SORTING OF COFFEE BEANS FOR 'POTATO DEFECT' IN EAST AFRICAN COUNTRIES

A Thesis

by

SHRADDHA PRAKASH WAIKAR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2011

Major Subject: Mechanical Engineering

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ABSTRACT

Sorting of Coffee Beans for 'Potato Defect'

in East African Countries. (May 2011)

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Since ancient times, coffee has been a savory drink for most of the world's population. It is the second most widely distributed commodity after crude oil in the world. Hence, there has always been a pressure on the coffee industry to produce more volume of good quality coffee. The coffee industry has not been able to meet this increasing coffee demand due to various reasons, such as low crop yield, high coffee rejection rate *etc.* Historically, the coffee production industry has had high rejection rates due to inadequate knowledge about the defects that plague coffee and the lack of research to detect and eliminate the defective coffee beans.

In this thesis, an attempt has been made to minimize the rejection rate of coffee beans due to a specific defect called "Potato Defect". Potato defect is very prominent in East African countries for reasons not yet known. It is caused by an increase in the concentration of 2-isopropyl -3-methoxypyrazine (IPMP), present in parts per billion concentration in coffee beans. In this thesis, various techniques have been evaluated to detect the increased concentration of IPMP, and then eliminate the 'potato defect' infected coffee beans. As these proposed techniques need to be implemented on an industrial scale, special care has been taken to keep the inspection time of coffee beans as low as possible to minimize its negative impact on the overall coffee production

rate.

Considering both sensitivity and time, non destructive methods such as ion mobility spectrometry, cavity ring down spectrometry and electronic nose were assessed for their suitability to identify low concentrations of IPMP in the complex matrix of coffee volatiles. Experiments were also conducted by Solid Phase Micro Extraction (SPME), followed by multidimensional gas chromatography with simultaneous olfactory and mass spectrometric detection (GC- MS-O) technology to validate information related to the 'potato defect'. GC-MS-O could detect IPMP present in whole green coffee beans while other researchers only detected IPMP in ground coffee.

The findings of this thesis opens the doors for the coffee industry to establish a non destructive, sensitive methodology to analyze further coffee aroma.

To People in Rwanda and Prof Bollfrass

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CHAPTER I

INTRODUCTION

This chapter provides a brief history of coffee growing, describes the current markets for the African coffee industry, outlines the need for this project and ends with a problem statement.

Coffee crops are grown in different parts of the world as shown in Figure 1[1].

Fig. 1. Coffee growing regions in the World

The journal model is *IEEE Transactions on Automatic Control.*

Amongst these regions, Africa is one of the leading coffee growing continents and contributes to around 10 percent of world's coffee production. The annual coffee production in Africa in the year 2009 is shown in Figure 2[2].

Fig. 2. Annual coffee production in Africa in 2009

Although coffee production is a major source of revenue for African countries, they lack leadership and do not have significant market share in good quality coffee production. The profit margin in the African coffee industry is too low due to low crop yield, insufficiency of the coffee distribution system and the high rate of coffee beans rejection. Farmers in African countries have not been able to make much headway in good coffee production due to inadequate knowledge and skills to grow coffee and overall lack of local research about coffee.

Good quality of coffee can also be produced by detecting and eliminating infected coffee beans. This would reduce the rejection rate of good quality coffee and also improve the yield of coffee production. The specific coffee defect discussed in this thesis is 'potato defect'. 'Potato defect' is endemic to the Great Lakes region of Africa *i.e.* Burundi, Kenya, Tanzania, Uganda and specifically Rwanda as seen in Figure 3[3].

Fig. 3. Map of Rwanda with neighboring countries

Rwandan coffee, although very famous for its strong acidic flavor, is less acceptable among coffee consumers because of the presence of unpredictable defects like the 'potato defect'. The Rwandan coffee industry, the government and the farmers want to eliminate this defect to the maximum extent possible.

The 'potato defect' is best described when a cup of coffee smells as freshly peeled potatoes. Apparently, consumers would be able to detect this awful smell only when roasted coffee is brewed. Sometimes this aroma is so prominent that one feels like sniffing the bad smell of tons of potatoes that were packed into a room for long time. In addition to the foul aroma, this defect also ruins the taste of coffee and leaves an unpleasant impression on consumers' mind. It also reduces consumers' desire to drink coffee for some time.

Hence, consumers hesitate to buy such a bad tasting coffee and may get diverted to coffee from other regions. Bad publicity of Rwandan coffee due to the 'potato defect' has affected the Rwandan coffee brand which was once famous for its special aroma. Insignificant physical difference between the 'potato infected' and good beans makes it difficult to sort the defected beans even by the best sorting practices available in Rwanda. A single 'potato defect' infected coffee bean can ruin many cups of coffee and the only solution is to throw away the entire brewed coffee and make a new one. Hence, consumers and coffee traders think twice before purchasing Rwandan coffee.

There are ongoing attempts from institutions like USAID(United States Agency for International Development), GCQRI (Global Coffee Quality Research Initiative) *etc.* to eliminate various defects such as the 'potato defect' detrimental to Rwandan coffee. Scientists currently are working on different aspects of coffee plant pathology, soil nutrients, tracking crop yield with respect to climatic changes *etc.* and small discoveries in any of these fields would bring a revolutionary change for the coffee industry and improve the social and economical life of farmers. This thesis aims to provide a solution for detecting and eliminating the 'potato defect' in coffee. This would save millions of dollars per year by reducing the potato defect related rejection rate of coffee beans.

'Potato defect or 'peasy flavor' in coffee beans is caused by a higher than expected concentration of '2-isopropyl-3-methoxypyrazine (IPMP)' in coffee beans. Identification of coffee beans afflicted by the potato defect is difficult before these beans reach the customers. It is believed that this defect can only be found after coffee is brewed. The origin of 'potato defect' is still undiscovered, since not enough research has been carried out about it till date. One of the reasons for the lack of research is that there are more than 800 compounds in coffee and it is a challenging task to isolate individual compounds and study their effect on coffee aroma. Scientists were more interested in analyzing the final coffee aroma rather than the contents of green or raw coffee beans. Hence, the available information is insufficient to list enough facts about this defect and its presence in green coffee. Similarly, all the chemical reactions responsible for the final coffee aroma have not been discovered yet. Therefore, it is difficult to implement solutions for the prevention of the 'potato defect' in coffee beans, because the origin of this defect is not yet been identified. Also, not all the information researched by scientists is published and the available information is scattered in different languages. With the available information, following problem statement was formulated.

A. Problem Statement

Provide a sorting method to eliminate the 'potato defect' infected coffee beans' without lowering coffee production rate and reduce the probability that these beans occur at the customers'.

Merits of the research : It will

1. increase the price and deepen the penetration of Rwandan specialty coffee in the world's coffee market

2. review the legitimate literature published on potato defect and its probable cause to help other scientists working on it.

3. compile and synthesize 'potato defect' related information for future reference.

4. document information on changes happening to coffee fruits or beans during processing to improve the quality of coffee

5. increase revenue from coffee crops for the African economy

In order to achieve the objectives of this research, different aspects such as coffee processing technology, composition coffee beans, applications of chemical, mechanical or sampling methods *etc.* were studied and are discussed in following chapters.

CHAPTER II

COFFEE PROCESSING TECHNOLOGY

A good understanding of coffee processing technology was required to trace origin and evolution of 'potato defect'. Chemical reactions involved during coffee processing would also have helped to find a catalyst, if exists any, responsible for increased concentration of IPMP in beans. Hence coffee farms and coffee stations in Rwanda were visited to comprehend coffee processing in depth and information is elaborated in this chapter.

Different regions have different coffee processing technologies depending upon types of coffee, geographical and climatic conditions, availability of the sun etc. Coffee fruits containing "beans" are produced by several species of small bush of the genus Coffea. Amongst several coffee species, two commonly grown species are Coffea 'arabica' and 'Coffea canephora'. There are several varieties of coffee arabica grown all over the world and are commonly called as 'arabica'. Where as most common variety of coffee canaphora is known as 'robusta'[4]. Coffee Arabica is superior and richer in flavor than robusta and thus used for high grade savory drinks. Where as robusta is usually used as filler in lower-grade coffee blends, instant coffee, and espresso blends to form "creama". Robusta is grown on the altitudes ranging from 200 to 800 meters where as Arabica is grown from 600 to 2000 meters.

Coffee fruits are processed through several stages as shown in Figure 4 to produce coffee beans. These coffee fruits are first plucked from plants coffee farms, processed at coffee washing stations and then finally get converted to green coffee beans at dry processing stations.

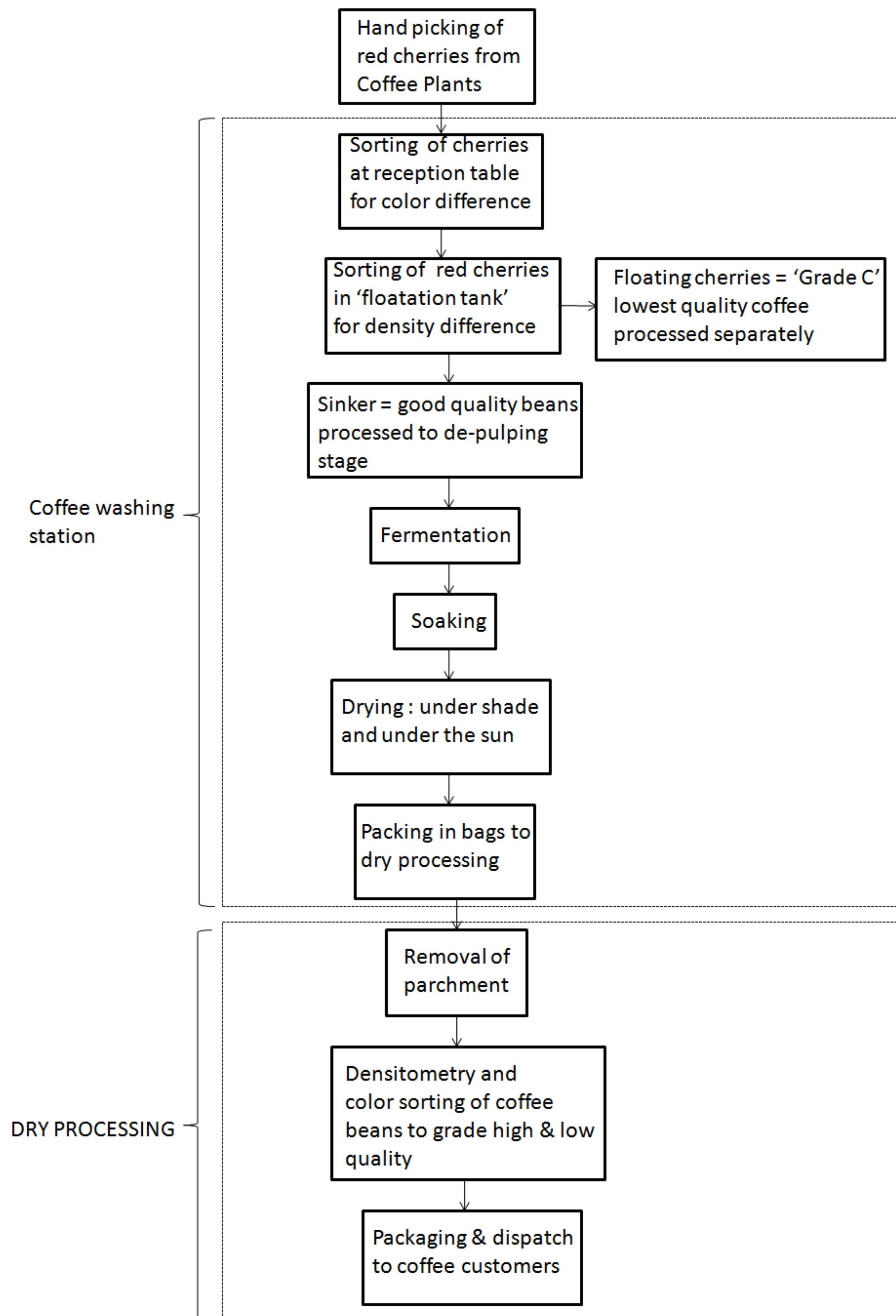

Fig. 4. Coffee processing flow chart

A. Coffee Processing Farms

Rwanda and neighboring regions are famous and admired for specialty Arabica coffee. When the coffee plants are few months old, they are transplanted from nursery to coffee farms. An average fruitation time for coffee plants is 2-3 years for coffee Robusta and 4-5 years for coffee Arabica after plantation in farms[5] . Arabica plants grown on hills in Rwanda are shown in Figure 5.

Fig. 5. Coffee plantation on hills in Rwanda

Coffee trees are planted down the slope of hills and hence mulching is done with dry grass to prevent draining off the soil by wind or rain water. During fruitation, coffee plants get loaded with coffee fruits called as 'coffee cherries'. Premature coffee cherries exhibit green color as seen in Figure 6.

Fig. 6. Premature green coffee cherries

After maturation green colored cherries transform into red as shown in Figure 7.

Fig. 7. Matured red colored cherries

Only red colored cherries are removed from plants for further processing as seen in Figure 8 and green colored cherries are left on plants to get matured. Cherries damaged by any agency like insects, fungus or of different color are not taken for coffee processing.

Fig. 8. Hand picking of cherries from coffee plants

The red colored cherries are transported by bikes or carts called as 'coffee bikes'to coffee washing stations for operations viz. fermentation, soaking and drying.

B. Coffee Processing at Washing Stations

A typical coffee washing station is shown in Figure 9.

Fig. 9. Coffee washing station

Cherries, not having desired red color, are not matured enough to be processed further. Hence they are again sorted at reception table at coffee washing station as seen in Figure 10. Only mature red colored cherries are transported to 'flotation tank'.

Fig. 10. Sorting of cherries for color difference at reception table

Ultimate aim of coffee processing is to deliver good quality coffee. Red colored cherries accepted at reception table may not always imply good quality coffee. Coffee quality is determined by density of coffee cherries or beans as there is a direct relation between them. Coffee cherries can get infected during maturation by diseases, insects and or micro-organisms such as bacteria, fungus etc. These defects reduce density of coffee cherries by making them hollow from inside. Hence denser the coffee beans better is the quality of coffee. Therefore coffee beans are graded based on their relative density during all possible stages at coffee washing station, first stage of which is 'flotation tank'.

Red colored cherries sorted at reception table are immersed in ‘flotation tank’ filled with water as seen in Figure 11 for initial density check.

Fig. 11. Flotation tank

Cherries which float on water are called as ‘flotants’ and considered to be lowest grade ‘Grade C’. These beans do not have special aroma. Farmers sell these ‘Grade C’ coffee beans in local market at lower rate rather than exporting them. Where as cherries which are relatively free from defects sink at the bottom of flotation tank.

A flowchart given in Figure 12 shows steps during coffee processing, performed up till now.

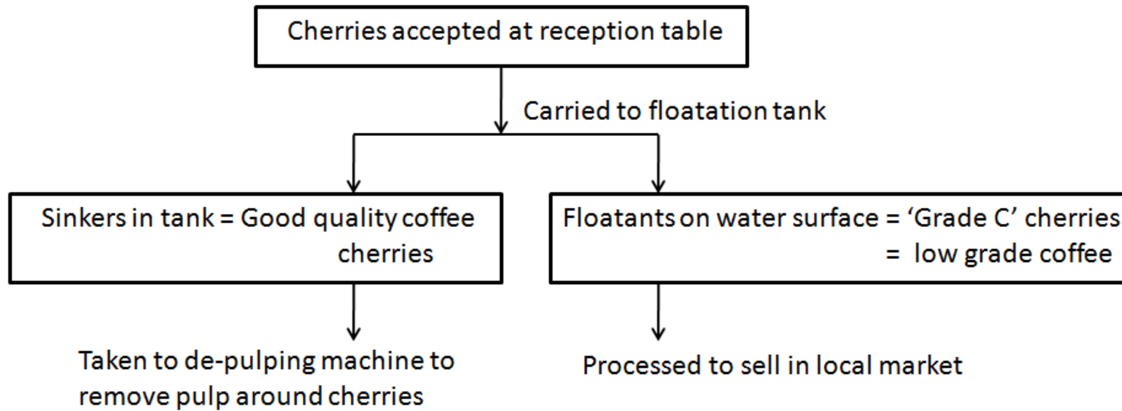

Fig. 12. Coffee processing steps uptill de-pulping stage

An understanding of structure of coffee cherry was also important to know which part of cherries is getting removed during further processing. Figure 13 shows bisected view of coffee cherries.

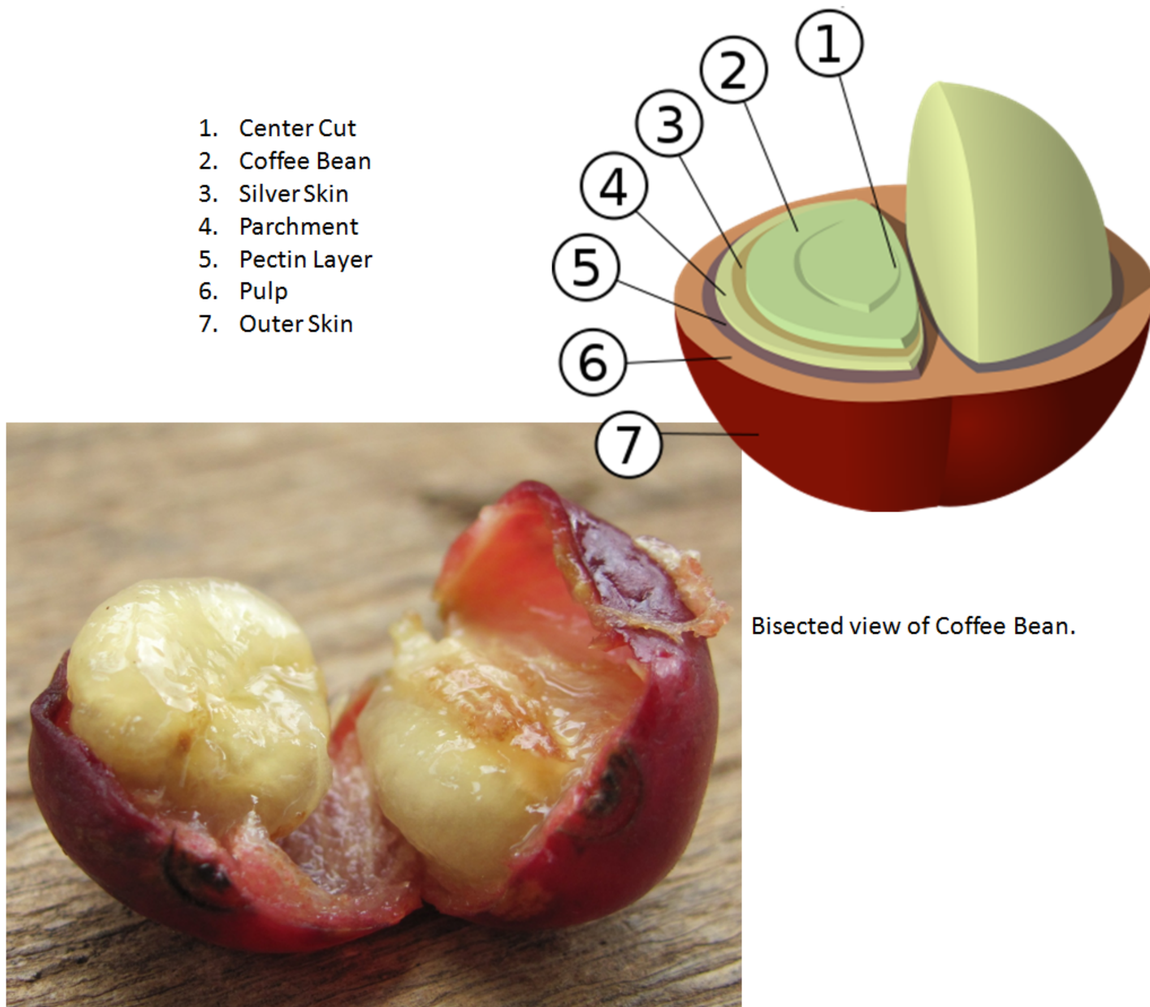

Fig. 13. Bisected view of coffee cherry

Sinkers separated at flotation tank are transported to 'de-pulping' station by channels as seen in Figure 14. Pulp and outer red colored skin of coffee cherries, part 6 and 7 in Figure 13, are removed at de-pulping machine and coffee beans are separated from each other. This is achieved by squeezing coffee cherry between a rotating cylinder or disk and a stationary surface called as breast.

Fig. 14. De-pulping stage

The distance between moving element i.e. disc or cylinder and breast is adjusted such that the space between them narrows as the fruit is carried through. The narrowing gap produces a squeezing action to detach pulp from the seed. The final distance between the gap is sufficient enough to allow seeds to pass through it without getting crushed. The rough surface of moving drum or disk forces most of the pulp away from the seeds. This pulp is used as waste byproduct to produce fertilizers. The seed is deflected onto a plate having ground sharp edges to separate beans from each other. This de-pulping is carried out in presence of water to facilitate squeezing of cherries and separation of beans. A vibrator, fitted to de-pulping machine, again categorizes coffee beans into Grade 'A1' and Grade 'A2' after removal of pulp. 'Grade A1' coffee beans are of higher density and thus higher quality than 'Grade A2'. These

two grades are processed separately because of quality difference between them.

These pulped cherries are covered with slippery mucilage which is removed by fermentation in the next stage of coffee processing. This mucilage consists of protopectin, pectin, pectin easter, sugar and some naturally occurring enzymes. Mucilage is insoluble in water and cannot be removed easily by simple washing processes. If not removed, mucilage causes the difficulty in handling coffee beans during drying process because of its slippery nature. In addition when mucilage is moist it provides favorable media for growth of micro organisms which produces several defects in coffee. Hence it is important to remove mucilage for which graded beans, i.e. 'Grade A1' and 'Grade A2' are transported to fermentation tank by channels.

Fig. 15. Agitation of coffee beans before fermentation process

These channels are built with slope of 1 in 100 to further sort beans according to their density. As seen in Figure 15, workers at coffee washing station agitate coffee beans and water in channels. During agitation, lighter beans cover a greater distance along the slope while denser beans settle down towards the start of the slope. Lighter density beans from 'Grade A1' are separated by this way and mixed with 'Grade A2' coffee beans for further processing.

Coffee beans undergo different densitometric sorting till this stage and Figure 16 shows a quick review of coffee sorting covered so far.

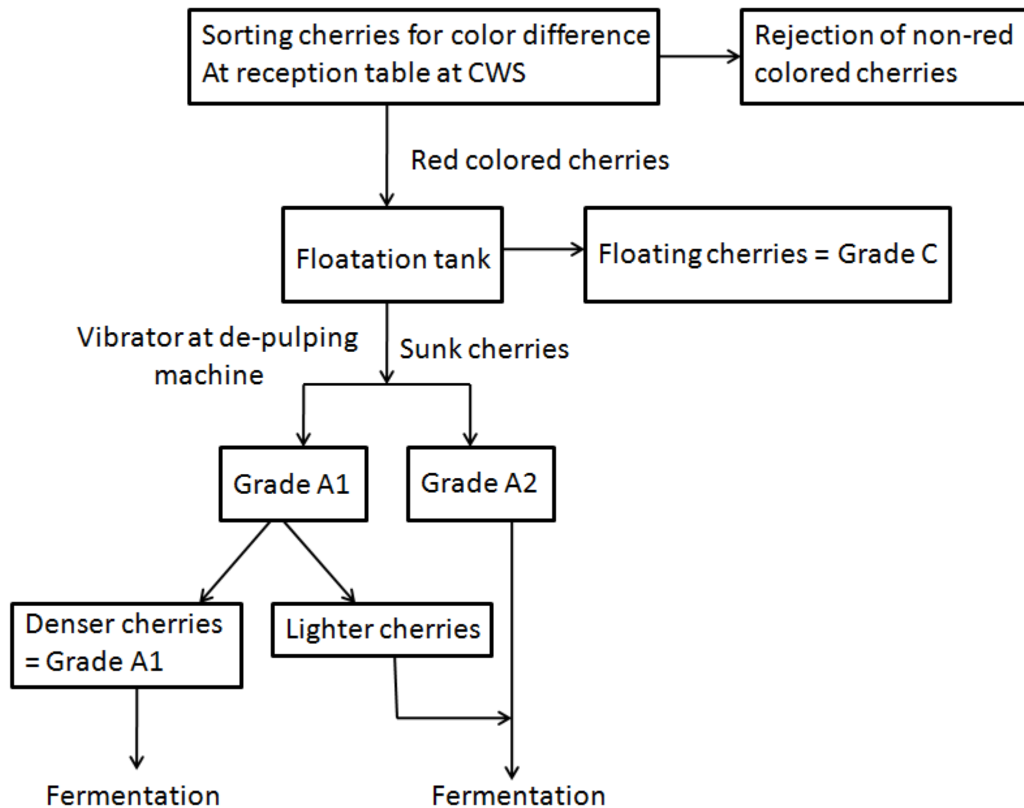

Fig. 16. Grading of coffee beans at different stages

At fermentation tank the water, which carries beans, is drained out and beans are covered with a clean sheet of plastic as seen in Figure 17. During fermentation, natural enzymes present in mucilage start digesting mucilage itself. Micro-organisms such as fungus, yeast, bacteria etc present in the air or tank secret additional enzymes by alcoholic and oxidative fermentation of sugars present in mucilage. It helps in the degradation of mucilage during fermentation. This fermentation can also be carried by added enzymes, chemical methods, warm water etc. The chemical changes happening in coffee beans during fermentation are quiet complex but were studied to find origin of potato defect. During study, IPMP was confirmed not to be a product of these reactions.

Fig. 17. Fermentation tank and fermentation process

Coffee beans, during fermentation, come in direct contact with tank surface, water and micro organisms. Hence maintaining sanitary conditions during coffee processing is really important. Contamination by any of these sources was thought to be a cause of increased level of IPMP in coffee beans. However technicians working at coffee washing stations verified the that the hygienic conditions are maintained as per the regulations and being followed. Hence contamination of water or by any media was ruled out as the possible cause of potato defect.

Typically the fermentation process takes 36-48 hours but over fermentation should be avoided to produce clean cup of coffee with desirable aroma. A conventional way to check completion of fermentation process it to rub few coffee beans against each other in hand. Fermentation is considered to be complete if beans do not slip over each other and make rubbing sound because of friction between them. Coffee beans, after loosing mucilage during fermentation process, are surrounded by white colored parchment and a layer of digested mucilage.

Coffee beans are soaked in water for almost 12 hours to remove this digested mucilage. Coffee beans would take extra day or two for drying if the digested mucilage around them is not removed. If a de-pulping machine removes around 95 percent of mucilage, fermentation stage is avoided and beans are directly taken to soaking tank as seen in Figure 18.

Fig. 18. Soaking process of coffee beans

Scientists are taking efforts to find out the effect of different types soaking process such as mechanical drying, terrace drying etc. on final coffee aroma. After soaking, beans undergo two types of drying processes viz 'Under Shade drying' and 'Sun drying' as seen in Figure 19.

Coffee beans are soaked in water at room temperature. These beans may develop crack and not deliver desirable coffee aroma if dried directly under the Sun after soaking in water. Hence beans are dried 'under the shade' for a day or two at the intermediate temperature. During 'under the shade' drying, parchment around coffee beans becomes translucent and starts showing surface defects which were not visible during earlier processes.

Fig. 19. Drying process of coffee beans

Coffee beans which exhibit visible defects are not of good quality and removed by workers at coffee washings stations as seen in Figure 20. These defective beans are sold in local market at cheaper rate rather than exporting. The defects identified during under the shade drying process include difference in color (like black, brown etc), variation in size, stripes on beans or damage to bean by any external agency.

Fig. 20. Hand picking of green coffee beans during under the shade drying

As described above, hand sorting is a manual monotonous process and may cause psychological and physical fatigues to workers, making the process inefficient. Hence, many times bad beans escape in good beans and it is taken care at the later stage by machine sorting technique.

After ‘under shade drying’, beans are spread over tables in continuous sun and allowed to dry further in open atmosphere. They are covered with transparent black colored cloth, as shown in Figure 21 to avoid contamination by wind or possibility of over heating by sun rays.

Fig. 21. Sun drying process of coffee beans

It is necessary to maintain a desired level of moisture in beans to avoid any bacterial or fungal growth during storage and produce desired coffee aroma. To monitor moisture content in coffee beans a moisture meter is used as shown in Figure 22. Around 50 g of beans are selected and fed to moisture meter. A reading of 12 percent moisture confirms completion of drying process. If moisture meter shows more than 12 percent, beans are dried further.

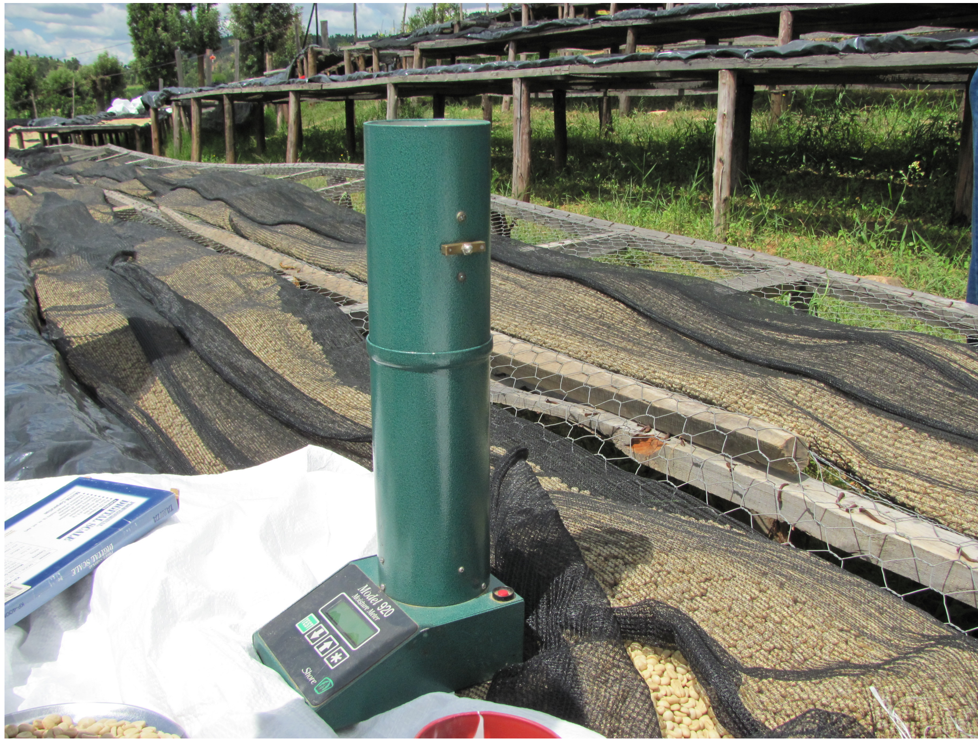

Fig. 22. Moisture meter to monitor moisture in coffee beans

After drying, coffee beans with parchment look as shown in Figure 23. They are are packed in bags and stored as shown in Figure 24. These coffee beans are not yet ready to be shipped in the market as 'Green coffee beans'. They need to be processed further at dry processing station to remove parchment and silver skin.

Fig. 23. Coffee beans with parchment ready for dry processing

Fig. 24. Storage of coffee beans ready for dry processing

C. Processing at Dry Processing Station

Coffee beans are transported to Dry Processing station by trucks. They are weighed and are stored at godowns as seen in Figure 25.

Fig. 25. Arrival and storage of coffee beans at dry processing station

These batches of coffee beans may not be always free from defect and thus are tested before accepting for dry processing. In order to decide the acceptance criteria, these coffee bags are pierced at three different locations and approximating 150 g of beans are selected from each bag. These beans are taken to coffee testing laboratory as seen in Figure 26.

Fig. 26. Sampling of beans and coffee testing lab

In coffee laboratory, parchment and silver skin around these beans is removed. These representative samples of beans from each lot are then roasted and ground and placed in four different cups for cupping as seen in Figure 27. Hot water is poured in these cups for the evolution of Coffee aroma through roasted coffee samples. Technicians are trained to identify coffee defects and quality of coffee by sniffing these samples during cupping.

Fig. 27. Preparation for cupping analysis of coffee beans

After preliminary sniffing crust of coffee powder floating in hot water is broken by spoon and then aroma beneath the surface is smelled as seen in Figure 28. Once sniffing is complete the ground powder is taken out from cups and then brewed coffee is sipped to detect any taste related defect. Thus, presence of defects is confirmed by sniffing as well as tasting. If any of the four cups is detected with defect, a corresponding bag is rejected at the entry of dry processing stage. Samples which show zero defects are allowed for further dry processing stages.

Fig. 28. Cupping analysis of coffee sample

Samples accepted by cupping results are taken to remove parchment around them as seen in Figure 29.

These coffee beans are then transported to remove silver colored skin around them. After removal of this last layer around bean, they are called as 'green coffee beans'. They are still not graded for their quality i. e. density and surface defects. Machine sorting is implemented to sort coffee beans for any surface defects and to take care of errors occurred during manual sorting processes in earlier stages.

Fig. 29. Removal of parchment around coffee beans

The machine sorting eliminates human intervention and grades coffee beans for color difference. The machine is pneumatically operated and works on laser technology. Coffee beans not confirming to characteristics of green coffee bean, like different color or presence of black stripes on surface etc are spotted by laser and are removed from the processing as seen in Figure 30.

Fig. 30. Machine sorting of coffee beans for color difference

The beans accepted by machine sorting are then transported to densitometric sorting process for final gradation based on their density as seen in Figure 31. This densitometric table is inclined at appropriate slope and vibrates with specified frequency to grade coffee beans.

Fig. 31. Densitometric sorting of coffee beans

After grading, these beans are ready to be shipped to customers and this completes the sequence of coffee processing steps.

This chapter describes the process of converting raw coffee fruits into green coffee beans ready for roasting and brewing. More detailed study was required to find contents of coffee beans, changes happening during roasting or coffee processing. Next chapter elaborated on the literature survey made to understand nature of coffee beans and potato defect.

CHAPTER III

LITERATURE REVIEW

As seen in previous chapter a generic description about the coffee processing steps does not give systematic answers to questions related to the 'potato defect' in coffee. Hence, information about the composition of green coffee and the aroma profile of the roasted beans was gathered. An attempt was made to look at 'potato defect' from all possible aspects to identify it's root cause. The literature survey, detailed in this chapter, includes research related to the contents of green coffee, changes in coffee during roasting and modes of synthesis of IPMP. 'Potato defect' in coffee has also been referred to as 'peasy off flavor' defect in some literature. Hence, both key words were used for the literature search.

A. Literature Review of Coffee Volatiles

Coffee aroma evolves during roasting and is a complex process in nature. Compounds responsible for coffee aroma have precursors in green coffee. A little imbalance in these compounds leads to a foul smelling cup of coffee. An increase in the concentration of existing compounds or the formation of new compounds adversely affects coffee aroma. Hence, researchers always attempt to find coffee volatiles and their concentrations in coffee.

Holscher et al. in their paper 'Aroma Compounds in Green Coffee'[6] performed liquid chromatography to extract volatiles from ground Colombian coffee. They analyzed the extracts by a Carlo Erba gas chromatograph and DB-Wax capillary column. They also studied different coffee volatiles and their sensory impact on green coffee by GC-Olfactometry. Their results confirmed that the smell of green coffee is mainly due to IPMP and isobutyl pyrazine (IBMP). An approximately 5 fold increase in

the concentration of IPMP generates a distinctive peasy flavor defect in coffees obtained from East African countries (this flavor is also known as the 'potato defect'). The authors also discussed the characteristics of methoxy pyrazines and the possible pathway of their formation.

Becker et al. found compounds responsible for the peasy flavor in central African coffee. They documented their findings in a paper 'Identification of the "peasy" off flavor note in central African coffees'[7]. The authors detected the peasy coffee samples by smelling whole coffee beans. This confirmed that IPMP, once its concentration exceeds a threshold value, can be identified by sniffing the coffee samples alone. Two dimensional chromatography allowed them to separate 2-methoxy-3-isopropylpyrazine and confirm that it was responsible for the peasy flavor defect in coffee. They also conducted GC-Olfactrometry analysis to sniff IPMP through a sniffing port to evaluate its sensory impact in peasy samples. Chromatograms of non peasy and peasy Rwandan coffee samples showed similar concentrations of other pyrazines such as IBMP. Hence other pyrazines (except IPMP) were ruled out as possible cause of 'potato defect' in coffee.

There is a possibility that IPMP is formed during roasting and this may cause the 'potato defect' in coffee. Czerny et al. in their paper "Potent Odorants of Raw Arabica Coffee. Their Changes during Roasting"[8] used aroma extract dilution analysis for raw Arabica coffee. They identified 3-isobutyl-2-methoxypyrazine (IBMP), 2-methoxy-3,5-dimethylpyrazine , ethyl 2-methylbutyrate , ethyl 3-methylbutyrate , and 3-isopropyl-2-methoxypyrazine (IPMP) as potent odorants of green coffee. This paper also concluded that the 'potato defect' is caused by a five fold increase in the concentration of IPMP. The authors studied the behavior of IPMP during roasting and confirmed the thermal stability of IPMP. They concluded that the concentration of IPMP in regular coffee, which is approximately 2.3 to 2.4 ppb, remains constant

during roasting. This helps to realize that the increased concentration of IPMP is present since the green stage of coffee beans and doesn't not evolve during roasting. Similarly since IPMP is present in all the coffee beans, identification as well as quantification of IPMP is required to identify 'potato defect' inflicted beans'.

Attention was also given to evaluate whether 'potato defect' is related to any plant disease. We tried to find out if IPMP is produced by biosynthesis in the plant or by some microorganisms such as bacteria. A search was done to understand the formation of IPMP by an external agency and this information is documented in the following sub-section.

B. IPMP from a Biosynthetic Perspective

CIRAD scientists worked on this defect to find the cause behind the 'potato defect'. Their hypothesis was that a bug called 'Antestia' (prominent in African countries) produces a hole in coffee fruits, giving an inlet to airborne bacteria to penetrate the cherries. These bacteria feed on cherry wall and produce an extra amount of IPMP as a metabolic byproduct. The bacteria identified by them were 'Enterobacteriaceae' [9]. The mechanism by which the concentration of IPMP is increased is unknown. Unfortunately they had to wind up their research because of the 1990 Rwanda Genocide.

Nancy N. Gerber in her paper 'Three Highly Odorous Metabolites from an Actinomycete : 2-Isopropyl-3-methoxy pyrazine, Methylisoborenol and Geosmin'[10] successfully proved for the first time that Streptomyces species of Actinomycete produces IPMP. This compound delivers 'musty flavor' or 'potato like smell' to substances on which these bacteria grow. But this paper doesn't provide any information about the media like soil or food on which these bacteria grow.

Gallois et al. in their paper 'Study of the biosynthesis of 3-Isopropyl-2Methoxy pyrazine by Pseudomonas taetrolens'[11] discuss the possible way of synthesis of IPMP by Pseudomonas bacteria. Murray et al proposed that IPMP in other vegetables such as peas, green pepper *etc* is synthesized by condensation of alpha amino acids with alpha , beta dicarbonyl compounds. According to the chemical reactions described by Murray, IPMP is derived from vanillin. Thus an increased amount of vanillin in a plant body could lead to an increased amount of IPMP. But Gallois et. al. proved this hypothesis wrong and proposed a new pathway for IPMP synthesis by Pseudomonas bacteria. Although it is well known by now that IPMP is produced by some bacterial species in different media such as fish, milk, stagnant water *etc.*, the literature does not give sufficient information about the presence of bacteria in soil or on plants to cause an increased concentration of IPMP.

Neta et al. in their paper 'Characterization of alkylmethoxypyrazines contributing to earthy/bell pepper flavor in farmstead cheddar cheese'[12] discussed IPMP as one of the contributors to earthy bell pepper aroma in cheddar cheese. They also mentioned that IPMP is a cause of musty, earthy flavor to fish, eggs, milk etc. IPMP is a metabolite in Pseudomonas perolans and Pseudomonas taetrolens. These authors used GC-MS-O coupled with solid phase micro extraction technique to gather the volatiles. Neta el al. found that only the part of cheese that was exposed to air during ripening exhibits musty flavor. Cheese that was vacuum sealed in polyethylene bags did not show the occurrence of bad flavor. This provoked the evaluation of water contamination during coffee processing as a possible source of increased IPMP concentration in beans.

The literature search showed the existence of family of bacteria which can produce IPMP as their byproduct. Help by a plant pathologist was required to culture the bacteria from at least one 'potato defect' infected bean. Unfortunately, adequate information about the bacteria 'Enterobacteriaceae' mentioned by CIRAD scientists was not available to conclude cause of 'potato defect' in coffee beans.

The literature survey lists some of the important facts which were helpful in finding a solution to detect 'potato defect' infected coffee beans. Figure 32 shows the information extracted from the literature survey and its usefulness in this thesis for quick reference.

FACTS	CONCLUSION
• More than 800 compounds present in coffee volatiles.	→ • Complexity of coffee headspace
• IPMP is present in 'healthy' and 'potato defected' coffee beans	→ • 'Identification' & 'Quantification' is required to see if IPMP is more than standard threshold
• IPMP is present in PPB concentration	→ • Sensitive method is required to detect IPMP in the matrix of 800 other coffee volatiles
• Earlier researchers used ground coffee beans and not whole beans	→ • It is not known if IPMP present in PPB can be found in whole beans by techniques and needs to be researched
• IPMP is thermally stable compound. Its concentration remains constant during roasting	→ • Higher concentration of IPMP is present since green stage of coffee & roasting of beans need not be done to find defective beans
• Tons of coffee beans are processed within less than a minute by automated processes in actual practice	→ • The process for sorting beans for 'potato defect' should cope up with timing to prevent hampering coffee processing.

Fig. 32. Review of literature survey

The next chapter provides information about the methods used to detect IPMP in a matrix of 800 other compounds.

CHAPTER IV

TECHNIQUES FOR DETECTION OF IPMP

It was evident from the literature research that sensitive techniques are required to detect IPMP present in ppb concentrations. In addition, these detection techniques should have response times as low as possible in order to avoid a negative impact on coffee production rates. With these requirements in mind, an extensive search was performed to find a sensitive, non destructive, rapid method for detecting IPMP in green coffee beans. Electronic nose (E-Nose), ion mobility spectrometry (IMS), cavity ring down spectrometry (CRDS) were deemed to offer the best possible solutions. Figure 33 shows the flowchart and overview of these three methods. Other methods considered are discussed at the end of this chapter.

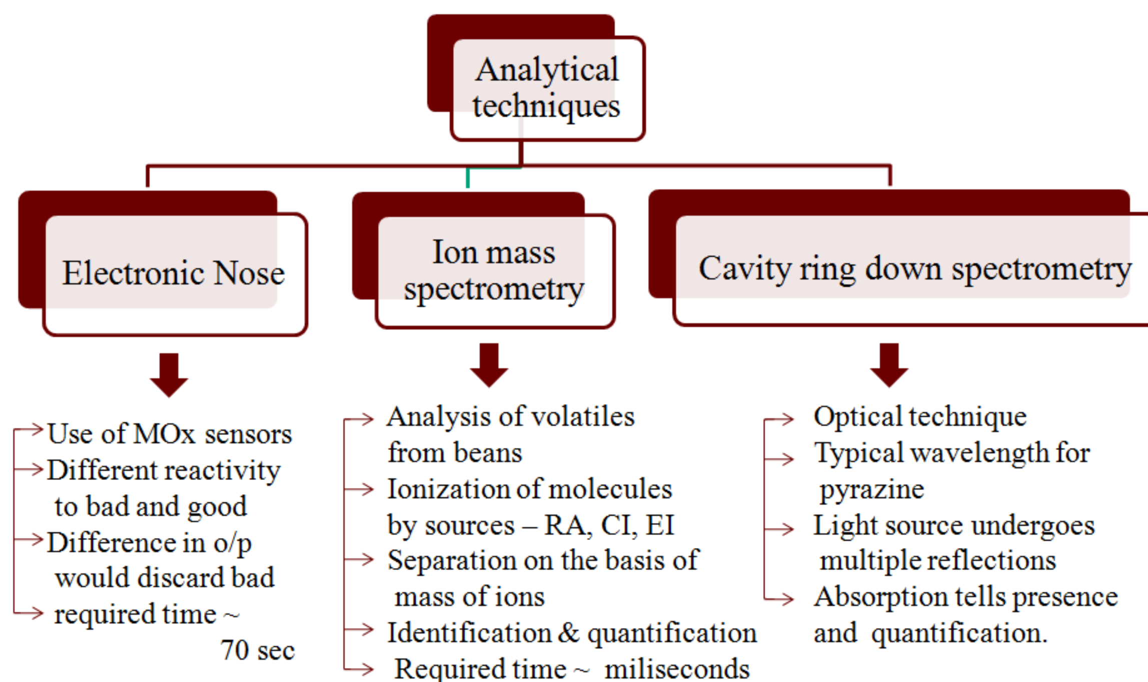

Fig. 33. Overview of detection techniques

Any of these methods, if found to work as a solution, would be implemented at the end of dry processing in order to avoid loss to farmers in terms of money and deal with sorting of a smaller number of high quality coffee beans. The following subsections discuss these techniques in detail along with their working principle.

A. Electric Nose

Electronic nose (E-Nose) is used to monitor volatiles coming from a substance. E-Nose is a rapid, real time technology that mimics biological nose to detect odors and vapors. There are already proven applications of E-Nose to detect volatiles from spoilt curd or characterize the quality of tea etc. Hence, it was interesting to explore the applications of E-Nose for detecting malodorous compounds in coffee.

1. E-Nose Literature Review

Nagle et al. did very concise comparison between E-nose and biological nose in their paper 'The How and Why of Electronic Noses'[13]. They explained the basic concepts of E-nose by briefly describing the working of E-Nose, the different chemical sensors used in E-Nose system, the advantages and disadvantages of these sensors, their sensitivities and methods of their manufacturing. This paper also guided the selection of the appropriate chemical sensor as per one's requirement.

Gutierrez-Osuna et al. evaluated the E-Nose as a replacement for a human panel for swine odor detection and documented the results in their paper 'Correlation Of Sensory Analysis With Electronic Nose Data For Swine Odor Remediation Assessment'[14]. They used 32 conducting polymer sensors in their E-Nose experiments and also validated the effect of humidity on E-Nose responses in long duration applications. Results from these experiments were positive and were corroborated by

the human panel establishing the reliability of E-Nose.

Tudu et al. in a paper ‘Electronic nose for black tea quality evaluation by an incremental RBF network’[15] evaluated the possibility of implementing low cost instrumental quality control for black tea. They established the potential of electronic nose for shop floor application to evaluate the quality of black tea. Five sensors of the TGS family from Figaro Japan were used for the experiments. During the experiments, a 50g sample of black tea was prepared at 60[1]C temperature for 30 seconds to generate a head space. The E-nose used by these authors could generate output in less than 120 seconds. This was helpful information for the on-line application of E-nose for the detection of coffee volatiles.

Schiffman et al. conducted experiments with 2 E-Noses, the NST 3320 and the Cyranose 320 along with three other sensing devices to find their suitability for odor detection. The results were described in a paper ‘Measuring Odor Intensity with E-Noses and Other Sensor Types’[16]. The other sensing devices included photo ionization detector, gold film sulphur sensor and infra red laser detector. Responses from these instruments were mapped to the findings of a human panel in a variety of realistic situations and it was shown that E-Nose can be used for detection of volatile components.

Rodrguez et al. in their article ‘Electronic Nose for Quality Control of Colombian Coffee through the Detection of Defects in "Cup Tests"[17] did preliminary study on the use of E-nose for detection and classification of defects in freshly brewed coffee. During the experiments, E-nose was tried on green coffee beans. Unfortunately, FIGARO and FIS type sensors did not respond to volatiles from green beans. It was an important point to consider while implementing E-Nose measurements for green

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[1]0

beans. The reason for the lack of response was not mentioned in this paper but it might be because of the large number of volatile components evolve during roasting in concentrations which can be detected by sensing techniques.

Amari et al. evaluated the performance of an E-nose as an instrument for the quality control of raw milk or meat stored at 4 degree centigrade. In their paper ‘Potential application of the electronic nose for shelf-life determination of raw milk and red meat’[18], experiments were conducted with an E-nose system comprising of 6 tin oxide based Taguchi sensors obtained from Figaro. Temperature and humidity sensors were also incorporated in the set up to evaluate response changes with respect to climatic conditions.

Botre et al. used an E-Nose to identify milk, rancid milk and yogurt odors. They conducted experiments with an E-nose comprising of 5 Figaro sensors and documented the results in their paper ‘Semiconductor Sensor Array Based Electronic Nose for Milk, Rancid Milk and Yogurt Odors Identification’[19]. An attempt to identify given or unknown odors by training a neural network was accomplished with an overall 96 percent success rate.

It was very evident form the literature survey that E-nose could be one of the possible solutions to identify coffee volatiles. A comprehensive study of the working principles of E-nose was done to design our own E-nose for the detection of coffee volatiles. The following subsection gives the working principle of E-Noses.

2. Working Principle of Electronic Nose

E-nose is a smart instrument designed to detect and discriminate complex odors using electrochemical sensors. An E-nose consists of an array of sensors, data acquisition and processing system as shown in Figure 34.

Fig. 34. Block diagram of E-nose

The workings of an E-nose can be better explained with the help of Figure 35. The sensors in this array are made of odor-sensitive biological or chemical materials. Sensors are the most important part in E-Nose as they come in direct contact with odors or volatiles and respond to these odors. Molecules from the head space of a substance are transported to sensors by an inert carrier gas such as nitrogen. These molecules adsorb on the sensors and change the characteristics such as the conductivity or mass of the sensors. Sensors in an E-nose are not selective to any particular compound present in volatiles. But a combined output from an array of sensors gives a signature of volatiles or odors. Patterns or fingerprints from known odors are used to construct a database and train a pattern recognition system to recognize given odors. An inert gas is passed over these sensors each and every time to delete the memory of earlier volatiles and try new ones. This helps in avoiding interference between two different odors.

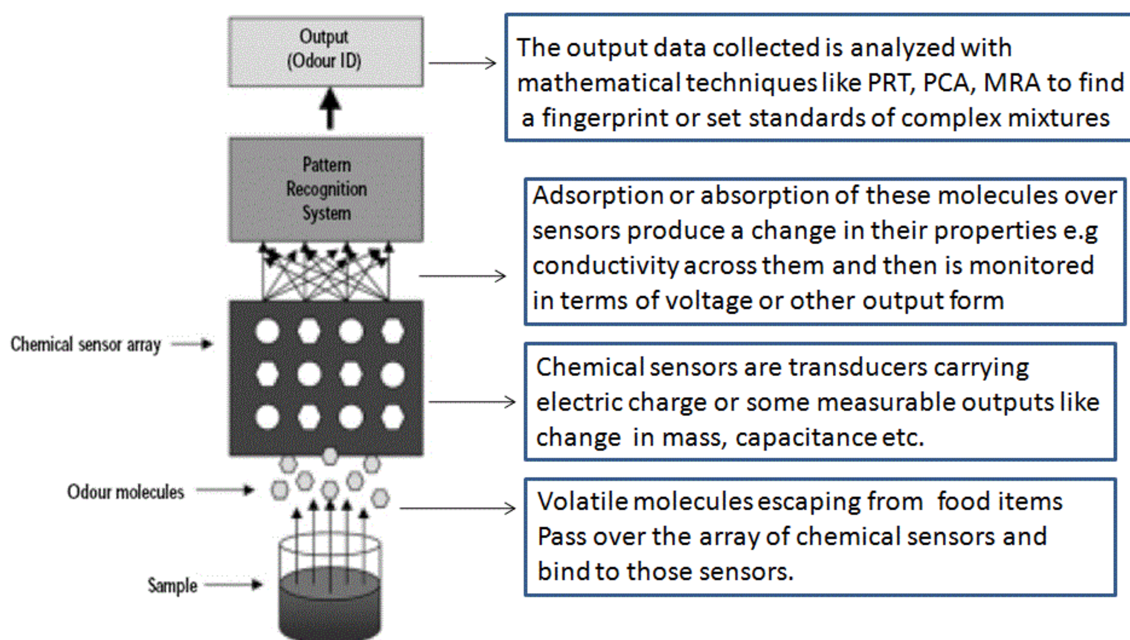

Fig. 35. Working of electronic nose

Different types of sensors such as metal oxide, optical sensors etc. are used in E-nose. Figure 36 shows a brief summary of the electrochemical sensors used in the application of E-nose[13].

As seen from the table, many sensors are still under research. Amongst the available sensors, metal oxide (MOx) sensors and conducting polymer(CP) sensors were the most suitable choices for the detection of IPMP present at ppb concentrations. These sensors are described in following subsection.

Principle	Measurand	Sensor type		Fabrication methods	Availability/sensitivity
Conductometric	Conductance	Chemoresistor	MOS	Microfabricated, Sputtering	Commercial, many types, 5-500 ppm
			Conducting polymer (CP)	Microfabricated, Electroplating, Plasma CVD, Screen printing, Spin coating	Commercial, many types, 0.1-100 ppm
Capacitive	Capacitance	Chemocapacitor	Polymer	Microfabricated, Spin coating	Research
Potentiometric	Voltage/e.m.f.	Chemdiode	Schottky Didode	Microfabricated	Research
	I-V/C-V *	Chemotransistor	MOSFET	Microfabricated	Commercial, special order only/ppm
Calorimetric	Temperature	Themal chemosensor	Thermister (Pyroelectric)	Microfabricated, Ceramic fab.	Research
			Pellistor	Microfabricated	Research
			Thermocouple	Microfabricated	Research
Gravimetric	Piezoelectricity	Mass-sensitive chemosensor	Quartz crystal microbalance (QCM)	Microfabricated, Screen printing, Dip-coating, Spin coating	Commercial, several types/1.0 ng mass change
			Surface acoustic wave (SAW)	Microfabricated, Screen printing, Dip-coating, Spin coating	Commercial, several types/1.0 ng mass change
Optical	Refractive index	Resonant-type chemosensor	Surface plasmon resonance (SPR)	Microfabricated, Screen printing, Dip-coating, Spin coating	Research
	Intensity/ spectrum	Fiber-optic chemosensor	Fluorescence, chemoluminescence	Dip-coating	Research
Amperometry	Current	Toxic gas sensor	Electrocatalyst	Commercial, ppb-ppm	

* I-V = Current - voltage

C-V = Capacitance - voltage

Fig. 36. Summary of sensors used in E-Nose systems

3. Metal Oxide Sensors (MOx)

MOx sensors work on the principle of variation in conductivity of sensors in presence of oxidizing and reducing gases as shown in Figure 37. Adsorption and absorption of molecules change the resistance of MOx sensors[20]. The magnitude of the response depends on the nature and concentration of the adsorbed molecules, and the type of the metal oxide layer on the sensors.

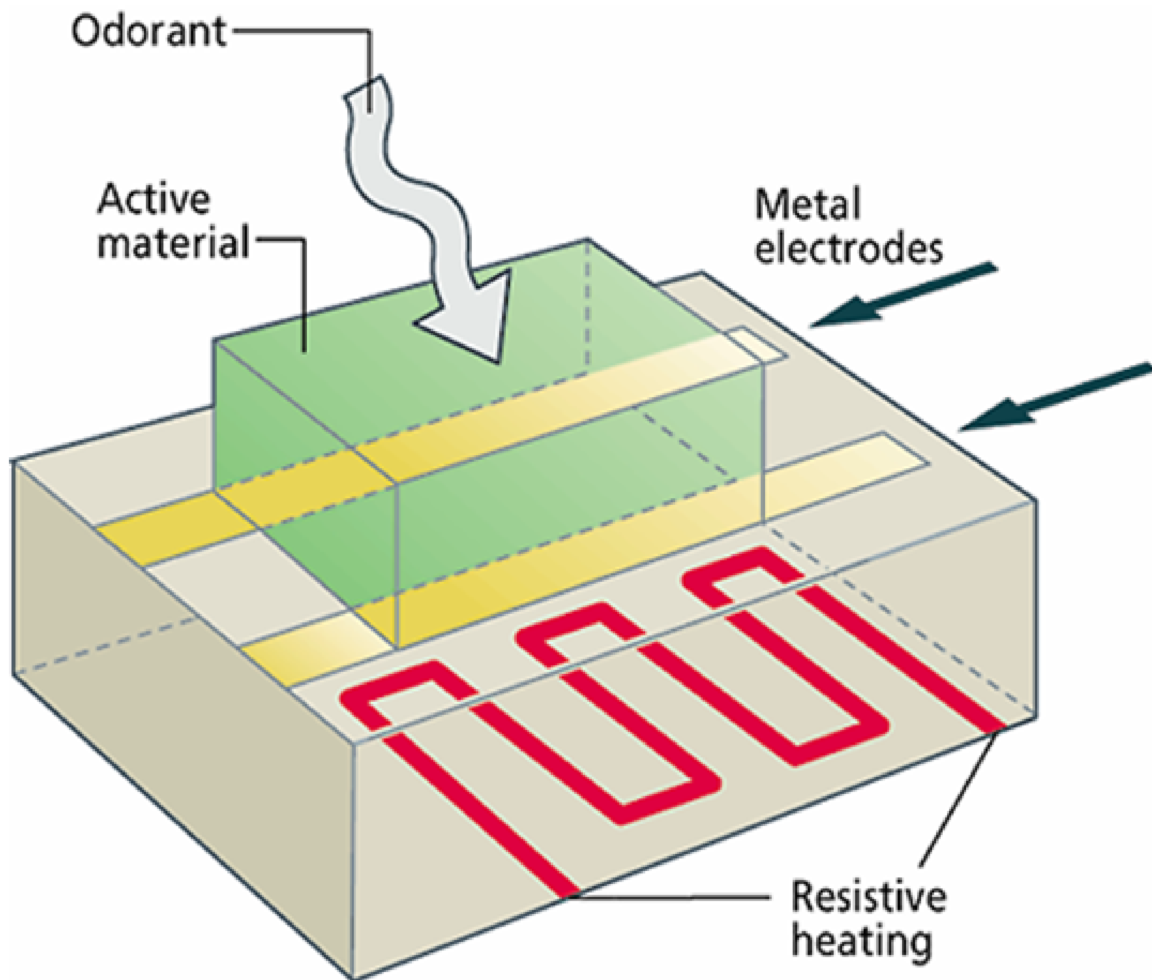

Fig. 37. Working of MOx sensor

Research is still being done on applications of MOx sensors to overcome shortcomings such as low selectivity, changes with changing atmospheric conditions during long term usage, low steady state stability, temperature dependent behavior etc.

To find a suitable MOx sensor having a preferential selectivity for IPMP is a challenging task. The difference in outputs from 'healthy' and 'potato defect' infected coffee beans was initially believed to be a good way to segregate defected beans. A number of samples of good coffee beans could be used to establish the signature of

healthy green beans and train the neural network or recognition system. Any deviation from this reference signature would confirm the presence of 'unhealthy' beans and these beans could be separated from the healthy ones. But this deviation from the standard signature would not necessarily mean that the concentration of IPMP is elevated because these sensors are not selective to any particular compound. The deviation could also be caused by any other defect or chemical present in the coffee beans. In addition, coffee beans are heterogeneous food commodity; concentrations of desirable compounds even will vary in good beans. Hence, such good beans might also show a different signature than the reference beans. It is also difficult to get consistent output from these sensors for a given odor as the sensors are affected by humidity and temperature. Taking these variables into consideration, there has to be more research to explore whether E-nose is a reliable solution to detect coffee volatiles.

Hence, even though the response time for E-nose with MOx sensors is as low as approximately 50-70 seconds, it is not a suitable solution to find 'potato defect' infected coffee beans.

4. Conducting Polymers (CP)

This is a second type of E-nose sensor suitable to detect IPMP present at ppb concentrations. These sensors are fabricated by electro polymerizing thin films across a narrow electrode gap. Adsorption of molecules onto the polymer films induces a temporary change in the electrical conductance of the film. This occurs because the population of active charge carriers in the polymer structure becomes altered. PC-based sensors have better selectivity than MOx sensors, because a specific functional group, preferentially interacting with the targeted compound, can be attached to the polymer. Usually it takes around 2-3 years to find a polymer selective for a certain

compound and there is no current polymer developed for the detection of IPMP.

Hence application of E-nose with conducting polymer sensors to detect IPMP in coffee is beyond the scope of this project. E-noses need to be explored in more depth for their robustness and suitability for recurring use, without becoming affected by external disturbances.

B. Ion Mobility Spectrometry(IMS)

Selection Criteria

1. Detection time : less than 30 seconds
2. Detection level : ppb

Ion mobility spectrometry works on the principle of converting analytes into ions in vacuum and then collection of these ions at the collecting region of spectrometer as shown in Figure 38[21].

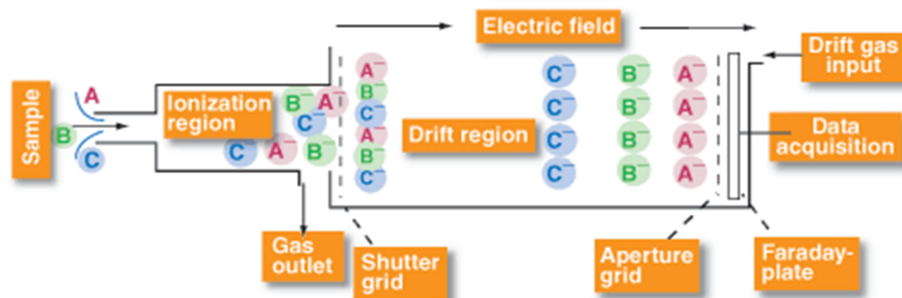

Fig. 38. Working of ion mobility spectrometry

An ion mobility spectrometer includes an ion molecule reaction chamber and an ionization source associated with this chamber. An ion or molecule injection shutter (Bradbury-Nielsen-Shutter) is placed between the ionization chamber and the ion drift chamber. Faraday plate is used as an ion collector.

Volatile molecules, present in the head space of an object, are transported by a carrier gas such as air or nitrogen, to the ionization chamber of the ion mobility spectrometer. The carrier gas and analyte molecules are charged by an ionization source such as radiation, lasers, discharge lamps etc . The charged molecules are accelerated by an electrostatic field gradient maintained between a counter electrode and the Faraday plate. The electric field forces the ions to travel toward the injection shutter interface of the ion drift chamber and then to the Faraday plate. The Faraday plate is connected to a data acquisition system to monitor its output signal[21].

The time between the introduction of the ions into the drift region at the electric shutter and their arrival at the collector plate is monitored because it can be different for different ionic species. The quantity of ions collected as a function of drift time is recorded as current and is used to quantify volatiles.

A source for testing coffee beans with IMS was located at Smiths Detection Laboratory, NY. They are the market leaders in producing instruments based on IMS techniques and have a facility to conduct experiments. 97 percent pure IPMP was tested initially to verify detection limit for IPMP in their IMS system. The lowest concentration of IPMP that the IM spectrometer could detect was 10 ppm. Hence, the instrument could not detect the required ppb concentrations of IPMP. The reasons for this are not yet clear, but they might be associated with the ionization source or pre-concentration of IPMP before injection into the ionization chamber. Hence, IMS was eliminated as one of possible options to detect 'potato defect' infected coffee beans.

C. Cavity Ring Down Spectrometry (CRDS)

Selection Criteria

1. Detection Time : milliseconds
2. Detection limit : part per trillion

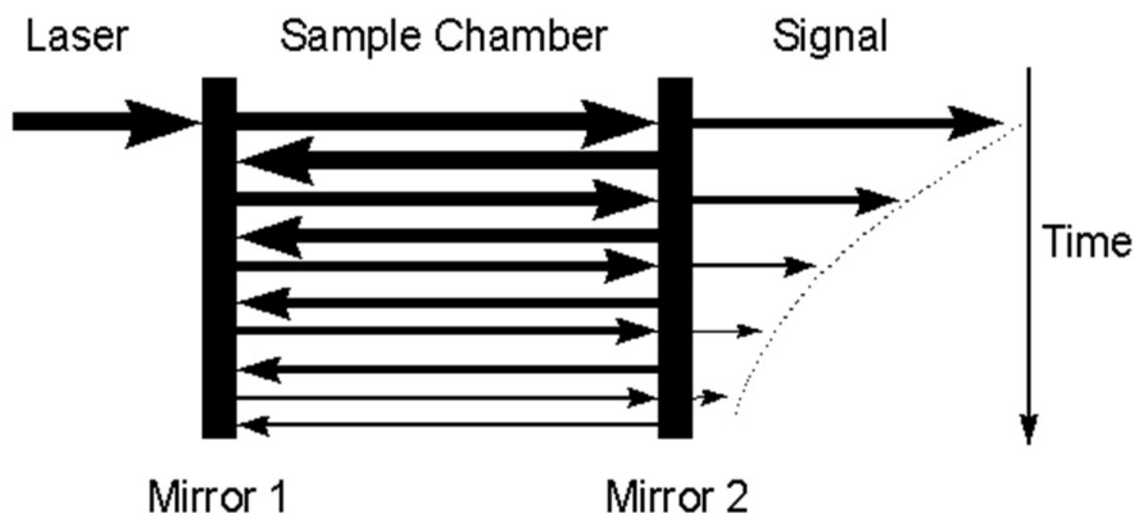

Fig. 39. Working principle of cavity ring down spectrometry

As seen in Figure 39, a cavity ring down spectrometer consists of a cavity created with a stainless steel chamber. The cavity is enclosed by highly reflective (99.998 percent reflective) mirrors at both ends. These mirrors are coated with a dielectric fused silica. A pulse of laser light is directed into an optical cavity. The sample is kept in the cavity and the laser is switched off when the amount of energy in the stainless steel cavity reaches a pre-set threshold. The light pulse bounces back and forth through the sample number of times. A small amount of light escapes through the second mirror during each pass due to optical losses. The intensity of light, escaping through the second mirror is monitored by a photo multiplier tube

(PMT). The ring down time in the cavity is related to the absorbance; hence the concentration of sample contained between the end mirrors. The long optical path results in a highly sensitive measurement technique.

A cavity ring down spectrometer was located at Texas A and M University and was used to carry out experiments with coffee beans. The absorption spectrum of IPMP had to be determined before starting experiments with the cavity ring down spectrometer. The test with 97 percent pure IPMP showed a broad absorption spectrum for IPMP. Many coffee volatiles would also absorb in this spectral range and it would be difficult to isolate IPMP from them. Hence CRDS, although highly sensitive and quick to respond, was not selective enough to detect IPMP and was ruled out as a possible solution.

From these experiments it was clear that a method which is efficient enough to isolate each and every compound in coffee volatiles can only be used to detect IPMP amongst 800 other coffee volatiles. The techniques considered so far, viz. E-nose, IMS and CRDS were not able to detect IPMP present at ppb concentrations. Further search was done for a suitable technique in a variety of fields as described in the following subsection.

D. Miscellaneous

1. As per the hypothesis proposed by scientists, bacteria responsible for the 'potato defect' enter the cherries through an opening produced by the bug called 'Antestia'. Hence, preventive measures such as using an anti bacterial pesticide by farmers was recommended. But Africa being one of the poor and under developed continent, farmers do not have the financial ability to invest in farming. Instead they would switch to other crops which are not expensive to grow.

Also, the species of bacteria, responsible for the potato defect, is not yet known to suggest an appropriate pesticide. So people working in the coffee farms in Africa were not very happy for this solution.

2. Any opening or mechanical damage to coffee cherries, in fact, would provide an inlet to bacteria which would result in 'potato defect' in coffee. Hence, improving the efficiency of the sorting process would help to eliminate damaged cherries and reduce the number of 'potato defect' infected coffee beans. This would have to be achieved by reducing the strenuous efforts of inspecting each and every cherry even for the smallest possible damage. A magnifying glass or mirror which would enlarge the bean surface for easy identification of any damage, could be placed under the sorting table. This would of course help workers to sort beans, but prevent the passing of 'potato defect' infected beans only if damages to cherries and subsequent bacterial infection are the causes of it. This approach would not work if the hypothesis proposed is not valid. Hence, before investing in making magnifying mirrors or glasses, validation of the hypothesis was important.

3. Lot of applications include the use of near infra red, mid infra red and ultraviolet radiation for on-line food inspection and quality assurance[22, 23, 24, 25, 26, 27]. Being rapid and proven techniques, they were thought to be used for detecting 'potato defect' infected coffee beans. But these techniques are not selective and can not identify a single compound from a matrix of other 800 volatiles. In addition, IPMP is present at ppb concentration levels, so according to Beer-Lambert's law, it would require long optical path lengths and thus higher coffee bean inspection time. Hence this solution was not considered for identifying 'potato defect' infected coffee beans.

4. A search was done to see if there exists any food safe chemical which would react with IPMP and shows some color difference. When coffee beans or cherries would come in contact with such a chemical, a change in their color would tell the presence of IPMP and the intensity of change in color would tell about the approximate concentration of IPMP. Even after extensive search, a chemical which is food safe as well as reactive to IPMP was not identified.

5. Ododrant Binding protein : This is an upcoming research area in the field of biosensors. Binding proteins from rat or cow' s mucosa are used to detect molecules present in odors[28, 29, 30, 31]. This technology would be highly sensitive as animals or humans can detect even slight traces of odors present. One of the concerns with this solution was the short shelf life of proteins; hence its use for on-line detection technique in industrial applications.

6. Animals such as dogs, which are very sensitive to odors could be used to detect the presence of IPMP in green coffee. But as IPMP is present in all coffee beans, identification of beans having higher concentration of IPMP, would be difficult. Similarly, some insects secret IPMP as pheromone and react to it. But this would lead to just identification of the presence but not quantification of IPMP.

As discussed in this chapter the problem of identification of 'potato defect' infected beans was approached from all possible directions. Still the problem was not solved. Hence additional experiments were conducted with coffee beans to find information about the 'potato defect' which might give leads towards the solution.

CHAPTER V

ANALYSIS OF COFFEE BEANS BY MULTIDIMENSIONAL GC-MS-O TECHNIQUE

As described in the previous chapter, the three most sensitive methods, viz. electronic nose, ion mobility spectrometry and cavity ring down spectrometry were unable to detect IPMP present at ppb concentration levels. Failure analysis of these methods conveyed the need for at least one 'potato defect' infected coffee bean sample to help us learn more about the 'potato defect'. Hence, additional experiments were conducted with Rwandan coffee samples using a multidimensional GC-MS-O method to identify and quantify IPMP present in coffee. Experiments were conducted at Iowa State University, Ames, Iowa with the help of scientists having expertise in olfactory analysis. Solid phase micro extraction (SPME), being a non-destructive, fast and sensitive method, was chosen to extract volatiles from the head space of coffee samples. The objectives of these experiments were as follow:

A. Objectives of the Experiments

1. Develop a sensitive analytical technique for detection of IPMP in green coffee beans at ppb levels

2. Establish signature of healthy and potato defected coffee beans received from Rwanda

3. Retrieve information from these experiments for designing a non destructive sorting technology for coffee beans.

Approximately 82 green coffee bean samples were received from MIG, KOAKAKA and BUF CAF coffee washing stations in Rwanda as seen in Figure 40. A portion of the coffee beans from each of these samples was kept for cupping analysis at coffee laboratories in Rwanda. The parchment around the coffee beans in the samples was removed and the beans were covered with silver colored skin. The samples were selected from the "under the shade drying" step of coffee processing.

Fig. 40. Arrival of coffee samples from Rwanda

Coffee beans were packed in vacuum sealed bags as seen in Figure 41. Details such as number of samples, name of samples, sample condition (vacuum sealed or partially sealed) and weight of samples were documented. The following section gives information about the different grades of coffee from which samples were collected in Rwanda.

Fig. 41. Individual coffee samples appearance

B. Details of Coffee Bean Samples Received from Rwanda

1. Green coffee bean samples were received in vacuum sealed bags.

2. Weight of the samples varied from 100 g to 250 g.

3. The samples contained coffee beans of different grades as described below.

   - Cherries sorted at the reception table : Coffee cherries sorted at the reception table at coffee washing stations for any visible defect or color difference are designated as 'M.Cerises'. These cherries were selected at random and processed for experiments.

   - 'Grade C' coffee beans : Cherries having relatively low density, float on water in flotation tank and are called as floatants or Grade 'C' beans.

- Defective ‘A1’ and ‘A2’ : ‘Grade A1’ and ‘Grade A2’ are sorted for any visible damage or defects during the ”under the shade drying” process. They are called ‘Grade A1 defective’ and ‘Grade A2 defective’ and randomly selected from different lots.

4. ‘Healthy’ green and roasted coffee beans were obtained from a Rwandan coffee retail shop to establish a reference sample.

The sample summary is shown in Figure 42. The numbers in the table indicate the number of green coffee beans samples from respective catagory.

Coffee Washing Station	‘Defective Grade A1’	‘Defective Grade A2’	‘Grade C’ (Floatant)	M.Cerises (cherries sorted at reception table)	Comments
MIG	11	11	11	8	A1: 9 double entries A2: 1 double entry C : 1 double entry
KOAKAKA	6	6	Not available	12	A1: 1 double entry
BUF CAFE	6	5	6	3	A2: 1 double entry
KABUYE	Data not yet available				

Fig. 42. Sample summary

C. Methodology for The Experiments

As seen in Figure 43 a multidimensional GC-MS-O instrument developed by Agilent Technologies was used for the experiments along with an auto sampler unit.

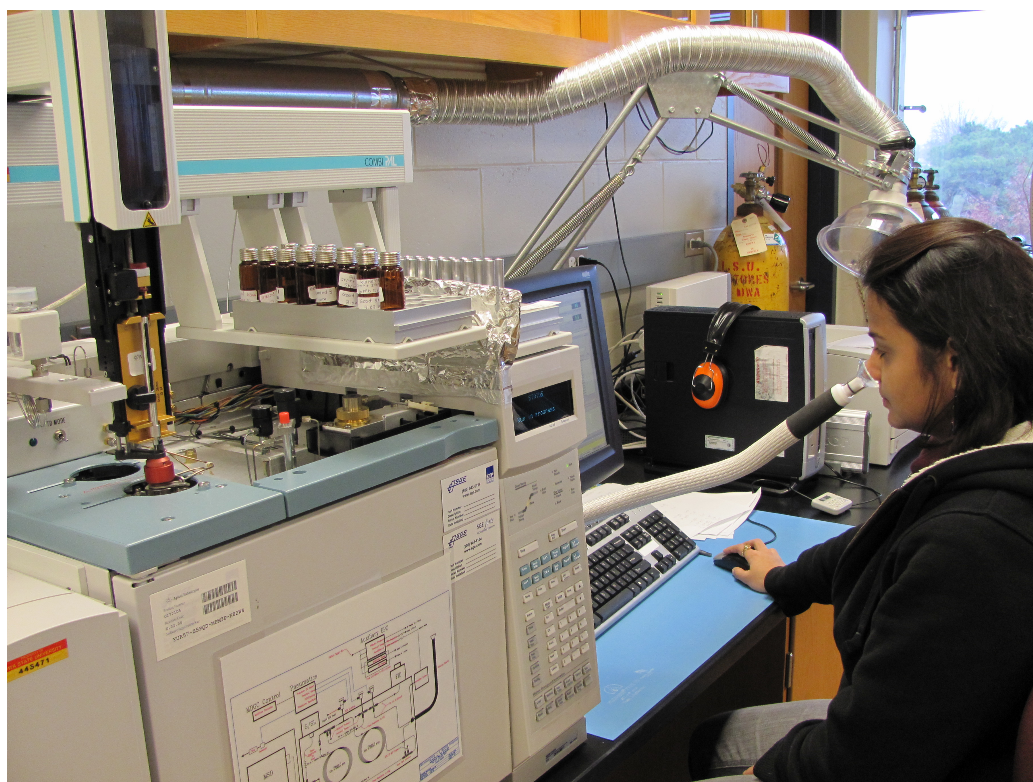

Fig. 43. Multidimensional GC-MS instrument with olfactory analysis

The nature of the sample such as whole or ground beans, agitation temperature and extraction time were important parameters for effective adsorption of the volatile components present in the head space of the coffee samples onto the SPME fiber. The coffee beans were ground as larger surface area would emit more volatiles than course particles. A paper by Oliveira et. al[32] was used as reference for selecting extraction time and agitation temperature. According to this paper, volatiles from head space of coffee samples were extracted by SPME at 70^0 C with a sample incubation time of 10 min and an extraction time of 30 min. Experiments were started with these parameters and were altered for further iterations depending upon the results of the first run.

The samples were made by grinding good green and roasted coffee beans. Amber colored and oven dried GC vials were used to minimize sample alteration avoid by light and water. A DVB/CAR/PDMS coated fiber was selected as the SPME fiber, because it is more sensitive to IPMP and IBMP[33]. 'Mr. Coffee' grinder was used to grind the coffee beans. The experimental apparatus is shown in Figure 44.

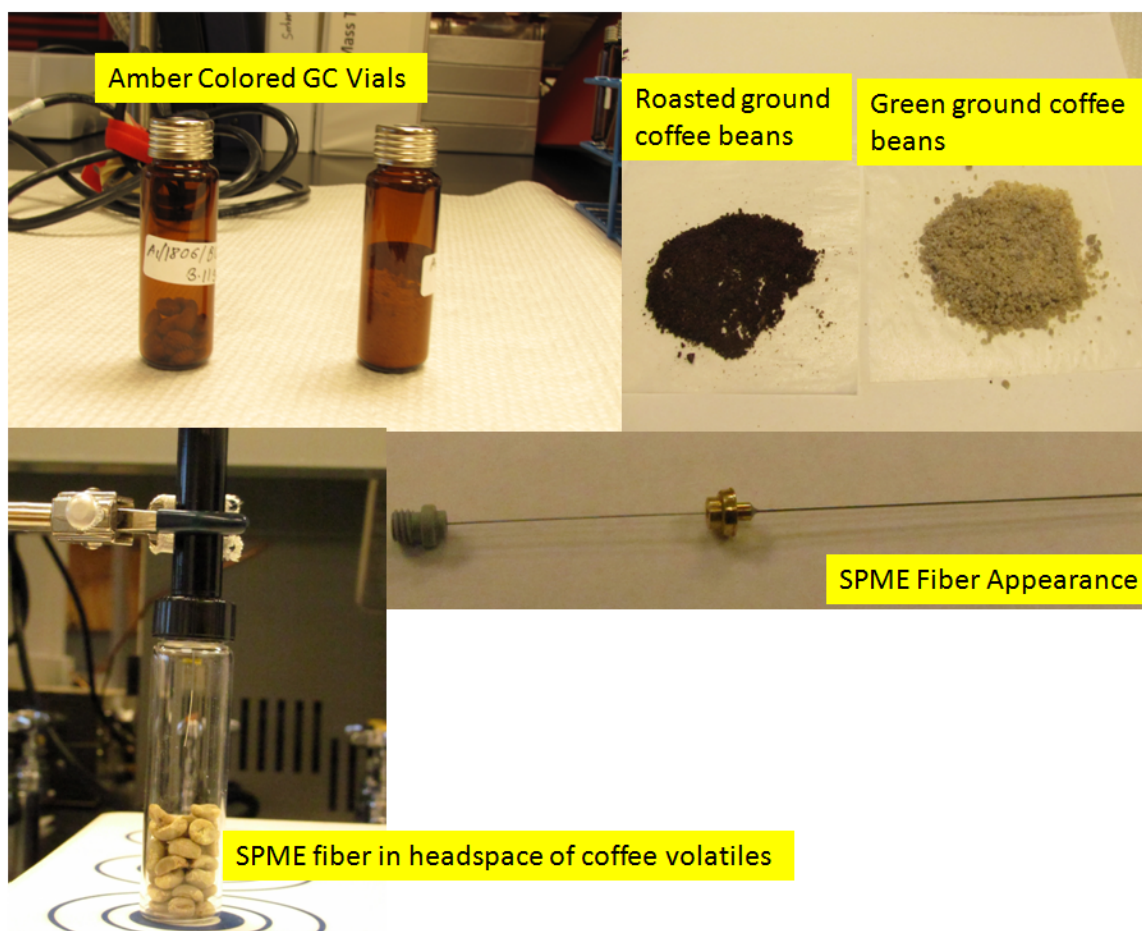

Fig. 44. Experimental apparatus

IPMP was not detected in the headspace of good beans with the extraction time and incubation temperature mentioned in the paper . Hence, the temperature was to 90^0C, incubation time to 30 min and extraction time to 40 min. 90^0C centigrade was below the coffee roasting temperature and would not burn the beans. Usually, coffee is roasted approximately 120 to 180^0C for 10-15 minutes. The revised procedure could identify IPMP in the head space of coffee samples and it was decided to proceed further with these parameters. As expected, the ground sample of good beans showed higher concentration of IPMP than the sample of whole good beans.

The next objective was to locate at least one 'potato defect' infected coffee sample amongst the 82 available samples. Identifying such a sample was like throwing an arrow in the dark. Cupping analysis at the Rwandan coffee tasting laboratories was not completed to help us in locating a 'potato defect' infected sample. Similarly it was difficult to detect the presence of 'potato defect' just by visual inspection of all the available samples.

Hence, a hypothesis was proposed when no information was available to find a 'potato defect' infected sample. As per this hypothesis, 'Grade 'C' coffee beans are more likely to be infected by the 'potato defect', because they are damaged or defective for other reasons. A Grade 'C' coffee sample was selected at random. A Grade 'A1' sample was also chosen to compare coffee volatiles with 'Grade C'. A physical difference between 'Grade A1' and 'Grade C' coffee beans was evident as shown in Figure 45. Only a few coffee beans from each of the 'Grade A1' and 'Grade C' samples were ground.

Fig. 45. Physical difference in Grade'A1' and Grade'C' green coffee beans

The average weight of the ground sample was kept at 3.05 grams with an intention to leave enough head space in the GC vial to accumulate coffee volatiles. SPME fibers were changed pro-actively as they were getting exhausted during experiments as seen in Figure 46. New fibers were preconditioned as per the recommendations of the SPME fiber manufacturer in order to obtain the best results. A grinder was washed with detergent and deionized water and then dried with care after grinding each coffee sample in order to avoid cross-contamination from other samples.

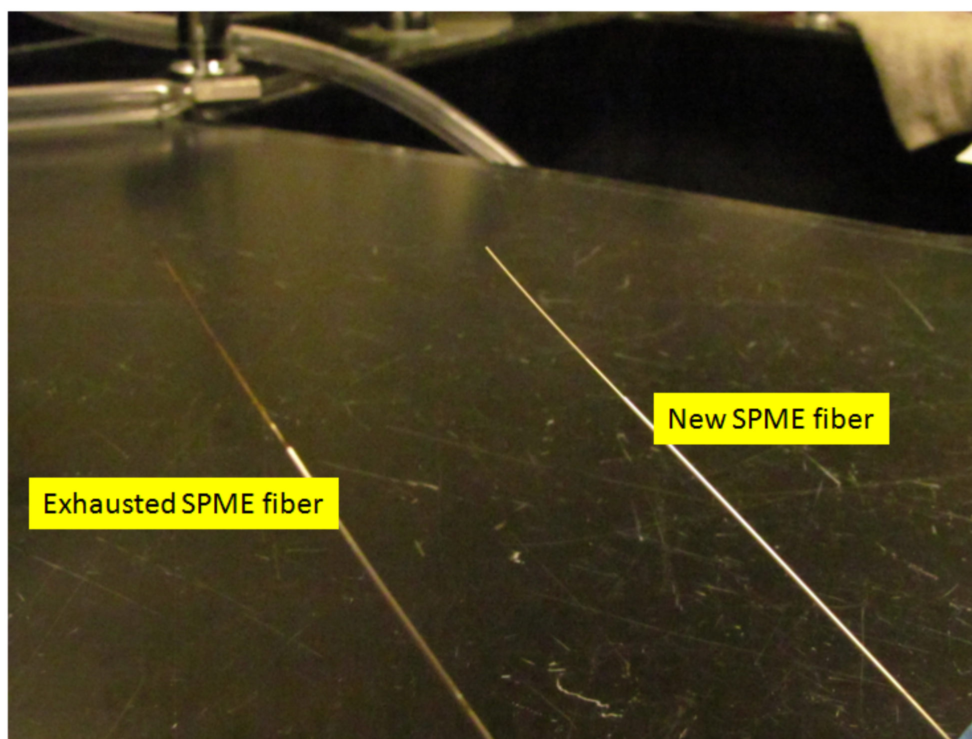

Fig. 46. Used and new SPME fibers

All samples, good green and roasted ,'Grade A1' and 'Grade C' showed almost similar concentrations of IPMP in their volatiles. This result contradicted the hypothesis that 'lower grade coffee was more likely to be infected by the potato defect than a higher grade coffee'. Hence, testing of the lower grade coffee beans, preferentially with respect to the higher grades, was no longer justified to find a 'potato defect' infected sample. A second option based on a sensory judgment test of the coffee beans was then adopted to find a 'potato defect' infected sample. According to the hypothesis the sensory judgment tests, the infected coffee sample would exhibit a different aroma profile than the standard green coffee sample due to presence of or increase in the concentration of some unfavorable compounds. In this attempt, green coffee bean samples were grouped on the basis of similarity of their aroma. Vacuum sealed packages of Rwandan coffee samples were opened and the head space accu-

mulated in the bag was compared by sniffing with that of good green coffee beans. The samples that smelled different than the standard green coffee sample were separated and again sub-grouped according to similarity of their smell. This way, rows or groups of samples smelling similar to each other were formed as shown in Figure 47. A representative sample from each row would give an approximate aroma profile for that group. A sample that would show an IPMP concentration higher than the standard threshold value would lead to a 'potato defect' infected group of samples.

Fig. 47. Rows of samples formed by sensory judgment

This might not be an appropriate way to sort coffee samples for the presence of the 'potato defect'. But it was suitable enough when we struggled to find at least some lead for 'potato defect' infected sample.

Two samples, M. Cerises/1506/MIG and A1/2006/KOAKAKA, showed much

higher concentrations of IPMP than the good green ground coffee sample. The peak area of IPMP was almost 4.5 times greater for M. cerises and 46 times greater for A1/2006/KOAKAA sample. This was called a 'first run' analysis of samples. It indicated that two samples were infected with the 'potato defect'. These samples were tested again to find the repeatability of the process. Chromatograms of samples that showed difference in the concentrations of IPMP are shown in Figure 48.

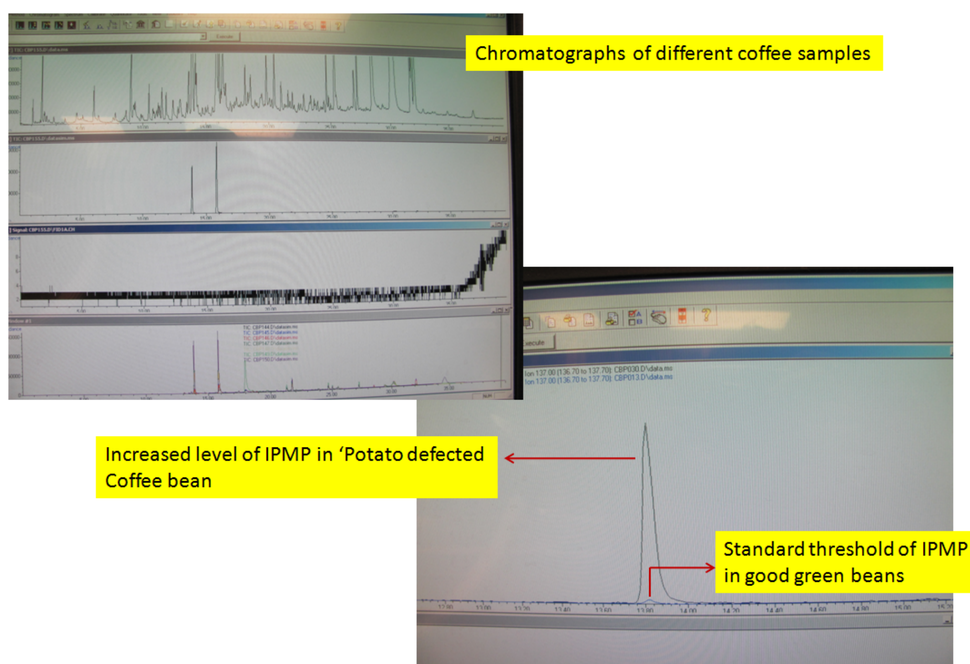

Fig. 48. Chromatograms of good and 'potato defect' infected green coffee sample

The instrument and experimental procedure used were confirmed to be reliable as they produced results that were similar to those in the first run for each of the samples M. Cerises/1506/MIG and A1/2006/KOAKAKA. In addition, there was the least possibility of contamination of any of these samples, though every possible care was taken to avoid it. Two vials of ground beans and a vial of whole beans from the samples M. Cerises/1506/MIG and A1/2006/KOAKAKA were prepared again

for further analysis.

All three newly made samples of A1/2006/KOAKAKA and two ground samples of M.Cerises /1506/MIG showed similar concentrations of IPMP as the sample of the standard green beans. The newly made whole coffee bean vial of the M.Cerises /1506/MIG sample showed an approximately 5 times higher peak area for IPMP than what was found in the chromatogram of the good ground beans. These results are shown in Figure 49.

Sample	First Run	Repeatability Of Instrument And Method	2 Newly Made Ground Coffee Bean Samples	Whole Bean Sample	COMMENTS
A1/2006/KOAKAKA	IPMP level > ground good coffee beans	yes	IPMP level ~ ground good coffee beans	IPMP level ~ ground good coffee beans	Results were not repeated for any of the newly made samples
M.Cerises /1506/MIG	IPMP level > ground good coffee beans	yes	IPMP level ~ ground good coffee beans	IPMP level > ground good coffee beans	whole bean sample showed higher amount of IPMP although expected to show lower than ground beans,

Fig. 49. Results of GC-MS

The results of the GC-MS analysis proved that an individual bean and not the whole sample was infected by the 'potato defect'. This infected bean did not spread its defect even when it was packed along with other good whole beans in the same package. Other beans exhibited this increased concentration of IPMP only when a defected bean was ground with them. This conclusion showed that a signature of a 'potato defect' infected bean can only be obtained, if and only if the inflicted bean gets incorporated into the test sample during the random selection of coffee beans for the experiments.

The isolation of the infected bean was an essential step and breakthrough to carry out further analysis. The infected single bean can be used to get answers for questions such as the origin and location of IPMP, remedies for the potato defect,

study of beans for infection, etc. This is an important step towards establishing a methodology to detect a single 'potato defect' infected bean that is not available today.

Hence, the group of whole beans which showed an increased concentration of IPMP (from the sample M.Cerises /1506/MIG) was segregated into individual vials. Chromatograms of the headspace from each of these vials were compared with that of a good whole bean. A single bean which showed a significant rise in the concentration of IPMP was detected as a 'potato defect' infected bean. This infected bean did not show any difference in physical appearance when compared to other healthy beans as shown in Figure 50.

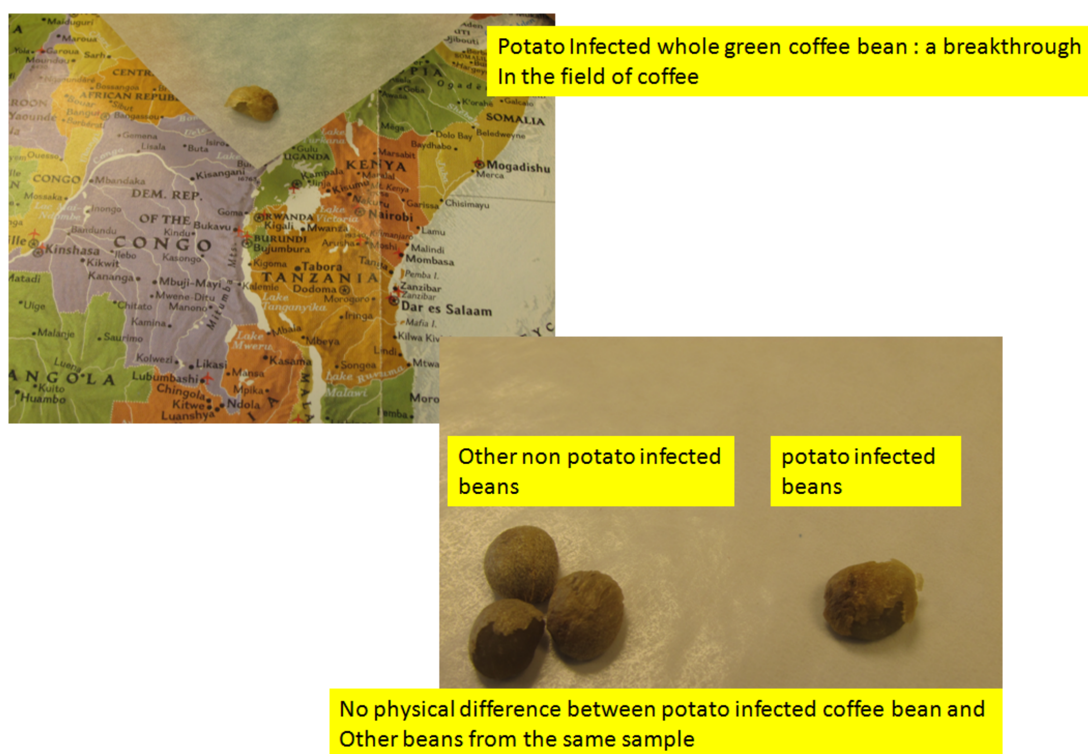

Fig. 50. 'Potato defect' infected whole green bean

Other scientists carried out experiments only with ground coffee beans. Hence, a

study of the earlier literature was not sufficient to evaluate the possibility of detecting the concentration of IPMP or the presence of the 'potato defect' in whole beans. Experiments conducted during this research showed that IPMP can be detected even in whole beans. Beans need not be ground or roasted for detection of the potato defect. This is an important finding because it indicates that a non destructive process might be implemented to sort coffee beans for presence of the 'potato defect'.

While these experiments were being conducted at Iowa State University, cupping tests were completed in the Rwandan coffee labs. Cupping testes were performed with coffee beans taken out from each of the samples. A table of these results is given in the Appendix.

The cupping results, in contrast to the GC-MS analysis, could not identify any potato defect in the M.Cerise/1506/MIG and A1/2006/KOAKAKA samples. Similarly, samples A1/1906/KOAKAKA and M.cerises/no7/KOAKAKA were identified by cupping results as strongly inflicted by the 'potato defect', yet they did not show higher concentrations of IPMP in the GC-MS analysis. It was hard to cross reference the results of the GC-MS analysis and the cupping tests, because there was not a single common sample that has shown the presence of 'potato defect'. Hence GC-MS analysis was again conducted for the samples which were identified by cupping tests as having 'potato defect'. This time, the entire coffee sample was analyzed, and not just a few coffee beans. The small GC vials were not large enough to accommodate the entire sample. Therefore, the experimental set up was altered to accommodate the increased sample size as shown in Figure 51. All beans from the selected samples were emptied into glass jars enclosed by lids with screw fitting. A heated water bath was used to increase the temperature of the glass jars to 90 degree centigrade.

A SPME fiber was inserted into the head space of the coffee jar through a circular opening provided on the lid. After sampling the head space for 30 min at 90 degree

centigrade, the SPME fiber was loaded into the injector of the GC column manually. This was an optimistic approach to find more beans with 'potato defect' in the whole sample.

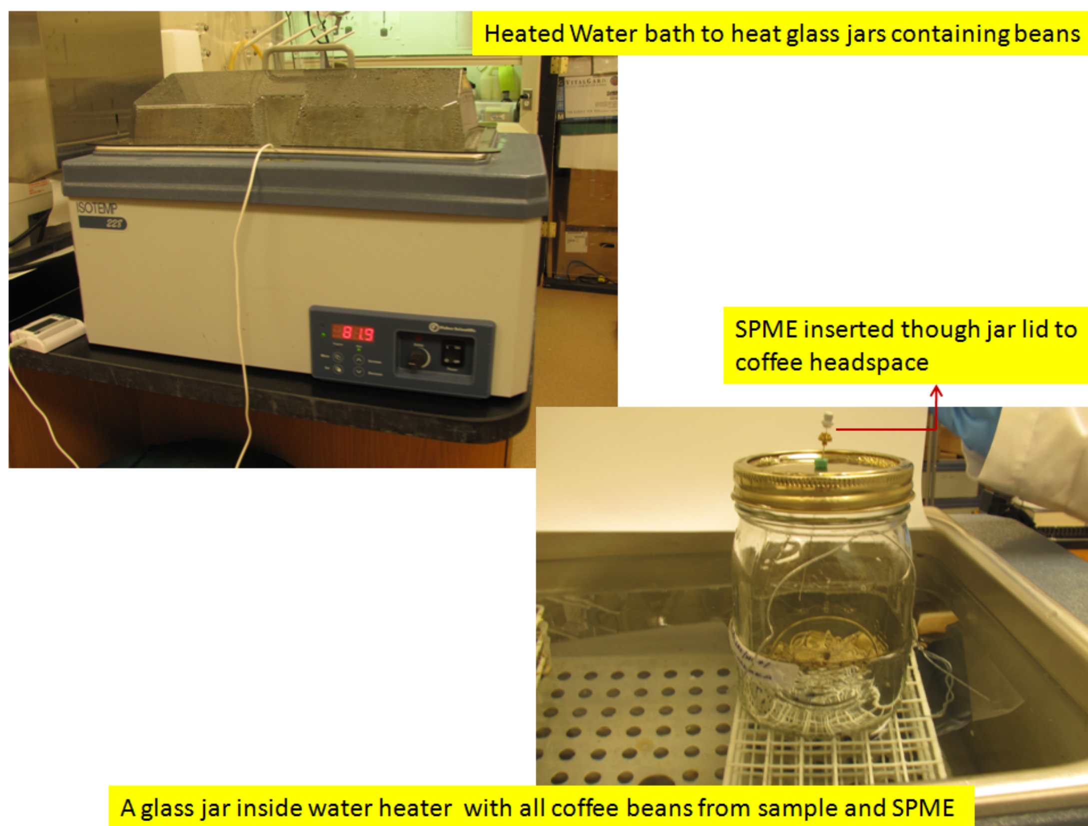

Fig. 51. Modification in experimental setup

Analysis of the chromatograms for the samples A1/1906/KOAKAKA and M.cerises/no7/KOAKAKA did not show an increased concentration of IPMP, although cupping tests indicated that these samples had the 'potato defect'. Each and every step during the experiments was a learning process. Chromatograms of the coffee samples showed similar concentrations for the other volatile components of coffee and thus it became clear that IPMP was the cause of 'potato defect' in coffee. In addition, since individual beans are infected, there is a high probability that the infected beans escape detection either during GC-MS analysis at Iowa State university or during cupping tests in Rwanda. Hence, cross referencing results from the two tests was difficult.

Same scenario must be repeated in Rwanda or other African countries. As mentioned earlier, the current process used to detect the presence of 'potato defect' in a bag of green coffee beans is not sophisticated. This includes collecting almost 150 g of beans by piercing a bag of beans at three different locations, taking the beans to cupping laboratories and then roasting, grinding and brewing these beans for cupping tests. The whole bag of green beans is rejected, if a cup of coffee, brewed from these sampled beans shows the presence of 'potato defect'.

Since the 'potato defect' inflicts individual beans, cupping a few coffee beans from a whole bag of beans is not a good representative test for the presence of the 'potato defect'. A bag of coffee beans declared to have 'potato defect' by cupping results may not have additional 'potato defect' infected beans left in the bag, because all that was present may have already been removed during cupping. Similarly, a bag of beans declared by cupping results to be without 'potato defect' may actually contain bad beans that simply escaped detection due to the sampling process used. Hence, an effective scientific sampling method would improve the results of cupping analysis by increasing the reliability of finding the presence of the potato defect on

the basis of cupping tests. The statistical method, which can help coffee technicians to access smaller groups of coffee beans would increase the probability of finding 'potato defect' infected coffee beans during sample collection for cupping. It would also minimize the rejection rate of good beans.

CHAPTER VI

PROPOSED FUTURE SORTING TECHNIQUES

As seen in CHAPTER V, a scientific statistical method would improve the results of cupping tests. The statistical method is required to map the IPMP concentration from sample to the whole batch of green coffee beans and predict more likely concentration of IPMP in the whole batch. This would reduce the rejection of good coffee beans and probability of occurrence of 'potato defect' infested coffee beans at the customers' end. In addition, conclusions from multidimensional GC-MS-O analysis of coffee beans showed that, a technical method, with a combination of rapid response time, sensitivity as well as selectivity towards different components would be the best choice to detect IPMP in coffee beans. Hence, two future techniques are proposed in following subsections to detect 'potato defect infected coffee beans.

A. Sampling Technique

A search was done for the existing sampling techniques implemented for green coffee beans. Currently, green coffee beans are sampled for a hazardous compound Ochratoxin A (OTA)[34, 35, 36]. This is a carcinogenic compound and should not be distributed through coffee beans beyond certain concentration. There is a similarity between the presence of OTA and IPMP in coffee beans. The distribution of OTA in green coffee beans is heterogeneous and only few coffee beans are infected with this toxin in the whole lot. Hence, it is difficult to predict true concentration of OTA in the whole batch. So, efforts are made to design a sampling plan for OTA. A reference can be taken from these papers to design a sampling plan for IPMP[34].

The steps to be followed for this design plan would be as follows :

1. The IPMP sampling plan would be defined by the accept or reject limit, which is nothing but the threshold concentration that separates good lots from bad lots. This threshold concentration can be decided by a regulatory agency or as per requirements.

2. The test procedure to detect IPMP in a sample can be decided. This test procedure would probably consists of sampling, sample preparation and analysis.

3. There will be uncertainties involved in quantifying IPMP in each of the test procedure steps. These uncertainties would hinder true prediction of IPMP concentrations in a coffee bean batch.

4. Variance with each of these uncertainty can be found out by experiments and total variance can be obtained by summing these individual variances in terms of concentration of IPMP in lot.

5. The true but unknown lot concentration can then be estimated by quantitating IPMP concentration in a test sample. The sample test result can then be used either as an estimate of the true lot concentration or to make a decision about acceptability of the coffee lot.

6. Theoretical distributions such as normal, log normal, negative binomial, and compound gamma can be evaluated to simulate the distribution of IPMP sample test results. The best fit curve can be obtained for the observed OTA sample concentrations.

7. Observed and predicted acceptance probabilities can then be computed. Observed probabilities can be computed by counting number of test samples having IPMP concentration less than accepted value and then dividing by total num-

ber of test samples. Predicted acceptance probabilities can be calculated by the best fit curve obtained in step 6.

8. As mentioned in step 1, an acceptance concentration for IPMP, Ca can be decided. The samples having IPMP concentration c would be compared with Ca. If c $\leq$ Ca, then only lot of green coffee beans would be accepted, otherwise rejected. The following equation would relate these variables. P(A) = probability (c $\leq$ Ca — C)

9. Operative characteristics curve can be obtained by plotting P(A) versus lot concentration of IPMP. This curve would be specific to given sample size and test conditions. It can be altered by trial and error and optimum parameters such as quantity of sample, number of samples can be decided as per the requirements.

B. Terahertz Spectroscopy

As seen in Chapters IV and V, E-nose, ion mobility spectrometry and cavity ring down spectrometry could not detect IPMP at ppb concentration levels. Experiments conducted by multidimensional GC-MS-O could find IPMP at ppb concentration levels even in the presence of 800 other compounds. This work, described in this thesis, gave us the direction to search for a probable solution for detecting IPMP in coffee beans. Requirements for the detection technique can now be visualized as shown in Figure 52.

Many of the techniques conventionally applied for analysis of volatiles such as FTIR, photo acoustic, mass and ion mobility spectrometry are immediately disqualified due to limited sensitivity or selectivity in a complex cluttered background.

The need for an even more sensitive and selective technique has been satisfied by terahertz spectroscopy. This approach is developed by Dr. Bevan and his group at

Rapid Detection { E-Nose, IMS, CRDS) +
Selectivity { GC-MS-O} + Sensitivity =
Most possible technique to detect
IPMP in coffee beans.

Fig. 52. Requirement from a possible detection technique

Texas A and M University and consists of integrating cavity-enhanced technologies and a terahertz. This technique has been used to detect and monitor the concentration of CO, NO, NH3 *etc.* at low concentration levels (parts per trillion) in gaseous samples at reduced pressures.

Dr. Bevan's group has applied this technology to detect CO at concentrations as low as 1 part in 10^{12} and at sample pressures of a few torr in air, within milliseconds without preconcentrating or destroying the sample. Looking at the sensitivity, selectivity and non destructive nature of the method it is thought to be a promising solution to detect IPMP in coffee beans.

Hence, this part would be carried out in future with 'potato defect' infected and good Rwandan coffee beans.

CHAPTER VII

SUMMARY

This research project started with developing an understanding of coffee, the terminology used in coffee processing and the different types of defects that can occur in coffee. This project helped our understanding of coffee ecology and the commerce of coffee in the world. A personal visit to Rwanda was helpful for gathering practical knowledge about coffee processing technology and details of coffee farming. This visit was important for learning about losses the farmers suffer due to different coffee defects, their effects on the social and economic life of farmers and the requirements of farmers in Rwanda. The cupping tests carried out in Rwandan coffee laboratories gave us an actual taste and aroma of the 'potato defect' in coffee.

In addition to learning about the coffee processing technologies, a literature review introduced us to the flavor chemistry of coffee. Chemical reactions involved in the evolution of coffee aroma helped us to find the origin of 'potato defect'. The literature survey made it possible to list important points such as the concentration of 3-isopropyl-2-methoxypyrazine (IPMP), its thermal stability and its presence in good as well as infected beans. This gave the lead to build a foundation for detection techniques to find the concentration of IPMP in the shortest possible time.

The detailed study of the different analytical methods such as electronic nose, ion mobility spectrometry (IMS) and cavity ring down spectrometry (CRDS) was done to evaluate their suitability for finding IPMP at ppb concentration levels in coffee beans. Learning the fundamentals of these methods was valuable to select a future path for developing coffee bean sorting technologies. In addition to these techniques, other methods were also surveyed for their utility in a sorting technology for 'potato defect' infected coffee beans. This part of the research was most valuable as different

methods from varied fields such as mechanical, chemical, statistical etc were learnt.

Experiments conducted at Iowa State University resulted in a major breakthrough in term of finding a single 'potato defect' infected coffee bean. This coffee bean will be used for future experiments and to solve the mystery of potato defect.

CHAPTER VIII

CONCLUSIONS AND FUTURE WORK

A. Conclusions

It is a challenging task to develop a technique to measure the concentration of IPMP at the ppb level in a matrix of 800 other volatile components. The three most sensitive methods, viz. electronic nose (E-Nose), ion mobility spectrometry (IMS), cavity ring down spectrometry (CRDS) were not sensitive enough to detect IPMP at the ppb levels. Multidimensional GC-MS-O could identify IPMP present in the matrix of 800 coffee volatiles. The major shortcoming of GC-MS is the slow speed of analysis : it took 90 minutes to analyze one coffee sample and generate a chromatogram.

A comparison showed that, GC-MS could separate most of the compounds present in coffee volatiles. It was also possible to detect IPMP at ppb concentration levels in the presence of 800 other coffee volatiles. The other mentioned methods i.e. E-Nose, IMS, CRDS were not sufficiently selective and sensitive to find the concentration of IPMP in coffee volatiles. In this scenario, a very upcoming terahertz technology seemed to be the possible solution, which would give us the combination of selectivity of GC-MS and low respond time of IMS or CRDS. In addition, a scientific statistical method can also be a good approach to design a sampling plan which would map a concentration of IPMP from samples to actual lots of green coffee beans. It would help to minimize rejection rate of good coffee beans and the occurrence of frequency of ‘potato defect’ infected beans at customer’s end.

B. Future Work

The present work opened wide avenues for further research in this field of 'potato defect' in coffee. Different aspects that could be covered in the future are listed below.

1. Analysis of a single 'potato defect' infected bean, identified by GC-MS experiments, from a plant pathology point of view could be done. A DNA signature or traces of any remains of infection could be analyzed to find the cause of potato defect with the help of plant pathologist. This would also test the hypothesis about bacteria being the cause of potato defect.

2. A commercial tera hertz spectrometer could be developed for use as the sensor guiding the sorting coffee beans for potato defect.

3. statistical survey could be completed to evaluate the occurrence frequency of potato defect in different grades of coffee in different farms and coffee washing stations in Rwanda to establish the major source of production of infected coffee cherries or beans.

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APPENDIX A

CUPPING TEST RESULTS FROM RWANDAN COFFEE TESTING LABS

Cupping Results – Potato Experiment 2010 (KOAKAKA CWS)

№	SAMPLES	POINTS	COMMENT
1	Triage sélection nº7 **M. Cerises N°7/KOAKAKA** **(Bad cherries N°7)**	72	Potato, in 2 cups strong, popcorn and nuts, bitter and harchy
2	Triage sélection nº11	79	Harchy, bitter, sour, solt, analype beans
3	Triage sélection nº10	77	Harchy, bitter, sour
4	Triage égouttage 2106A2	82	Sweet clean lemon milk chocolate
5	Triage égouttage 1806A1	78	Harchy, bitter, sour
6	Triage égouttage 2006A1	80	Popcorn, bitter and harchy
7	Triage égouttage 1806A2 **Déchets A2/1806 KOAKAKA**	74	Potato in 1cup
8	Triage égouttage 2006A2	76	Over fermantation, bitter, sour
9	Triage sélection nº08 **M. Cerises N°8/KOAKAKA** **(Bad cherries N°8)**	74	Potato in 1cup
10	Triage égouttage 2206A2	79	bitter and harchy
11	Triage égouttage 2106A1	75	Solt,harchy
12	Triage séchage nº4 **M. Cerises N°4/KOAKAKA** **(Bad cherries N°4)**	74	Potato in 1 cup
13	Triage Table de Sélection nº1	77	bitter and harchy
14	Triage Sélection nº06	75	Harchy, bitter
15	Triage Sélection nº05	76	bitter and harchy,analype beans
16	Triage Sélection nº09 **M. Cerises N°9/KOAKAKA** **(Bad cherries N°7)**	72	Potato in 1 cup
17	Triage Table de Sélection nº2	78	bitter and harchy
18	Triage égouttage 2206A1	70	Harchy, bitter, sour, over fermentation
19	Triage égouttage 1906A2	77	Harchy, bitter
20	Triage Table de Sélection nº3	78	Nuts, bitter,solt
21	Triage égouttage19006A1 **Déchets A1 1906 KOAKAKA** **Defects A1 1906 KOAKAKA**	68	Potato in3 cups 2 very strong
22	Triage égouttage2306A1	76	Harchy, bitter
23	Lot 0406A2k NG G 2	83	Clean,citric acid,orange flavor,medium body and sweet
24	Triage égouttage	79	Harchy, bitter

N.B: Names of Cuppers: Uzziel Habimana and Emerthe Mukabavugirije (Nov 9th, 2010)

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