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Mycotoxin Prevention and Decontamination

HACCP and its Mycotoxin Control Potential: an Evaluation Of Ochratoxin A in Coffee Production[1]

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The views and opinions expressed in this paper are those of the author and do not necessarily represent those of FAO, WHO or UNEP.

Abstract

Coffee is produced in over 50 nations that contain well-watered tropical highlands and constitutes an important source of income in agricultural districts as well as hard currency for the producer nations. A revision in the scientific view of the significance of ochratoxin A (OTA) to human health has led coffee consuming nations to consider legislation to prevent the importation of coffee containing more than minute amounts of this toxin. We have concluded two years of field and laboratory study aimed at providing the information required to develop a HACCP model to prevent accumulation of OTA in coffee. Below we describe coffee production as a world wide activity and present a HACCP analysis, the preferred method of safety assurance (EC directive (91) 525) in the food industry, of coffee production and a model ready for field testing and verification.

Introduction

Ochratoxin A

1. OTA is a renal toxin that is carcinogenic, teratogenic, immunotoxic and may be genotoxic. It is known to cause nephropathy in pigs and is likely to be involved in the aetiology of the human kidney disease 'Balken endemic nephropathy' and urinary tract tumors. Since the publication of data indicating genotoxicity, which if proven would put OTA on a risk par with aflatoxin, the origins of OTA in the diet have moved up the public health agenda.

2. In Europe OTA enters the human diet most importantly in cereals and cereal products. It has also been reported in meat and meat products, wine, pulses, beer, dried fruit and coffee. Best current estimates show a mean OTA intake in Europe of between 0.2 and 4.6 ng/kg b.w./day based on dietary analysis and blood serum analysis but regional differences are notable. It is fairly heat-stable and survives baking though significant degradation in the higher temperatures of coffee roasting has been reported. OTA is labile in high pH conditions by virtue of its lactone ring.

3. A large list of *Aspergillus* and *Penicillium* species are reported to produce OTA but most are the result of misidentifications in these difficult genera. The most important source of the toxin in the North Temperate Zone is *Penicillium verrucosum* and that in the tropical and sub-tropical regions is *Aspergillus ochraceus*. When present at all, the amounts of toxin reported in food products are generally in the range of a few tens of ug/kg down to the detection limit which is now less than 1 ug/kg. Occasionally extreme amounts are found and experimentally there is no difficulty in producing OTA at a level of hundreds of **mg**/kg, thus there is the potential for much higher exposure under poor conditions.

The coffee Plant

4. Commercial coffee production can only be conducted in well-watered tropical and sub-tropical highlands that are nominally frost-free. Two species are of commercial importance, *Coffea arabica* (arabica coffee) and *Coffea canephora* (robusta coffee). They are shallow-rooted low trees that occupy a forest under storey niche in the wild. Flowering intensity is regulated by light intensity and the onset of flowering is initiated by the first rains following a period of drought and further rains after flowering are required to ensure good

fruit-set. Sensitivity of the plant to fruit-bearing stress can be seen in the biannual cropping cycle where a high yield season is followed by one of low yield. Ripening is markedly uneven and often ripe and green fruit (referred to as 'cherries') can be seen together with flowers or pin-head stage fruit on the same tree. Harvesting is extended over no less than three months, as a consequence, and can be nearly year-round in places with well-distributed rainfall. The seed has no resting stage and a rapidly declining viability. The seedlings are difficult to raise and this is done in specialist nurseries to a stage beyond the tiger ear (cotyledonary leaf) stage.

5. Of the two, *C. arabica* is a smaller (up to about 5m) and more slender tree with smaller leaves. It requires higher input costs to cultivate and in addition has a shorter life in production than robusta. The flowers are more or less evenly distributed on lateral shoots and are self- pollinated. Eight or nine months are required for fruit maturation. This species is indigenous to the highlands of southwest Ethiopia where it forms extensive dense expanses beneath an upper storey of *Albezia* species, *Cordia abisynica* and *Croton* species, amongst others. Although it will grow at sea level, commercially it requires the climate found at 700 to 2500m and with good husbandry can remain productive for up to about 40 years.

6. *Coffea canephora* is indigenous to central Africa. Its dense and fibrous root system is often visible at the soil surface, and the large leaves have a characteristic drooping habit. It is taller than *C. arabica* at 5 to 6m and more heavily wooded with the flowers borne in clusters at the nodes. This species requires cross-pollination and fruit maturation requires nine to eleven months. As the name implies, robusta is generally the more vigorous species with resistance to several diseases that are serious problems in arabica coffee, most notably rust (*Hemileia vastatrix*), and it has lower cost implications in cultivation. Robusta is generally cultivated at altitudes between 400 and 1500m. The author has seen trees of 80 years and more, still bearing well.

Coffee processing

7. Before coffee beans are ready for roasting, the surrounding fruit tissues must be separated from them. One of two approaches to accomplish this is employed. ‘Wet processing’ is the generic term for processing methods that include a fermentation step. ‘Natural processing’ refers to methods in which whole fruits are sun-dried. Within these two generic terms lie important regional and local variations in how they are conducted. Arabica coffee is produced by the wet process with the very important exception of most of Brasilian and Ethiopian production. Robusta coffee is produced by the natural process, with the exception of a part of Indian production. Much of coffee processing takes place on the farm; that which is not so processed is taken daily to a local processing facility often owned collectively by the farmers.

8. In regions where natural processing is practised, either the bulk of the crop is allowed to dry on the tree and all of the fruit harvested in one pass (strip picking) or only the ripe cherries are picked (finger picking) over several passes according to the cost and availability of labour. After strip picking the tree-dried fruit (boia) is floated away from the ripe and unripe cherries by water buoyancy in a process referred to as ‘semi-washed’ where water supply and capital investment permits. This step ensures that high and low moisture content fruit are dried separately. Drying is variously conducted on compacted earth, cow dung plaster, brick, or concrete patios or on tables and sometimes mechanical drying is employed. Coffee prepared in this way is called cherry coffee at this stage and it can be transported and stored as is (en casca) or shelled and stored as green coffee (the naked bean prior to roasting).

9. In wet processing, the fruit must be uniformly ripe so finger picking and hand sorting (garbling) must be employed. The skin and much of the underlying pectinaceous mesocarp of this ripe fruit is removed mechanically (pulping) and the remaining mesocarp that adheres to the parchment (a tough integument that encloses the bean) is removed by fermentation. The fermentation is followed by a washing step, sometimes a soaking step, and then sun, or exceptionally machine, drying. Coffee prepared in this way is called ‘parchment coffee’, named for the integument that encloses the bean. It is normally transported and stored in this form until the final, pre-sale preparation.

10. There is a tendency to make an erroneous identity between cherry coffee prepared as described above and 'mbuni' a term that represents the dried cherries rejected from wet processing. In many regions the ripe fruit that floats in water, an indication of the presence of a diseased or aborted bean in the fruit, is removed from the main production. These ‘floats’ and the unripe and overripe fruits separated by garbling are dried together as ‘floats coffee’ or ‘mbuni’. The low quality of 'mbuni' is attributable to the lack of care in drying and not, as is sometimes implied, to the procedure of drying <u>cherries</u>, *per se*.

11. Once the bulk green coffee is produced some process of grading and sorting will be conducted. These vary widely in the different producing nations but in broad terms visibly and physically discernible defects will be sorted out and graded into low quality classes and sorting into size classes will be conducted. Price at auction will be determined by the size (larger bean size fetches higher price) and quality class and by the results of cup testing of the beverage brewed from a sample of the lot. Due to certain organoleptic considerations, arabica coffee is more sought after in the roast and ground market and commands a higher price than robusta. But robusta yields dissolved solids in far greater amounts than arabica when brewed and is therefore essential to the soluble coffee market.

12. Coffee and cocoa are perhaps unique amongst commodities in that their value and desirability is entirely attributable to organoleptic features and there is no single formulation of attributes that can suit the varied forms in which coffee is drunk. Coffee is never discarded with the result that low grades are consumed in the producing countries. Any coffee that may be deemed unfit for human consumption for whatever reason has no alternative use such as in animal feed or alcohol production.

Developing HACCP Protocol
The Process Model

13. We have examined production chains in six nations on three continents (Brasil, Ethiopia, India, Indonesia, Kenya and Venezuela) to provide the diversity in production procedures necessary to develop an accurate process model of coffee production. In detail the procedures differ markedly, as noted above, but by concentrating on the *purposes* of the various activities, they can be understood and unified. A diagrammatic representation of a unified process model for coffee production can be found above the dynamic model in figure 1.

14**.** **setting and development of fruit:** Features such as soil fertility, pH, rainfall and rainfall patterns, availability of labour and capital, size of the farm, altitude, physical aspect of the land, markets for secondary crops and traditional practices all contribute to the determination of horticultural practice in the coffee orchard. The goal is to consistently produce sound fruit in good yields. Yield must always be evaluated against inputs but in gross terms, unmanaged forest coffee may yield 200 kg of green coffee/ha while high density, unshaded orchards may yield ten times this figure. Managed forest coffee reduces inputs by exploiting natural (free) mineral cycling and water management systems as well as providing secondary crops and habitats encouraging biological control agents but coffee yield rarely exceeds about 800 kg/ha.

15. **harvesting and sorting:** The method of processing, wet or natural, exerts an overriding influence on how these activities may be conducted. The underlying problem is the extreme unevenness in the ripening of the fruit. In all cases the goal is to group fruit into classes that are uniform - ripe separated from non-ripe in wet production and high water content fruit from low water content fruit in natural processing. The removal of fruit containing defective beans from the main production chain is also accomplished in some protocols by water buoyancy separation. Floats coffee can comprise more than 20% of the harvest in bad years.

16. **drying and separation of tissues:** This is the most complex and varied area of the process although it is fundamentally the method of processing, wet or natural, that determines how these activities are conducted. Rapid and uniform drying of the beans represents the most concise formulation of the objective of this link in the production chain. The elimination of the fruit tissues and thereby the associated microbial load, is also a significant aspect of these procedures.

17. Verified in several countries, the normal sun-drying rate on patios is a drop of about 0.06 in equilibrium relative humidity (erh) per day. This applies to both parchment and cherry coffee although cherry coffee requires about two days on the patio before drying (erh reduction) commences. The use of drying tables, in place of patios, slows drying rates so that the time required for drying is increased from about two weeks to three but their use makes protecting the commodity from rain simpler.

18. **grading out of defects:** The frequency of defects gives a coffee lot its classification. Removal of defects by hand or machine can improve the classification and thereby the value (although reducing the weight) and this is the object of these procedures. The removal of defects, which are well described characteristic faults, could be involved in OTA occurrence in the final product if any defect corresponds or tends to correspond to the activity of the fungi that produce OTA.

19. **storage and transport:** Normally coffee is 'rested' for some three months after drying in the producing areas, on farms, in co-operatives or curing works, before transport to auction. Coffee for export invariably spends some time in the tropical lowlands and then the north temperate zone with all the temperature and humidity changes implied by the transit from tropical highlands. The objective of all transport and storage is to maintain a low and uniformly distributed moisture content in the commodity (11-13% ≅ 0.55-0.65 erh), under which conditions it is microbiologically stable. In a model system we have shown water can be rapidly redistributed via the gas phase in a mass of green coffee beans.

Summary of the Mycological Findings

20. We have found nothing to alter the traditional view that *Aspergillus ochraceus* growing in bean tissue is the source of OTA in coffee. None of the *Penicillium* isolates

and only one of seventy-five niger group aspergilli tested produced OTA in culture whereas about 80% of 42 *A. ochraceus* isolates did so. Also, it was always possible to isolate *A. ochraceus* from OTA-containing coffee samples collected from farms. This is not always the case in samples from long-term storage, however. Not all *A. ochraceus*-containing samples also contained detectable OTA and this observation is an important one relating to the distinction between the presence of the toxin-producing fungus and that of the conditions necessary for toxin production.

21. It proved possible to characterize fungal communities associated with the stages in coffee production that were remarkably uniform internationally although significant local deviations from the 'typical' were noted (Table 1). We attribute this to the existence of mutualistic interactions (all participants benefit) producing stable selective conditions in coffee-growing habitats, which include the coffee tree itself, of course.

Table 1. A generalised view of the occurrence of the most common fungi in the coffee production chain. Early is up to about 6 months, long term is after about 18 months.

Crop Development	Processing	Post Processing		
		Early	Late	Long Term
Fusarium stilboides *Candida edax* *Cryptococcus spp.* *Cladosporium spp.* *Aureobasidium pullulans* *Penicillium brevicompactum* *P.citrinum* (in Kenya)	in general: *Cryptococcus spp.* *C.edax* *F.stilboides* only in wet proc.: *Kloekera apiculata* only in natural: *P.brevicompactum* *Cladosporium spp.* in tree-drying: *Eurotium spp.*	*Cryptococcus spp.* *C.edax* *F.stilboides* *P.brevicompactum* aspergilli of the: niger group ochre group flavus group *Eurotium spp.*	aspergilli of the: niger goup ochre group *Eurotium spp.* *Wallemia sebi*	*Eurotium repens* *E. rubrum* niger group aspergilli *A.penicilliodes*

22. Four types of experiments were conducted to assess the effect of the process on fungal communities. The first is a sampling experiment wherein a single day's harvest is sampled every few days as it is processed. The second is also a sampling experiment in which several samples of coffee at different stages of processing are made on one day. The third is a direct comparison of different processing methodologies where a sample of fresh cherries is divided and processed in the laboratory. Lastly, a fresh cherry sample can be manipulated in the laboratory to assess the consequences of hypothetical circumstances such as a heavy superficial contamination of a particular fungus or removal of the natural external microbial flora. In only the last of these protocols is the initial condition, the disposition of the fungal community at the time of harvest, controlled. An extensive programme of OTA analysis of field samples was also conducted.

23. Some of the more pertinent conclusions to emerge from these studies fo llow.
a) *A. ochraceus* and other aspergilli with similar physiological properties are sometimes present but appear to be uncommon in coffee at harvest.
b) The most likely source of this early *A. ochraceus* contamination is the coffee rhizosphere but the route of infection has not yet been elucidated.
c) Plant disease, per se, does not generally increase the fungal infection rate of beans though some specific diseases can do so.
d) *A. ochraceus* can apparently become established in a local region as shown by its consistent presence in coffee of certain locales throughout two seasons.

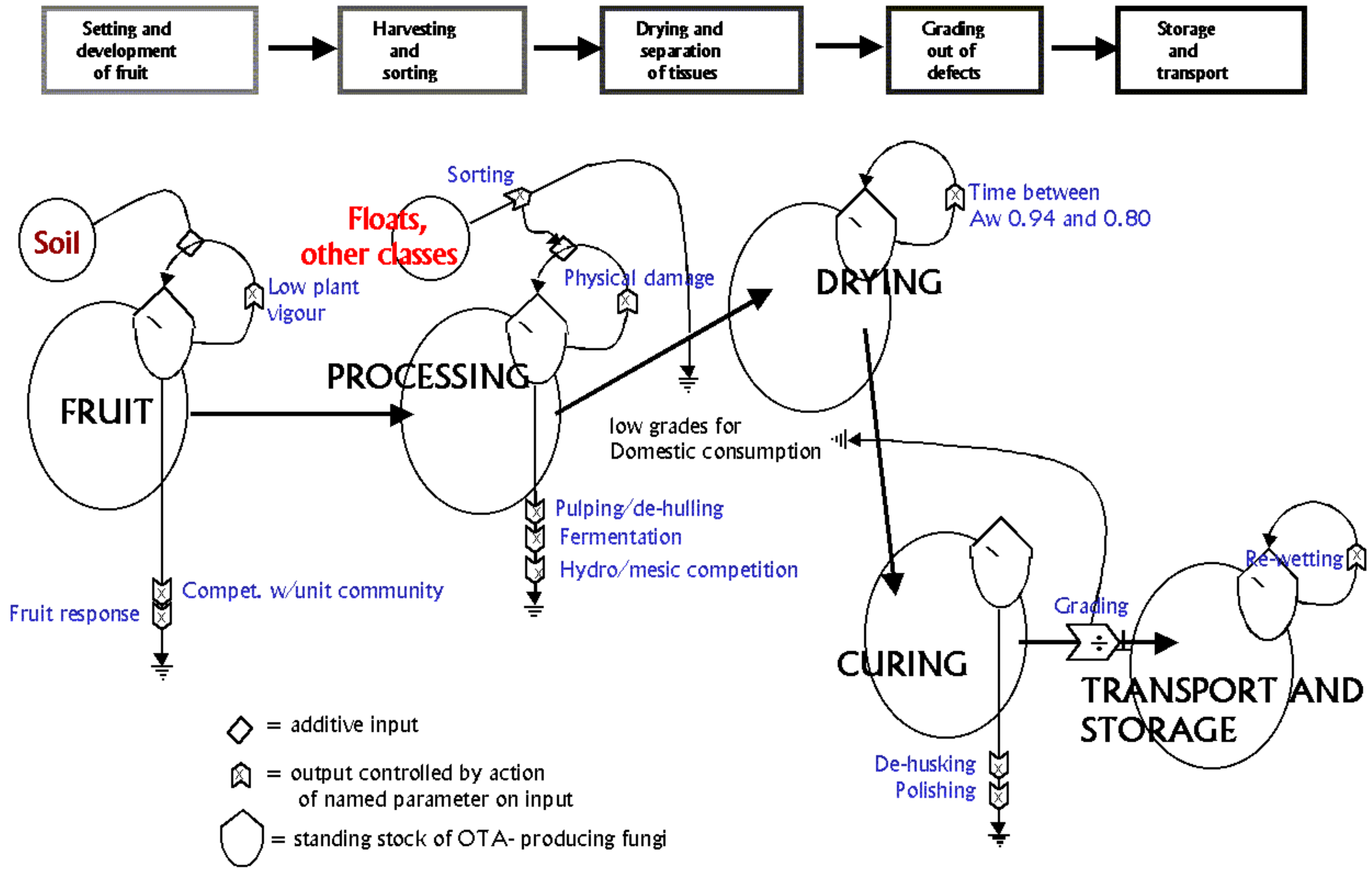
e) Even gross contamination of fresh cherries or of fermentation liquor does not result in *A. ochraceus* infection of bean tissue or OTA accumulation in beans under normal processing conditions.
f) The natural microbial flora of the cherry has a protective influence with respect to *A. ochraceus* invasion from the outside.
g) Cherries in a wet state are not prone to *Aspergillus* development.
h) In some samples *A. ochraceus* was observed for the first time or observed to increase in frequency during drying even when this was conducted in circumstances where *de novo* contamination was unlikely (i.e. the laboratory)
i) Coffee berry borer (CBB; an obligate insect pest of coffee)-affected beans are not more prone to general fungal infection but are observed to have a higher *A. ochraceus* infection rate in samples where this fungus is present at a significant frequency.
j) During fermentation there is typically a reduction in the biological load of filamentous fungi in favour of yeasts.
k) If the fungal population at the beginning of parchment or cherry drying is predominantly fermentation yeasts or hydrophilic moulds, a significant reduction in fungal infection will occur. If relative xerophiles are present, however, an increase is likely.
l) A high **frequency** of *A. ochraceus* infection does not necessarily indicate a high degree of fungal development if OTA accumulation is taken as a measure of development. We have identified the period of time the coffee spends in a partially dried condition as the key to converting the potential for mycotoxin production into occurrence of the toxin.
m) Our data do not show 'Floats' coffee to be more likely to contain OTA if dried properly but carelessly dried 'mbuni' samples can contain disturbingly high OTA levels.
n) Outer fruit tissues more frequently contain *A. ochraceus* and OTA than beans but the concentration of the toxin is not always greater in these tissues than in the bean.
o) The size grades do not have OTA implications.

The Integrated (HACCP) Model

24. In figure 1 the findings of the study are presented as a model that shows the significant elements identified in the study simultaneously with their probable relation to each other. These are briefly discussed in HACCP terms below.

25. The soil appears to be the source of OTA-producing fungi for infection. The model identifies low plant vigour as a contributory factor to increasing bean infection but not all causes of loss of vigour are equally effective in this. The two most important factors in reducing or preventing bean infection are competition with the adapted microbial community of coffee fruit and the 'immune' response of the plant directed against invasion by other organisms. Thus far, no feature of horticultural practice has been identified as leading specifically to OTA contamination thus no critical control points have emerged in the orchard. Good Agricultural Practice (GAP) action points are clearly numerous, however. Aspects such as drought or nutrient deficiency should be examined in future.

Figure 1. Integrated model amalgamating the process model (reproduced above it for reference) with the biological studies. The model can be employed to predict performance (what if) to verify its elements and to ascertain where the critical control points lie.



26. The model treats out-sorted classes separately from the main crop and allows for cross contamination if there is a failure of effective sorting. Steps should be taken to protect the local people from some of these classes – one such sample contained OTA at 1050 ppb. Where the cherries are tree dried this sorting step is performed by air floatation of green coffee rather than water buoyancy of fruit. Direct tests have refuted the significance of physical damage and cross contamination in OTA accumulation under good drying conditions but taking precautions to reduce these is a sensible activity in case poor drying conditions follow. The outer fruit layers, especially after drying, were found to contain OTA and OTA-producing fungi more frequently than did the beans thus the removal of this material (pulping, dehulling) reduces the biomass of these fungi. Fermentation is adverse to the development of *A. ochraceus* as represented in the model which we consider to be due to competition from yeasts and filamentous fungi better adapted to high moisture conditions. The same phenomenon applies to the first days of cherry coffee drying.

27. The best-established critical control point lies within the drying procedure. The field and laboratory work has strongly suggested that most if not all contamination (frequency of contaminated beans) has already taken place by the end of the first few days on the drying yard and probably well before. Further growth of *A. ochraceus* (and other species with similar physiological properties) and OTA production is restricted to a fairly narrow window of moisture content which we estimate to be between A_w values of 0.94 and 0.80. The critical control parameter is to maintain a drying rate such that residence in this A_w window is not more than three days. This mechanism explains the observation that some farms produce coffee with a fairly high *A. ochraceus* contamination frequency but coffee that is OTA-free when drying conditions are good.

28. At the curing works de-husking and polishing is well established to reduce OTA content in contaminated batches so it is likely there is a concomitant reduction in biological load of the producing organisms as well. sorting will eliminate the more obvious defects including some resulting from mould growth from the main production. We were unable to identify any pattern relating OTA to the traditional defects, size grades nor to parchment grades (a system of grading of the parchments before fermentation) except as noted above in the case of CBB damage and *A. ochraceus* frequency.

29. Once the product has reached the stage of storage and transport any existing spore load on the outside or, especially, mycelial development within the bean tissue present a potential rather than an actual problem. As the model shows, re-wetting is the trigger to produce an incident. We have shown that water vapour can migrate at a significant rate in a mass of beans. The change in temperature experienced in transit from the tropics to the north or the temperature differential in a container positioned so that there is a hot side and a cool side could cause a redistribution of water leading even to condensation. The principle of this aspect as a critical control point is clear – maintain stable, dry conditions during transport and storage but much more detail is required before this area is fully understood.

General Conclusions

30. The elucidation of the role of Aspergillus flavus and parasiticus in aflatoxin contamination of maize and peanuts took well over twenty years from the identification of aflatoxin as the active agent of 'turkey x disease'. Among the reasons for this slow

progress was the lack of a systematic approach to the problem. The HACCP approach is a systematic approach that draws attention to gaps in knowledge during the model-building process. We believe this approach has an exciting broad potential in agricultural problem-solving applied to any problem arising from biological (including human) interaction. This includes spoilage and plant disease as well as mycotoxin occurrence.

31. The initial model, which is analogous to an ecological model , embodies the interrelations of all the identified elements and contains the means for its own improvement through an iterative process of prediction and evaluation of outcomes. Human activity, including economic motivation as an impetus for activity, can be accommodated in the same way as other animal activity might be accounted for in a natural ecosystem once adjustments for the difference in the currency of interaction are made.

32. Following refinement and field validation the means to minimize OTA contamination will be available as well as an understanding of how best to go about implementing the procedures.

Discussion points

33. Points pertaining narrowly to the case in coffee:
a) Coffee inter-cropping systems vs. intensive cultivation systems
b) Coffee marketing systems and motivation for changes in practice
c) Wet vs. dry processing and market demand
d) Significance of uneven ripening to coffee processing
e) Farm scale as an economic factor in coffee production
f) Monitoring of moisture content and appropriate technology
g) Responsibility and risk related to legislated contamination limits

34. Points pertaining broadly to the application of HACCP to agricultural problems:
a) Application of HACCP to outdoor (uncontrolled) processes
b) Informing HACCP models with socio-economic factors
c) Agricultural systems as special cases of ecological systems and their evaluation
d) Identification and potential of 'natural (free) farm inputs'
e) Concept and relevance of relative adaptation and microbial interaction

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