Are food and environmental toxicants 'overdetected' by bioassay?

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The definition of clean bioprocessing of foods should relate to the discharge of clean effluents that do not disturb functional ecosystems in the environment. Clean effluents should not pollute aquatic or terrestrial environments by increasing the levels or determined bioavailibility of reactive oxygen species (ROS), traces of heavy metals (e.g. arsenic, mercury, lead, cadmium) or radionuclides or other ecotoxicants such as the endocrine disrupting chemicals (e.g. xenoestrogens), herbicides and pesticides. Some saleable foodstuffs can contain very small amounts of potentially toxic components. Strategies dealing with potential toxins should be aimed at targeting remedial bioprocessing to safe limits as stipulated by regulatory agencies, rather than trying to eliminate all toxic components of food so that they can no longer be detected by bioassay or other highly sensitive techniques. The ability to detect even the tiniest amounts of toxicants may not be necessary. This 'overdetection' could lead to inappropriate courses of action in some situations.

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Many food-additives are antitoxicants (and are often antioxidants) that act mainly by opposing the deleterious effects of reactive oxygen species (ROS). Vitamins C and E are the main dietary antioxidants and the polyphenolic antioxidants protect biomembranes from ROS. The opportunity now exists for processed foods to be 'multi-functional foods', that is, contributing to health benefits in the long term, while at the same time having their dysfunctional – or toxic – components eliminated using antitoxicants. Food-derived environmental pollutants could be subjected to clean-up technology, for example the activated-sludge microbial biorestoration of domestic sewage. Moreover, gut microfloral metabolites add to the loading of sewage discharge. Therefore, successful clean food-bioprocessing could benefit from identifying 'green chemistry' pathways of conversion that avoid the inadvertant appearance of bioprocess or gut-generated environmentally undesirable chemicals in drinking water. Other sources of ecotoxicants, such as the ubiquitously distributed traces of industrially derived xenoestrogens [1], can lead to the adventitious partial feminization of male fish and other susceptible species in aquatic environments. It is fortunate, therefore, that enzymic processes usually produce fewer side-products than do microbial or chemical processes, and that the enzymes involved do not usually contaminate the food product when they are immobilized to solid supports, such as crystalline cellulose, especially if covalent bonding of enzymes is used [2].

Enzymes added in food processing

Simple hydrolases have often been the preferred choice in food bioprocessing. More selective procedures, however, are being developed for bioprocessing foods that use oxido-reductases, such as particular isoforms of cytochromes P450 [3]. These isoforms are chosen from among the 750 known to occur naturally, or they can be made in recombinant-DNA yeast. However, a few isoforms (cytochromes P450 1) have the potential to activate carcinogens such as benzo(a)pyrene (traces of which are dietary contaminants formed at high temperatures during toasting and flaming). Carcinogenic metabolites are highly reactive and usually combine with food components before they injure tissue DNA: antitoxicant biomolecules are abound in some foods, including vitamins E and C (and polyphenolic antioxidants).

Avoidance of ROS production in foods

The deterioration of foods, especially those that are rich in lipids, can cause food to become rancid. This is caused by peroxidised lipid, leading to the generation of ROS [4]. Production of ROS is best avoided by adding appropriate antioxidants to the food, including enzymes such as glucose oxidase (plus catalase), that reduce the level of oxygen (required for generation of the ROS) to very small amounts in the presence of excess glucose. Build-up of ROS in reused (recycled) cooking oils in high-temperature cooking could constitute a health risk. However, flavour and odour changes in rancid cooking oils, fats and lipid-containing foods are likely to deter people from using them, thereby lowering the risk-to-hazard ratio. Another cause for concern is pesticide residues in some fruits and vegetables [5]. Bioprocessing of such foodstuffs could benefit from the inclusion of a recycle stream that applies antitoxicant procedures, such as the addition of enzymes to remove adventitious auto-oxidation products, before sale and consumption. Biomonitoring and neutralisation of toxicants using antitoxicants requires further research but even more-sensitive bioassays that often rely on biomolecular recognition (e.g. of specific antibodies) are already emerging.

Are toxicants 'overdetected' by biomolecular-recognition assay techniques?

Techniques of biomolecular recognition of toxicants in foodstuffs usually comprise affinity binding, which can be either natural or can involve the use of

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antibodies produced in animals or cell cultures of animals. The natural method can involve using an enzyme substrate or inhibitor, biotin (a β-vitamin) for avidin (a protein obtained from raw egg-white), and mannose (or glucose) for concanavalin A (a lectin protein obtained from the Jack-bean). The antibody procedure would use the analyte as hapten group bound to antigenic proteins. The antibodies, produced by polyclonal and monoclonal techniques, have an extremely high specific ligand-binding affinity within the range 10^6–10^{14} (with a dissociation constant of 10^{-6}–10^{-14} M for the liganding analyte). Because of this remarkably high affinity, it is likely that only a few hundred molecules of ligand (analyte) would be sufficient to trigger the assay-positive response in foods or at environmental biomonitoring sites, which often then results in the initiation of bioremediation. Although biomonitoring of the environment serves to alert authorities to initiate bioremedial procedures, over-reaction sometimes occurs because the sensitivity of biomarker detection systems is so high. Therefore, a threshold concentration of ecotoxic analyte that would have to be reached before bioremediation is deemed necessary must be decided *a priori* on a case-by-case basis. Such decisions would need to be made even for cases in which it is suspected, on theoretical grounds, that a no-injury level is absent. For example, a no-action policy for bioremediation has been recommended in some situations, such as marine oil-spillages [6], because the food-based species ecowebs can return the ecostat to the pre-spill situation.

Examples of analyte 'overdetection' can be found for female hormone-like biomodulators (endocrine disrupting chemicals) such as xenoestrogens of industrial origin: some extremely weak oestrogen mimics, such as phthalates, have been detected in the environment but their effect on male neonates is unproven. Nevertheless, the observation of hermaphroditism in fish, polar bears, alligators and seagulls does merit serious consideration. These *in vivo* bioindicators might prove to be more important in assessing the impact of toxicants on the environment than using sensitive biomarkers such as in oestrogen-mimic liganding to oestrogen-receptor protein in binding-assays (using human oestrogen-receptor produced in recombinant yeast). In addition, xenoestrogens, dietary phytoestrogens (and their gut metabolites) might require distinctly different policies in relation to bioremediation. In the future, assay of ecotranslators as a measure of species modulation might well require ecotranscriptor-assay as a predictor to recognise stresses such as oxidative stress by ROS [7]. Habitats can be species-stabilised by food-chain ecowebs comprising as few as five species [8]. This quasi-equilibrium state might be a reflection of a steady-state ecostasis controlled perhaps by the limiting presence of one species in an ecostat situation. Disturbance of ecostasis might also be observed in mixed-culture fermentations *in situ* (aquatic and terrestrial) and *in vitro*. Trade-off substitution of species (ecovirement) might become more acceptable as the biomonitored level of pollutants such as the xenoestrogens (hormone-like biomodulators, endocrine disrupting chemicals) rises. Similar problems can arise in the 'overdetection' of toxicants in foodstuffs, causing them to be wrongly classified as dysfunctional.

Retaining functional components of food for a cleaner environment

The due recognition of functional foods that promote long-term health benefits has moved one of the focuses of food technology into novel areas of food-function-bioprocessing (nutritional biotechnology). Considerations of gross digestibility of modified carbohydrates, cross-linked proteins and non-digestible fats have been augmented by concern for the bioavailability of micronutrients, in addition to the known vitamins and minerals. Micronutrients in foods must now include antioxidants such as phytoestrogens (e.g. in soya) and the redox polyphenols (e.g. in tea and apples). Natural oestrogens (excreted in faeces) could be a gender-hazard to fish. Thus, due regard must be given to human tissue metabolites of micronutrients formed by biotransformation, for example those formed by the ubiquitous enzymes, cytochromes P450, found mainly in the liver and brain [3]. Bioconversion by these and other enzymes also occurs in meat, fish, fruit and vegetable bioprocessing owing to the release or activation of endogenous enzymes (as in fruit ripening) or by possible side-effects of affordable crude-enzyme 'mixed-catalytic preparations' added for other technical benefits.

Clean-bioprocessing might, in future, define the limits for permitted effluent components both from bioprocessing and after human digestion [9]. Nutritional biotechnology, therefore, could become a part of the biostrategy of reducing environmental pollution through the consumption of designer foods that have retained functional components but are nevertheless low in recognised toxicants (natural or ROS-generated) that could contaminate effluent. In addition, more attention could be given to designing cleanly digested foods and beverages.

ROS-created toxicants in food: avoiding retention or discharge

One of the dangers of food technology is that foodstuffs could lose some of their nutritional value during bioprocessing. The reduction in the nutritional value of food is often caused by ROS, which are ubiquitous because their generation from the oxygen essential for energy production in aerobic biota is universal, ranging from environmental flora and fauna to *Homo sapiens*. Lowered yields of product in fermentation processes used to manufacture human foods (including alcoholic beverages) might be caused by ROS-induced damage to enzymes and cells. Such damage can occur despite the protective addition of selective antioxidants [10] (when oxygen mass transfer considerations will allow this). Antioxidants could be added after the logarithmic phase of growth, before the production of required secondary metabolites, for example in fungal antibiotic production or in brewing with yeast. This approach might be useful for protection of human therapy proteins, such as insulin, specified by recombinant-DNA in yeast, by glutathione peroxidase (contains selenium) and a variety of other enzymes that afford protection *in vivo* against ROS, such as superoxide dismutase. This enzyme converts the superoxide free-radical anion to oxygen and hydrogen peroxide; catalase (contains iron) can remove the hydrogen peroxide (a form of ROS that is not a free radical) by converting it to oxygen and water [4].

Antioxidant enzymes such as glutathione peroxidase [10] offer a measure of protection and this enzyme is therefore an important component of the body's defences against ROS. Selenium is required for the production of glutathione peroxidase and is an integral part of the enzyme. The mineral is beneficial in the diet at ~50 µg per day, but much higher doses are toxic, and therefore selenium can be referred to in the context of hormensis [11] as a 'benemin' (beneficial in small doses only). Lipid peroxides, which are also toxicants, are formed by oxygen uptake in rancid oils and in cellular phospholipid membranes in chain reactions that produce ROS, among other things [1]. Antioxidants can prevent bioinjury of cellular macromolecules such as DNA, RNA, proteins and phospholipids [12]. Dietary antioxidant-protective biochemicals include the phytoestrogens such as the soya-derived genistin and daidzin, which are polyphenolic in structure. They are glycosides that are converted to the corresponding aglycones, genistein and daidzein in the gastrointestinal tract by microorganisms (further metabolites are then generated *in situ*) [12]. Notwithstanding this protective armoury, even mild chronic toxic insult can cause unrepairable damage to essential proteins, often leading to disturbance of regulatory and signalling mechanisms needed for good health and retention of youthfulness. In addition, mutagen-induced damage to DNA that is not repaired can lead to carcinogenesis, especially in older people. Functional foods, such as soya offer some promise of benefit, as do vitamins (especially vitamins C and E) and appropriate minerals in the diet.

Bioprocessing of functional foods will also need biomonitoring, especially if isoforms of cytochromes P450 (oxidoreductase enzymes) are used [3]. Isoform 1A, for example, can activate benzo(a)pyrene present in 'burnt' food to form mutagens that might be carcinogenic unless removed by reaction *in situ* before the processed food is ingested. Furthermore, these mixed-function oxidase enzymes can generate ROS from the molecular oxygen that they use, especially during futile cycling at low substrate concentrations. Bioprocess ROS stem from the use of atmospheric oxygen: this highly reactive gas is biologically derived from the photolysis of water by photosynthesis in green plants caused by the absorption of sunlight at red wavelengths. It is noteworthy that photosynthesis removes a greenhouse gas, carbon dioxide, in the formation of carbohydrate (this qualifies, therefore, as a natural 'green-chemistry' process).

Concluding remarks

Special care is clearly needed in the handling and storage of lipid-rich foodstuffs. Rapid development of efficient antioxidant biochemicals and enzymes is desirable to combat the generation of lipid alkoxides and peroxides by the action of ROS during food bioprocessing, as part of the clean approach. Continuous monitoring of these free radicals and their toxic products should be beneficial in controlling the consumption and excretion of oxidised lipids, DNA (RNA) and proteins (peptides) in human and animal food. It is clear that a realistic appraisal of clean food-biotechnology needs to balance carefully the retention of mainly ROS-generated toxicants (owing to biomolecular injury of cellular membrane components and other cellular macromolecules [12]) against allowing these toxicants to contaminate the effluent stream from the bioprocessing [13]. Value judgements will have to sit alongside legislative requirements and economic considerations. Technological problems will need to be addressed to achieve the fine balance, which will to be judged in relation to public opinion.

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