

## A Look to the Future Regarding Present Food Concerns

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### Food Safety

It can be anticipated that advanced technology and its automation will be increasingly applied to a number of problems associated with foods. Large scale product recalls can be expected to occur in the near future involving fresh and ready-to-eat foods such as ground beef, soft cheeses, in addition to fruits and vegetables. There are several microorganisms of primary concern. *Escherichia coli* O157 and its various H serotypes harbor the potentially lethal shiga toxins I and II that can cause hemolytic uremic syndrome (HUS) and resulting death. The presence of various serotypes of *Salmonella enterica* on fruits and vegetables is notably hazardous since such foods are frequently consumed raw, and can result in salmonellosis. *Listeria monocytogenes* is a potentially lethal organism resulting in a high incidence of abortions among pregnant women stricken with listeriosis. Foods involving such infection have recently included soft cheeses and fruits. Twenty years ago the number of reported salmonellosis outbreaks in the USA was ~200,000 annually and has now been reduced to ~50,000 annually. This reduction is due to an intense focus “by USDA on “Hazard analysis and Critical Control Points” more frequently referred to as HACCP”, particularly with respect to sanitary practices involving red meat and poultry processing.

Molecular methods are presently available for the rapid, near real-time detection of such microorganisms utilizing the polymerase chain reaction (PCR), and Real-time PCR (Rti-PCR). Both of these methods allow detection and quantification of relatively low numbers of targeted cells, down to ~0.1 colony forming units (CFU) per gram of food sample after enrichment for 4 to 12 hrs. Without enrichment, 0.5 – 1.0 CFU per gram of food can now be detected with a complex food matrix such as ground beef with a total assay time of no more than 4.5 hrs. [1]. This is sufficiently rapid to allow detection of a targeted infectious organism well before shipment and distribution. Four major problems arise with such direct methodology that excludes enrichment cultivation. One concerns the fact that with a product

such as ground beef, with a notably high fat content, microorganisms tend to partition primarily into the lipid phase, making it difficult to capture them in the aqueous phase after stomaching. This problem can be overcome with the use of beta-cyclodextrin [2], soluble starch [1], or enzymatically modified starch [3]. All three of these carbohydrates have the ability to hydrophobically capture and bind the terminal methyl groups of individual fatty acid molecules in triglycerides or fats. A second major problem concerns the removal of large amounts of PCR inhibitors such as hemoglobin and myoglobin. Minicolumns do not have sufficient capacity to remove all of these potent PCR inhibitors derived from a 25g sample of a complex food matrix such as ground beef. The use of gram quantities of activated carbon appropriately coated with bentonite so as to prevent the binding of bacterial cells to the surface of the activated carbon particles, while still allowing soluble PCR inhibitors to be bound by the submicroscopic pores, has been found to be notably effective [4]. A third problem associated with the direct molecular detection of low number of food born pathogens concerns the ability of the PCR and Rti-PCR to indiscriminately amplify a DNA target sequence from both dead and viable targeted bacterial cells. This problem, however, can be circumvented by exposing the extracted and concentrated cells immediately prior to lysis to ethidium bromide monoazide (EMA). EMA is a bifunctional planar DNA intercalating agent that enters only dead cells with damaged cytoplasmic membranes [5]. On exposure to strong visible light the reactive ends of EMA then covalently link both DNA strands so as to prevent thermal denaturation which is required for DNA amplification. Such selective DNA amplification is ideally suited to leafy vegetable processing, particularly, lettuce and spinach which are now frequently treated with ~100 ppm sodium hypochlorite, which imparts extensive cytoplasmic membrane damage when killing bacterial cells [6]. A fourth problem involves the cost of thermal cyclers which for conventional PCR can now be purchased for ~3,000 - \$4,000. Rti-PCR thermal cyclers cost ~ \$30,000.

However, the cost of thermal cyclers can be circumvented with the use of isothermal DNA amplification, using a constant temperature of 62 °C - 65 °C. This requires only a simple temperature controlled water bath. Such isothermal DNA amplification is exemplified by “Loop-Mediated Isothermal DNA Amplification” (LAMP). LAMP reactions involve 4, 5 or 6 DNA primers and the use of the DNA polymerase *Bst* III from *Bacillus stearothermophilus* which of necessity lacks 5' to 3' processing possessed by Taq polymerase [7]. LAMP reaction are completed in 60 min. and yield ~1,000-times more amplified DNA than either the PCR or Rti-PR. Positive tubes can be detected using a variety of fluorescent methods in addition to visual or photometric assessment of turbidity from extensive precipitation of magnesium phosphate. As these methods are further refined and automated, the incidence of major food recalls due to contamination by bacterial pathogens should notably decrease over the next several decades, reflecting a safer food supply.

## Identification of species and the presence of genetically modified organisms (GMOs)

Molecular biology has also made it possible to detect what species of animals or plant are present in various animal and plant food products in addition to the presence of GMOs [8]. For example, a recent study on herbs [9] has indicated a significant level of unlabeled species substitution. Herbs are not regulated by the Food and Drug Administration and for most herbs, the complete spectrum of biologically active components has yet to be identified. This would appear to be a fertile area for analytical chemists able to undertake HPLC/mass spectroscopy analyses.

## Safety of dietary supplements

The recent increase in the incorporation of caffeine into dietary supplements is of grave concern. There is clinical evidence that the consumption of ~480 mg of caffeine can be lethal. The average cup of coffee contains 75 – 200 mg of caffeine. This is sufficient to stimulate most people and to keep some awake at night. At least one commercial caffeine drink of 2.5 oz. contains a total of 350 mg of caffeine which is stated to consist of two doses. The consumption of two such containers over a short period of time clearly exceeds the potentially lethal dose of 480 mg. This is a classic situation where the pharmacologically active dose for humans is just below the lethal dose. The present oral LD50 for rats is considered to be 22 mg/Kg. For a 150 lb human this equates to ~1.5g which is at least a 3-fold higher dose than clinical deaths from caffeine overdoses would suggest. The recent availability of pure caffeine and its growing use by bodybuilders has also resulted in known cases of death from cardiac failure. Hopefully, such products will be regulated by the FDA with poten-

tial hazards clearly stated on the labels.

The Journal of Food and Nutrition should serve as an ideal venue for dealing with forthcoming advances involving these and related areas.

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