultry and eggs, the predominant pathogens are salmonella and campylobacter. excellent control of all these organisms can be achieved with doses in the range of 1-3 kGy (Patterson, 2005; World Health Organisation, 2005; Ziebkewicz et al., 2004).

(d) Shelf Extension

The same dose levels appropriate for food borne pathogens can also significantly extend the shelf life of the products just discussed by reducing populations of spoilage bacteria, moulds, and yeasts. For example, a dose of 2.5 kGy can extend the shelf life of chicken and pork by as much as a few weeks,, while the shelf life of low –fat fish can be extended from typically 3-4 days without irradiation to several weeks with 5 kGy (Patterson, 2005). In addition, the shelf life of various cheeses can be extended significantly by eliminating moulds at doses of less than 0.5 kGy. Finally, shelf life extension for strawberries, carrots, mushrooms, papayas and packaged leafy vegetables also appears to be promising at dose levels of a few kGy or less (Bibi et al., 2006; Hammad et al., 2006). Irradiation of mushrooms at 2-3 kGy inhibits cap opening and stem elongation and can be increased at least by two fold (by storage at 10 ℃). Treatment of strawberries (which are spoiled by Botrytis sp.) with a dose of 2-3 kGy, followed by storage at 10 ℃ can result in a shelf life of up to 14 days (Ahari and Safaie, 2008). Ripening in bananas, mangoes, and papayas can be delayed by irradiation at 0.25-1 kGy. It is important to irradiate them, before ripening start (Hammad et al., 2006; Lagoda, 2008; Marcotte, 2005).

(e) Spice Irradiation

The fresh plants from which spices are derived are almost always contaminated by microorganisms from soil, with blown dust and by bird droppings. During the drying process, these microorganisms can grow to population densities exceeding 106 organisms per gram of material (Marcotte, 2005). When used as seasonings in the manufacture of processed foods for which the manufacturing process does not include a satirizing step, these organisms can cause rapid food spoilage and can lead to food borne illness. Since moist heat treatment is not generally suitable

for such dry products, spice producers in the past routinely used fumigants for disinfestations'. Producers are now increasingly turning to ionizing radiation. In fact, the commercial irradiation of spices has been approved and practices in many countries for several years. Doses of 5-10 kGy usually give quite satisfactory results (elimination of bacteria, mould spores, and insects) without negative impact on chemical or sensory properties (Farkas, 2004; IAEA, 1996; Koopmans and Duizer, 2004).

High –Dose (>10 kGy)

Some foods such as fresh fruits and vegetables deteriorate when subjected to high radiation doses. However, other foods including meat, poultry and certain sea foods do maintain good quality, provided that certain precautions are taken. As a result, it is possible to effectively sterilize these foods with doses in the range of 25 -45 kGy (Stewart, 2004a, 2004b; The Institute of Food Science and Technology, 2006). To prevent off flavours resulting from lipid oxidation, oxygen must be excluded by vacuum packaging and the irradiation must be performed at low temperatures (-20 ℃ to -40 ℃). Foods which are preheated to inactivate enzymes can be commercially sterilized such as it occurs in canning. These products can be stored at room temperature almost indefinitely. While these additional procedures and high doses significantly increase costs, these products are nonetheless important for hospitalized patients with suppressed immune system and NASA astronauts (Patterson, 2005 Scott, and Suresh, 2004).

9.3 General Analysis

9.3.1 Global Perspective

Over 90, 000 tonnes of dried herbs, spices, and vegetables seasonings were irradiation in some 20 countries in 2000, with around half of this quantity being

irradiated in the USA. Food irradiation is classified as a food additive in USA legislation. Since 2002, beef has also been irradiated in the USA and sold to a growing market. One major E-Beam facility in the USA overestimated the expected uptake of irradiated beef for the school Lunch Programme and went out of business in 2004. However, a Texas – based investment firm purchased the assets in June 2005 and in late December, the plant began processing about 40 000 pounds per day of animal feed for mills in the US Midwest. Fermented pork sausages (Nam) usually consumed raw in Thailand have been irradiated since 1986 (Food Standard Agency 2004). A survey of the extent to which foods are irradiated in the EU, carried out by the commission of the European Communities. (EC. 2004) found: Belgium irradiated 6.613 tonnes (frozen 'frog' legs, frozen seafood and spices/seasonings were the principal products) Germany irradiated 795.3 tonnes (dried aromatic herbs and spices and herbal tea-for expert to Poland comprises the majority of products) France irradiated 5,129 tonnes (mechanically recovered chicken meat, spices and frozen frogs' legs were the principal products) The Netherlands irradiated 7,114.4 tonnes (dehydrated vegetables, spices and herbs, frozen poultry and foods intended for export to third countries comprises the majority No food was irradiated in the UK.

In the UK very few, if any, irradiated food products are on retail sale. A survey was carried out in 1996 and repeated in 2002 to investigate whether irradiated food is on sale in UK but not labelled as such (Food Standards Agency, 2002). In this country, 543 samples without declared irradiated ingredients covering three food categories (203 herbs and spices, 138 dietary supplements and 202 prawns and shrimps) were analysed. These three food categories were selected because a number of reports had claimed that these products were likely to have been irradiated and unlabelled. One of the herbs and spices (ground nutmeg), five prawns and shrimps and forty – four dietary supplements (42%) were found to be irradiated ingredients without appropriate

labelling. The analytical methods used PSL (photo stimulated luminascene) as a screening procedure while TL (thermolumunescence) was used to confirm those samples which gave ‘positive’ or ‘intermediate’ (suspected irradiation) when analysed by PSL. Comments were elicited from all the retailers or suppliers of the offending products. These varied from a declaration that the company does not knowingly sell irradiated food to queries over the accuracy of the analytical results or that an excipient ingredient (talc) may have been responsible for the false positive. The food (Control of irradiation) Regulations came into force in 1991 and was amended in 2001. On 15 June 2004, the UK Food Standards Agency (FSA) issued an alert (Food Alert for Information (FAFI) on noodle based snacks due to undeclared presence of irradiated ingredients contained in the vegetable seasoning mix of the dried spicy soup.

9.3.2 Consumer Attitude to Food Irradiation

The introduction of irradiated foods has many analogies with the introduction of pasteurisation of milk over a century ago-one of the most significant advances ever made in food safety. The principal allegations advanced against the introduction of thermal pasteurisation of milk and food irradiation (cold pasteurisation) are very similar (Satin, 1996). Opponents of both thermal pasteurisation of milk and cold pasteurisation of foods by irradiation have claimed:

- Nutritional value will be diminished.
- The price will be increased.
- Possibly unsafe.
- Will be used to mask filthy products.
- Legalises the right to sell stale food.
- Is unnecessary.

- Is meddling with nature.
- Will take the 'life' out of the product.

Many surveys have been carried out (mostly in the USA to assess consumer attitude to food irradiation e.g. Bruhn and Schutz 1989; Resurrection et al 1995; Fox 2002; Nayga et al. 2005). Results have consistently shown that many consumers have misconceptions about the technology and believe that it makes food radioactive. When consumers are given information about the process and a chance to try irradiated products for themselves they are much more likely to accept technology. Market trials have also met with success. One of the most successful market trials of irradiated foods was carried out in 1991 in a small food store in Chicago, USA. Clearly labelled irradiated strawberries, oranges, and grapefruits outsold their non-irradiated counterparts by a ratio of 9.1. In the following season, irradiated unirradiated product. This positive experience encouraged approximately 60 stores in India, Illinois, and Ohio to sell a variety of irradiated foods (Pszczola, 1992).

In one study, the sensory characteristics and consumer acceptance of E-Beam irradiated (at 1, 2, and 3 kGy) RTE meats (frankfurters and diced chicken) were evaluated by a consumer panel of 50. After 18 days of refrigerated storage for the chicken and 32 days for the frankfurters the acceptability of the irradiated products was significantly higher than for the non-irradiated (Johnson et al, 2004). The same authors compared attitudes towards irradiated foods over ten years 1993 to 2003. Consumer awareness was no higher in the 2003 study than in 1993 but more consumers were willing to buy more irradiated foods in the 2003 than in 1993 (69% and 29% respectively). Consumers in both studies showed more concern over pesticides and animal residue, growth hormones, food additives, bacteria and naturally occurring toxins than irradiation. The slight concern expresses regarding irradiation has decreased significantly among

this group. Approximately 76% prefer to buy irradiated pork and 68% prefer to buy irradiated poultry to decrease the possibility of illness from *Trichinella and Aalmonellae* respectively (Johnson et al, 2004).

In another study, 113 consumers who were over 18 and consumed ground beef at least once a month were selected for a trial in Mesa, Arizona to examine the effects of products exposure and consumer education about irradiation. Products exposure was found to exert no effect while educating consumers had most significant impact on their views of food irradiation. Sensory evaluation showed that consumers could not differentiate between irradiation and non-irradiated ground beef either at the beginning of the study or after months frozen storage. Groups that receive irradiation education were more accepting of the technology and more consumers in these groups changed their perceptions of irradiation in a positive way (Hamilton et al, 2004).

A similar study (Nayga ety al. 2005) carried out in 2002 in four Texan towns (Austin, Houston, San Antonio, and Waco) involved face to face interview with 484 customers intercepted at random at supermarkets entrances. Each respondent was initially asked to say to which of four consumer segments they belonged "strong buyer", "interested", "doubter" or "rejecter" of irradiated foods. They were then presented with two informative statements, the first pertaining to the nature and benefits of food irradiation. The second statement described the two different processes of irradiation (gamma rays and E – Beam) and also involved watching a short video illustrating the E-Beam process. The results are presented in the accompanying table. Males were more inclined to change their view than females and switch towards the segment more likely to purchase irradiated foods.

Table 9.2 *Effects of Consumer Education on Consumer Attitudes.*

	Strong buyer	Interested	Doubter	Rejecter
initially	8.4%	73.8%	13.9%	3.8%
after first statement	28.3%	66.6%	3.4%	1.7%
after second	42.2%	54.0%	3.2%	0.6%

These results strongly support the thesis that supplying digestible information can be highly effective in shifting consumer attitudes in favour of purchasing irradiated foods. The participants were also asked about their perception of the Radura symbol: 67.1% considered it an assurance of quality. 5.5% considered it a warning signal and avoided the product. 17.1% indicated it did not affect buying decisions. 10.3% did not recognise the symbol.

Consumer acceptance of irradiated foods in the USA is being reinforced by three key – drives; these being (i) growing public awareness of the risks from bacteria in meat products, (ii) growing levels of educational media coverage on food irradiation and (iii) fear of bioterrorism on centralised food production. (Delay, 2002). In the EU Member States the use of irradiation inorganic food expressly prohibited (EU Regulation 2093/91). This sector is enjoying the fastest growth of any sector of the foods industry in the UK and many other European countries and thus represents an expanding market sector not open to irradiated foods.

9.4 Actualisation

9.4.1 The Case of Carcinogens and Their Relation to Irradiation

Reduction of Volatile N-Nitrosamine and Nitrite Content with Irradiation

Human exposure to carcinogen N-nitrosamines occurs through endogenous and exogenous sources such as foods and beverages (Chung, 1996, 2000). The major

N-nitrosamines found in food systems are notrosodimethlamine (NDMA) and nitrosopyrrolidine (NPYR) (Lijinsky, 1999). Low levels of biogenic amines in food are not considered a serious risk. However, if the amount consumed is high enough, or normal pathways of amine catabolism are inhibited, various physiological effects may occur, such as hypotension or hypertension, nausea, headache, rash, dizziness, cardiac palpitation and emesis, and even death (Rawless et al., 1996). The formation of N-notrosamines in foods occurs due to an addition of nitrite, smoking, drying with combustion gas, salting, pickling, fungal contamination, or food contact materials (Thicker, 2000). Nitrite is an essential addictive for developing typical cured meat colour, flavour, and texture.

Table 9.3 *Effect of Irradiation on Protease Inhibitors in Various Products.*

Food Type	Gamma Irradiation Dose (kGy)	Irradiation Effect	Reference
Soybean seeds	0, 1, 5, 10, 20, 40, 60, 80, and 100	Inhibition of 25.4% trypsin inhibitor activities and 16.7% chymotrypsin inhibitor activities were found when the soybean seeds were irradiated at 100 kGy.	Hafez et al., 1985
Safflower oilcake	0.07 0.1	The trypsin inhibitor was inactivated at 0.42 kGy, whereas the chymotrypsin inhibitor remained active, even at the much higher dose of 0.1 kGy. The *in vitro* digestibility values also showed a significant improvement after irradiation.	Joseph and Dikshit, 1993
Karanja oil seed residue	1, 5, 10, and 50	Trypsin and chymotrypsin inhibitor activities retained in the cake on exposure to 50 kGy dose were 22 and 16%, respectively.	Rattansi and Dikshit, 1997
Broad bean	0, 2.5, 5, 7.5, and 10	Irradiation treatment reduced the trypsin inhibitor of irradiated seeds. In subsequent dose of irradiation, the decrease in trypsin inhibitor was proportional to the irradiation dose.	Al Kaisey et al., 2003
Soybean grains	2, 4, and 8	Radiation with dose of 2 kGy promoted reduction of 11.19% on average in trypsin inhibitory activity, and a dose of 4 kGy reduced 28.59% and that of 8 kGy reduced 37.60%.	de Toledo et al., 2007

Several methods have been developed to inhibit nitrosamine formation with the use of green tea (Yang and Wang, 1993), ascorbic acid (Vermeer et., 1999), and phenol compounds (Bartsch et al., 1988). Fiddler et al. (1981) found that irradiation sterilization (30 kGy) reduced residual nitrite in bacon prior to frying,

thereby reducing volatile nitrosamines after frying, and destroyed performed volatile nitrosamines in the bacon before irradiation. Hu and Song (1988) reported that g-irradiation could reduce nitrite from 41.2 to 21.0 ppm in eel at 5 kGy of irradiation. Jo et al. (2003a) studied the packaging and irradiation effect on pork sausage. Emulsion –type cooked pork sausage was made with (156ppm) or without $NaN0_2$ and packed at 4 ℃ in aerobic, vacuum, and CO2 (100%) conditions. The samples were irradiated at 0 and 5 kGy. Residual nitrite content was the lowest in the sausage with CO_2 packaging, but no irradiation effect was found at 5 kGy. The 5 kGy irradiation eliminated the nitrodopyrrolidine (NPYR) in the sausage with vacuum or CO_2 packaging at 0 weeks. At 4 weeks, the NPYR content in the sausage regardless of packaging. Moreover, irradiation at 5 kGy significantly reduced the NDMA content regardless of packaging method. The characteristics of nitrite radiolysis with g0rays were investigated by Ahn et al. (2003a). Sodium nitrite in deionized distilled water was irradiated at 0, 5, 10, 20, 25, 30 and 40 kGy. The sodium nitrite was reduced approximately 50% by 10 kGy irradiation, and complete degradation was shown over 40 kGy. When nitrite was nitrosated at different pH ranges (2, 3, 4, and 6) after irradiation, the irradiated nitrite could not form the carcinogenic N-nitrosodimethylamine. The authors concluded that g-irradiation could be effectively used for reducing nitrite, and radiolytically destroyed nitrite could not form carcinogenic N-nitrosamine, even in a model human stomach condition. Ahn et al. (2002a) studied the reduction of carcinogenic N-nitrosamines and residual nitrite in model system sausage with irradiation. Sausages were packed under air and under vacuum and irradiated at 0, 5, 10, 20, and 30 kGy. The residual nitrite levels were significantly reduced with g-irradiation, and in vacuum packaging the reduction was done dependent. Vacuum packaging proved to be more effective than aerobic packaging for lowering the nitrite levels. In aerobic packaged sausage, NPYR levels were not

affected by irradiation. However, after weeks of 20 and 30 kGy of irradiation, NPYR levels were reduced 47.7 -51.0% in VP compared to non-irradiated sausage. NDMA levels in non-irradiated aerobic and VP samples were significantly higher than those in 20-kGy irradiated samples. A significant difference was found between non-irradiated samples and samples irradiated with a 10-kGy or higher dose in aerobic packaging.

In conclusion, for reduction of NDMA and NPYR in sausage, 20 kGy of irradiation or higher was needed. Gamma irradiation was applied for the breakdown of the volatile N-nitrosamines, NDMA, and NPYR. NDMA and NPYR were dissolved in distilled water, dichloromethane, or ethanol and irradiated at 2.5, 5, 7.5, 10, 15, 20, and 25 kGy. The NDMA in dichloromethane was broken to the level of 448 ppb (mg/1) at 2.5 kGy, and NPYR was completely broken at the same dose. The NDMA required a dose of 10 kGy or higher of g-irradiation to achieve 99% breakdown. NDMA and NPYR dissolve in ethanol were comparatively stable to g-irradiation. At the dose of 20 kGy, NDMA and NPYR showed 95 and 100% breakdown, respectively. NDMA and NPYR dissolved in distilled water were easily broken down with g- irradiation, and all of the volatile N-nitrosamines were undetectable at 5 kGy or higher. NDMA and NPYR displayed 65-84% breakdown at 2.5 kGy, and NPYR was the most sensitive to g-irradiation. The results indicated that volatile N-nitrosamines in distilled water were easily decomposed with g-irradiation at doses of 5 kGy or higher (Ahn et al., 2002b). Ahn et al. (2003b) studied salted and fermented anchovy sauce spiked with or without NDMA and NPYR. Samples were irradiated at 0, 5, 10, 15, and 20 kGy. NDMA and NPYR reduction with irradiation was not observed in non-spiked samples at 0 weeks, whereas a significant reduction was observed after 4 weeks of storage at 15 ℃. NDMA and NPYR levels decreased with irradiation at 5 kGy or higher after storage at 15 ℃. After storage, the degraded nitrosamines with irradiation were

not recombined. The impact of different doses of irradiation on NDMA content of pepperoni sausage, packages under air, and fermented anchovy sauce at Week 0 is shown in Figure 9.1. Byun et al (2004a) studied volatile NDMA and NPYR in irradiated pepperoni and salami sausages. These fermented sausages were packed under vacuum air, 100% CO_2, 100% N_2or

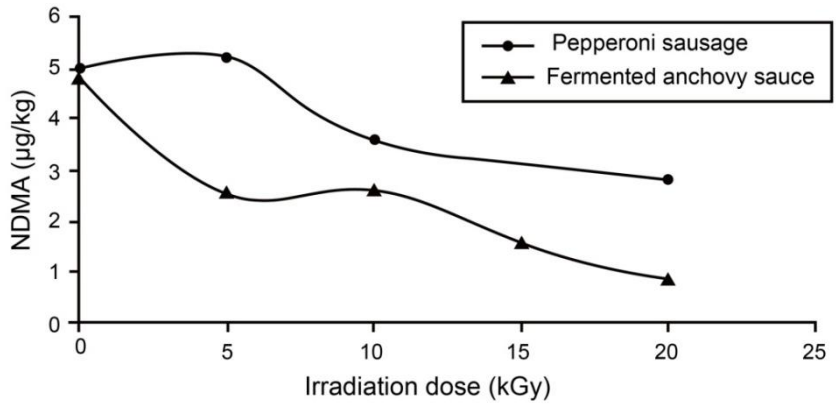

Figure 9.1 *Effect of different doses of irradiation on N-nitrosodimethylamine (NDMA) content of pepperoni sausage, packaged under air (Byun et al., 2004a), and fermented anchovy sauce at Week 0 (Ahn et al., 2003b).*

25% CO_2/75% N_2 and they were irradiated at 0, 5, 10 and 20 kGy and then stored for 4 weeks at 4 ℃. Irradiation significantly reduced the NDMA in the salami sausage at 0 weeks, whereas the NPYR was not detected in the sausage irradiated over 5 or 10 kGy. Regarding the pepperoni sausage, the VP showed lower nitrosamine content than that of the air packed. After storage for 4 weeks, the irradiated salami showed low NDMA and NPYR contents compared to non-irradiated ones. Results indicated that high dose of irradiation (>10 kGy) was required to reduce the carcinogenic N-nitrosamines in the fermented sausages. The effect of different doses of irradiation on NPYR content of pepperoni sausage, packaged under air, and fermented anchovy sauce is displayed in Figure 9.2. Ahn et al. (2004c) investigated the combined effects of irradiation and modified

atmospheric packaging (MAP) on residual nitrite and NDMA in sausage during storage. Sausages were packed under air, vacuum, CO_2, N_2 OR CO_2/N_2 packaging and irradiated at 5 kGy. Residual nitrite was reduced by irradiation, and the contents were lower under vacuum or MAP than aerobic ones. Furthermore, NDMA was significantly reduced with a 5-kGy dose. Ahn et al. (2004b) investigated the irradiation effects on cooked pork sausage during storage at 4 ℃. Sausage with aerobic or vacuum packaging was irradiated at 0, 5, 10, or 20 kGy. It was found that irradiation treatment reduced the nitrite contents of sausage, and especially under vacuum, nitrite content decreased with g-ray dose in adose – dependent manner. Irradiation at 20 kGy reduced the residual nitrite contents to 31 and 17% under aerobic and vacuum packaging, respectively. After 4 weeks of storage, a decrease in residual nitrite content was reported in all samples, and the irradiation effect was still found. Residual nitrite contents of sausage irradiated at 5 kGy or higher were lower than those of non- irradiated control for both packaging conditions. NDMA contents in sausage with VP were decreased by irradiation at 10 and 20 kGy, whereas no different was found.

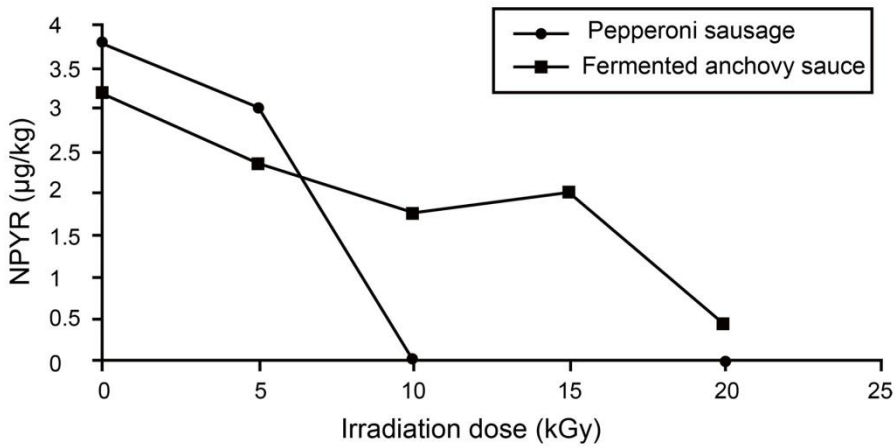

Figure 9.2 *Effect of different doses of irradiation on N-nitrosopyrrolidine (NPYR) content of pepperoni sausage, packaged under air (Byun et al. 2004a), and fermented anchovy sauce at Week 0 (Ahn et al., 2003b).*

In aerobic packaging at 0 weeks. Irradiation reduced NPYR contents in sausage with aerobic packaging, and NPYR was not detected by irradiation at 5 kGy or higher. After 4 weeks of storage, irradiation decreased NPYR contents in sausage with VP, whereas the packaging effect was not found during storage.

9.5 Discussion

More than 100 years of research that have gone into accepting of the safe and successful use of irradiation as a food safety method is more than any other technology used in the food industry today, even canning (Scott and Suresh, 2004). The safety and efficacy of the technology has been continually considered and judged accepted on available confirmation. This has resulted in international bodies including the World Health Organisation (WHO), the Food, and Agriculture Organisation (FAO) the International Atomic Energy Agency (IAEA) and Codex Alimentarius commending the process. Irradiation is very successful against living organisms which contain DNA and / or RNA but do not cause any significant loss of macronutrients. Proteins, fats, and carbohydrates undergo little modification in nutritional value through irradiation even with doses over 10 kGy, though there may be sensory changes. In the same way, the essential amino acids, essential fatty acids, minerals, and trace elements are also unchanged. There can be a decrease in certain vitamins (mostly thiamine) but these are of the same order of magnitude as it occurs in other manufacturing processes such as drying or canning (thermal sterilization) IAEA, 1996; Landgraf et al., 2006; World Health Organisation, 2005). Consequently, with modest radiation doses (1-5 KGy), it is probable to successfully destroy the organisms responsible for food borne disease and spoilage with no effect on the nutritional and sensory qualities of foods (Lagoda 2008, Miller 2005). A maximum overall average dose of 10 kGy was measured adequate for the majority of food applications. To date, over

50 countries have given agreement for their irradiation of over 60 foods and food products on either a conditional or unconditional basis and these figures are growing annually. Spices, dried herbs and vegetable seasonings are most common food products to be treated with over 90 000 tons (being irradiated in 2000). Irradiation of hamburgers in the USA rose from 6.800 tons in 2001 to over 22,000 tons in 2003 (Koopmans and Duizer, 2004; Miller, 2005). About 9 million tonnes bees and papaya were irradiated (in Hawaii) in 2003.1,754 tons of herbs and spices were irradiated (in South Africa) during 2004 (The Institute of Food Science and Technology 2006). There is growing public consciousness on food safety and quality combined with current incidences of food borne pathogens (Patterson, 2005; The Institute of Food Science and Technology, 2006). The rate of irradiation ability is becoming plainer as food security and consumers' safety questions are discussed. Advertising trials of irradiated food have been conducted over the past several years in countries such as France, Hungary, USA, Holland, Belgium, Argentina, Chile, China, Poland, Thailand, Indonesia, Bangladesh, India, Pakistan, and the Philippines, all with favourable outcome.

9.6 General Recommendations

The steps required to exploit the benefits of irradiation involve standardization, communication, and education. WHO, in collaboration with FAO and IAEA should:

- Coordinate the preparation of documentation and the drafting of appropriate technical language for adoption of standards by the Codex Alimentarius Commission.
- Prepare appropriate brochures and documents that integrate food irradiation into existing guidelines and rules governing the safe production,

distribution and handling of food (in order to minimise the spread of biological contamination and incidence of food borne illness).

- WHO should take lead in advising international spread of pathogens in food irradiation, for preventing the international spread of pathogens in food and animal feed, for controlling food borne illness and for enhancing their availability of safe and nutritious foods.

- Organise and participate in appropriate training courses and workshops that educate food regulators and food workers about the role food irradiation could, and should, play as a control measure in the framework of application of the HACCP (Hazard Analysis and Critical Control Point) system.

9.7 Conclusion

Those in favour of food irradiation have a number of pros to support their stand. After more than 40 years of studies of the effects of irradiation on approved foods reflect that disease causing microorganisms are either eliminated or reduced. This includes E. Coli, salmonella, and a number of more dangerous food borne illnesses. These illnesses are not only health threatening, but also economic losses created are in the billions.

Irradiation on foods greatly decreases the loss of harvest due to bacteria, insects and spoilage and it reduces use of chemical pesticides, some that are very environmental harmful. Irradiation does not affect the environment as the radioactive materials are fully enclosed and then returned for recycling or disposal. There is also a good safety record for irradiation facilities. Also, food does not become radioactive like some fear.

Irradiation delays ripening and sprouting so food can be longer and nutrient losses from irradiation, which do occur to some extent, have been found to be no worse than loses from cooking and the levels recommended for irradiation does not result in significant loss of vitamin or create any deficiency. Those who are not in favour of irradiation state that since the amount of irradiation allowed does not eliminate all pathogens that the remaining are then radiation resistant and may create strains of hard to delete pathogens.

Also those against irradiation feel that there is not enough known about potential health problems associated with food that has been irradiated. Some people also fear possible devastating accidents at the irradiation facilities and if more foods are approved for irradiation more facilities will have to be built, increasing the risk of accidents. The prices for irradiated foods are slightly higher than other foods.

Food irradiation is done on a constant basis, food growers and sellers say that food irradiation is safe and is means of extending the shelf life of fresh foods. The benefits are high according to food sellers; irradiation kills disease-causing organisms. It also increases the safety of foods for people with low immunity system. It promotes longer life of vegetables in stores; it also prevents the vegetables from sprouting. It is also beneficial for grains; it kills or sterilizes insects that can be found in grains. It also allows fruits to be picked early and delay the ripening of the fruit until it gets to the supermarkets.

Irradiation is the process of exposing fresh foods to low amounts of x-rays to sterilize and prolong its life. Food suppliers say that it is safe and does not make foods radioactive. But the general public has problems with this observation; the general public believes that any radiation exposure holds a threat of health hazard. They also believe that consuming these x-rayed foods on daily basis will pose a threat of developing mutant organisms within the body.

There are government regulations in place that insure the use of certain irradiation processes. The process of x-ray irradiation is allowed, because x-rays travel through an object without leaving radioactive material behind. Food may be irradiated by exposure to cobalt and certain caesium isotopes these methods are considered cold sterilization. There is an exception to meats, x-ray is still allowed, but it takes higher doses of radiation to kill the parasites, salmonella bacteria, and other organisms. There is a warning, meats that are irradiated by irradiation are darker, fish and seafood become mushy, and irradiation of grains destroy the fats found in grains and make them taste sour.

In conclusion, the pros of use of irradiation outweigh the cons and humanity should now move to embrace the use of this novel preservation technique to enable enhanced global food security and safety.

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