Kasetsart J. (Nat. Sci.) 49 : 32 - 41 (2015)

Microbiological Study During Coffee Fermentation of *Coffea arabica* var. *chiangmai* 80 in Thailand

Rujikan Nasanit[1,*] and Komate Satayawut[2]

ABSTRACT

This study investigated the microbial communities during coffee fermentation using the wet method for *Coffea arabica* var. *chiangmai* 80 which has been widely grown in northern Thailand. The coffee cherries and the depulped coffee samples were collected during fermentation from four processing areas. The fermentation tank pH decreased from 6.27 to 4.00 after 48 hr fermentation. Bacteria were the most abundant microorganisms found throughout the process. The most common bacteria were members of *Enterobacteriaceae* such as *Enterobacter agglomerans*, *Erwinia dissolvens*, *Escherichia coli* and *Klebsiella pneumonia*. In addition, lactic acid bacteria were frequently found throughout fermentation and included *Leuconostoc mesenteroides, Lactobacillus brevis, Lactococcus plantarum* and *Enterococcus casseliflavus*. Gram-positive spore forming bacteria such as *Bacillus subtilis* and *B. cereus* were also found during fermentation. The number of yeasts increased after 24 hr fermentation. *Candida, Pichia, Debaryomyces, Kluyveromyces* and *Saccharomyces* were the most common yeast genera. Filamentous fungi were minimal during the fermentation. *Penicillium* was the most common fungi in this study. The genera and species identified include members known to have pectinolytic activity. These microorganisms may be used to improve coffee fermentation or to produce pectinolytic enzyme for industrial purposes. Moreover, some had the ability to produce a special aroma.

Keywords: *Coffea arabica*, wet process, coffee fermentation, mucilage degradation

INTRODUCTION

Coffee is one of the preferred beverages consumed worldwide (Farah, 2009). In addition, it is an economic plant that is grown widely throughout southern and northern Thailand to produce *robusta* and *arabica* coffee, respectively (Office of Agricultural Economics, http://www.oae.go.th/ewt_news.php?nid=13577). In general, coffee beans are produced from coffee cherries by one of three methods—wet method, dry/natural method and semi-dry method (Avallone *et al.*, 2002; Masoud *et al.*, 2004; Jackels and Jackels, 2005; Silva *et al.*, 2008a; Vilela *et al.*, 2010). The wet method has been used normally for *arabica* coffee as it has a thicker pulp than *robusta* coffee (Clarke and Macrae, 1987. Briefly, the coffee cherries are depulped and placed into the fermentation tank to remove the mucilage layer of coffee beans using natural microbes for 12–60 hr depending on the climatic temperature and the maturity of the coffee cherries (Avallone *et al.*, 2002; Masoud *et al.*, 2004). Then, the coffee beans are sun-dried on a platform for 7 d to reduce the

1 Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University, Nakorn Pathom 73000, Thailand.

2 Post-harvesting and Processing Research and Development Office, Bangkok 10900, Thailand.

* Corresponding author, e-mail: rujikan@su.ac.th

Received date : 15/08/14 Accepted date : 04/02/15

moisture content to 12%. The parchment coffee is stored with good ventilation before hulling in order to prevent moisture absorption in the green beans. In the dry or natural method, the coffee cherries are sun-dried for 10–25 d on the ground in a 10 cm thick layer, heaped at night and then respread each day (Silva *et al.*, 2008a; 2008b). This process has been widely used for *robusta* coffee; however, in Brazil it has been used to process *arabica* coffee (Silva *et al.*, 2000). The semi-dry method is a modification of the wet process as the depulped coffee beans are naturally fermented without water. However, the coffee quality is not as good as from wet processed coffee (Jackels and Jackels, 2005; Vilela *et al.*, 2010).

The cup quality of coffee is a highly complex trait, and depends on physical and sensory qualities, with the coffee variety, climatic conditions during plant growth, processing method and storage conditions being factors that can affect the coffee quality (Joet *et al*., 2010; Bertrand *et al*., 2012). The presence of microorganisms in coffee processing is also an important factor that can affect the final beverage quality as some pectinolytic microorganisms are associated with the degradation of the pulp and mucilage layer producing acids and other metabolic compounds that can diffuse into the coffee beans (Silva *et al*., 2013).

Bacteria, yeasts and filamentous fungi have been detected during coffee processes from previous studies (Avallone *et al.*, 2001; Masoud *et al.*, 2004; Silva *et al.*, 2008b). However, most of the previous studies were in Central and South America where the highest production of coffee occurs (Chanakya and de Alwis, 2004. In Thailand, *Coffea arabica* var. *chiangmai* 80 was selected by plant breeding to exhibit resistance to coffee leaf rust disease and has been introduced to farmers and widely cultivated in northern Thailand (Office of Agricultural Research and Development Region 1, Department of Agriculture, http://www.oard1.org/index.php?option=com_content&view=article&id=75&Itemid=85). However, there has been no report on the microbial communities present during coffee production with this coffee variety. Therefore, the current study aimed to determine the microbial communities of *Coffea arabica* var. *chiangmai* 80 during fermentation by the wet method.

MATERIALS AND METHODS

Sampling

Coffee cherries (*Coffea arabica* var. *chiangmai* 80) and mucilage coffee beans during fermentation were collected from four sites on agricultural research centers in Chiang Mai (Khun Wang), Chiang Rai (Wa Wee), Phetchabun (Khao Kho) and Tak (Musoe) provinces in January 2011. Samples of coffee cherries were collected randomly in three different areas of each coffee plantation. The depulped coffee beans were collected every 12 hr over 48 hr fermentation by collecting the samples from three different points at the middle depth of the fermentation tank and mixing. The temperature at each collection point was measured with a portable electronic thermometer.

Measurement of pH and dry weight of depulped coffee beans

Depulped coffee beans (20 g) were gently swirled in distilled water and the pH of the supernatant was measured (Avallone *et al.*, 2001). The dry weight was determined on samples that had been oven-dried at 60 °C until constant weight.

Microbiological analyses

The coffee cherries (20 fruits) and fermented coffee beans (20 g) were transferred to a flask containing 180 mL of sterile peptone water (1% peptone, 5% weight per volume NaCl) and swirled gently for 20 min. Peptone water was used to make a serial dilution (1:10) for plating on plate count agar (PCA) for a total plate count. Eosin methylene blue (EMB) agar was used to detect *Enterobacteriacea*, de Man, Rogosa and Sharpe

(MRS) agar with 0.25% volume per volume (v/v) nystatin (Sigma-Aldrich; MO, USA) was used to detect lactic acid bacteria (LAB) and dichloran glycerol (DG18) agar (Oxoid; Basingstoke, UK) was used to enumerate yeasts and filamentous fungi. Plates were incubated in triplicate at 28 °C for 48 hr for bacteria and 5 d for yeast and filamentous fungi. Colony-forming units (CFUs) were counted, and data were expressed as the mean of the decimal logarithm of the CFU per gram of fermented bean dry weight. For each type of medium containing isolated colonies, numbers of colonies were taken at random for identification. Isolates were purified and stored at -80 °C in 20% (v/v) glycerol. Strain frequencies were calculated as the ratio of the respective population density.

Identification of microorganisms

Bacteria were identified based on bacterial colony morphologies, Gram-stain, endospore-stain and biochemical analysis based on Bergey's manual of determinative bacteriology (Holt *et al.*, 1994).

Yeasts were identified based on morphological and biochemical methods such as colony morphology, spore formation, fermentation on different carbon sources and assimilation on different nitrogen sources (Kurtzman and Fell, 1998; Barnett *et al*., 2000).

Filamentous fungi were identified based on the morphology of colony, color, appearance and spore formation (Barnett and Hunter, 1987; Pitt and Hocking, 1997).

RESULTS

pH and temperature during coffee fermentation

Sample collection was undertaken under atmospheric, exposed conditions every 12 hr during 48 hr fermentation. The average pH values and temperatures of four fermentation tanks were determined as shown in Figure 1. As can be seen, the pH decreased gradually during the fermentation period of 48 hr from 6.27 to 4.00. Temperature in the fermentation tanks fluctuated since the atmospheric temperature varied between day and night.

Microbial trend during coffee fermentation

The average quantity of aerobic bacteria, lactic acid bacteria, *Enterobacteriaceae* and yeast/filamentous fungi were determined in the four sampling tanks during fermentation as shown in

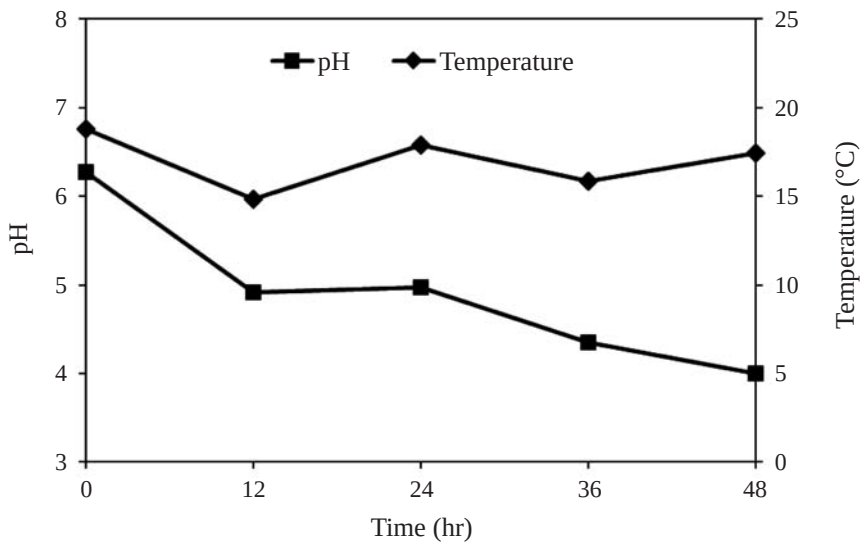

Figure 1 Changes of pH and temperature in tank during 48 hr wet fermentation of coffee beans.

Figure 2. The quantity of aerobic bacteria, lactic acid bacteria and *Enterobacteriaceae* increased from the start of fermentation up to 24 hr. These microorganisms decreased slightly after 24 hr of fermentation. Yeast and filamentous fungi were minimal compared to bacteria during the fermentation. However, yeast and filamentous fungi increased gradually during fermentation and reached their maxima in the final stage of the fermentation process.

Qualitative study of microflora during coffee fermentation

In total, 681 isolates were taken randomly from the four media for identification. All isolates were successfully identified to at least the genus level. Of this total, 79.4% (541 isolates) were bacteria, 15.8% (108 isolates) were yeasts, and 4.7% (32 isolates) were filamentous fungi. The predominant bacteria detected on the surface of coffee cherries and fermented coffee beans were members of the family *Enterobacteriaceae* (241 isolates, 44.5%), followed by lactic acid bacteria (237 isolates, 43.8%), and Gram-positive spore forming bacteria (46 isolates, 8.5%) which belonged to *Bacillus subtilis* and *B. cereus*. Seventeen isolates of Gram-negative bacilli were also detected in the coffee samples; the bacilli belonged to the two genera of *Pseudomonas* (*P. fluorescens* and *P. delafieldii*), and *Aeromonas* (*A. schubertii*).

Bacteria in the *Enterobacteriaceae* belonged to nine genera consisting of: *Enterobacter* (*E. agglomerans*, 147 isolates), *Erwinia* (*E. dissolvens*, 42 isolates and *E. herbicola*, 4 isolates), *Escherichia* (*E. coli*, 25 isolates), *Klebsiella* (*K. pneumoniae*, 12 isolates), *Citrobacter* (*C. freundii*, 7 isolates), *Salmonella* (*Samonella* sp., 1 isolate), *Yersinia* (*Y. mollaretii*, 1 isolate), *Leminorella* (*L. grimontii*, 1 isolate), and *Proteus* (*P. penner*, 1 isolate). The lactic acid bacteria belonged to five genera consisting of: *Leuconostoc* (*Ln. mesenteroides*, 75 isolates), *Enterococcus* (*E. casseliflavus*, 59 isolates), *Lactobacillus* (*L. brevis*, 44 isolates and *L. plantarum*, 36 isolates), *Lactococcus* (*L. lactis*, 17 isolates) and *Streptococcus* (*S. faecalis*, 8 isolates). Furthermore, acetic acid-producing bacteria were not detected in any of the fermentation tanks. The strains isolated with the highest frequencies from cherries and during the fermentation period were *E. agglomerans*, *E. casseliflavus* and *Ln. mesenteroides* (Table 1). In addition, *E. agglomerans* and *Ln. mesenteroides* were

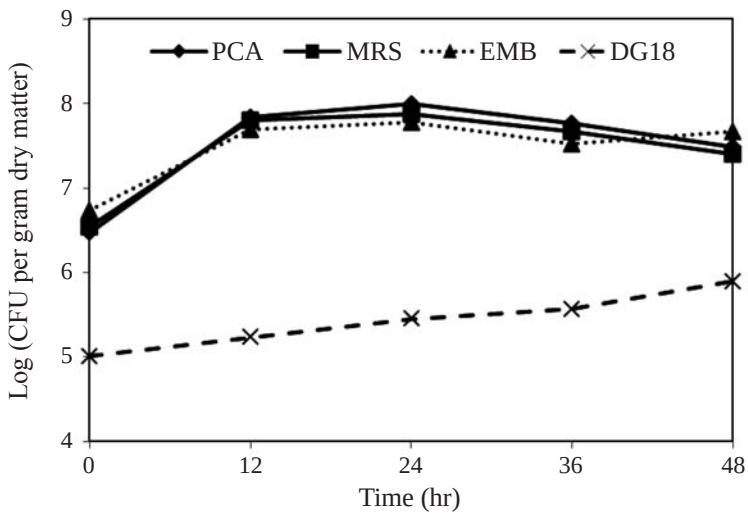

Figure 2 Microbial counts (CFU = Colony forming units) during coffee fermentation: for aerobes (PCA), lactic acid bacteria (MRS), Enterobacteriaceae (EMB) and yeasts and fungi (DG18).

the common species detected at each stage of fermentation and on the surfaces of coffee cherries.

Yeasts were isolated and identified based on morphological and biochemical methods. One hundred and eight isolates of yeasts were identified to the genus level, which belonged to seven genera—*Pichia* spp. (28 isolates), *Candida* spp. (25 isolates), *Kluyveromyces* spp. (23 isolates), *Saccharomyces* spp. (19 isolates), *Debaryomyces* spp. (10 isolates), *Hanseniaspora* spp. (2 isolates) and *Schizosaccharomyces* sp. (1 isolate). Yeasts were detected on the surfaces of cherries and coffee beans throughout fermentation. The number of yeasts started rising after 12 hr fermentation as the pH of the fermentation tank decreased to around 4 to 5 which was appropriate for yeasts to grow. The frequencies of yeasts detected during the

Table 1 Main species of bacteria detected on coffee cherries and coffee beans during coffee fermentation.

Time (hr)	Bacterial species	Frequency[a] (%)
Cherries	*Enterobacter agglomerans*	29.4
	Leuconostoc mesenteroides	15.7
	Erwinia dissolvens	9.4
	Lactobacillus brevis	9.4
0	*Enterobacter agglomerans*	23.2
	Erwinia dissolvens	13.6
	Lactobacillus brevis	12.3
	Bacillus subtilis	10.9
	Lactobacillus plantarum	8.2
	Leuconostoc mesenteroides	8.2
12	*Enterobacter agglomerans*	27.6
	Lactobacillus plantarum	12.7
	Bacillus subtilis	11.7
	Lactobacillus brevis	7.4
	Leuconostoc mesenteroides	7.4
24	*Enterobacter agglomerans*	35.3
	Leuconostoc mesenteroides	14.6
	Enterococcus casseliflavus	12.1
	Erwinia dissolvens	9.7
36	*Enterobacter agglomerans*	31.0
	Leuconostoc mesenteroides	18.3
	Enterococcus casseliflavus	16.0
	Lactobacillus brevis	10.3
48	*Enterobacter agglomerans*	18.1
	Enterococcus casseliflavus	16.3
	Leuconostoc mesenteroides	15.4
	Bacillus subtilis	10.0
	Lactobacillus plantarum	8.1
	Klebsiella pneumonia	7.2

[a] Frequency related to the total bacterial population at each stage.

fermentation and on the coffee cherries are shown in Table 2. Four genera of yeast were mainly detected during the fermentation—*Saccharomyces* spp., *Pichia* spp., *Kluyveromyces* spp. and *Candida* spp.

Filamentous fungi were the least common of the microorganisms found in coffee samples (Table 3) with only 32 isolates detected in this study. Of this total, *Penicillium* spp. (14 isolates) was the most common genus, followed by *Acremonium* spp. (11 isolates), *Cladosporium* spp. (5 isolates), *Aspergillus* sp. (1 isolate) and *Fusarium* sp. (1 isolate). Filamentous fungi were mostly detected in coffee cherries.

Table 2 Main yeast genera detected on coffee cherries and coffee beans during coffee fermentation.

Time (hr)	Yeast	Frequency[a] (%)
Cherries	*Candida* sp.	57.1
	Pichia sp.	42.8
0	*Saccharomyces* sp.	42.8
	Kluyveromyces sp.	28.5
12	*Pichia* sp.	30.7
	Saccharomyces sp.	30.7
	Kluyveromyces sp.	30.7
24	*Pichia* sp.	38.0
	Candida sp.	19.0
	Saccharomyces sp.	19.0
36	*Kluyveromyces* sp.	33.3
	Candida sp.	27.3
	Saccharomyces sp.	22.2
48	*Kluyveromyces* sp.	30.3
	Candida sp.	24.2

[a] Frequency related to the total yeast population at each stage.

Table 3 Filamentous fungi isolated from coffee cherries and coffee beans during coffee fermentation.

Time (hr)	Chiang Mai	Chiang Rai	Phetchabun	Tak
Cherries	*Penicillium* sp. (1), *Acremonium* sp. (1)	*Cladosporium* sp. (3), *Acremonium* sp.(2), *Penicillium* sp. (1)	nd	*Penicillium* sp. (1), *Cladosporium* sp.(2)
0	nd	nd	nd	nd
12	*Penicillium* sp. (1)	*Acremonium* sp. (5), *Penicillium* sp. (1)	nd	nd
24	*Penicillium* sp. (2)	*Fusarium* sp. (1)	nd	nd
36	*Penicillium* sp. (3), *Aspergillus* sp. (1)	nd	*Penicillium* sp. (2)	*Acremonium* sp. (1)
48	nd	nd	*Penicillium* sp. (2)	*Acremonium* sp. (2)

nd = Not detected.

( ) = Numbers in parentheses indicate number of isolates.

DISCUSSION

Coffee quality depends on various factors. One of the major factors that affects the coffee quality is the presence of microorganisms in the coffee process, since they produce organic compounds which the green coffee can absorb (Franca *et al*., 2005). In previous studies, bacteria, yeasts and filamentous fungi were detected during the coffee producing process (Avallone *et al.,* 2002; Masoud and Jespersen, 2006; Silva *et al*., 2008a; Vilela *et al.,* 2010). The role of microorganisms in coffee cup defects is a matter of debate. The most serious defects are 'fermented taste', 'sour' and 'stinkers', which are often attributed to problems during fermentation (Wootton, 1961; Franca *et al*., 2005). Therefore, to gain information on the microbial community during the coffee production process is important. In the current study, the depulped coffee was fermented in tanks for 48 hr to remove the mucilage layer composed of pectin. The results showed that bacteria were the predominant microorganism found throughout the fermentation period and on the surface of coffee cherries. Bacteria in *Enterobacteriaceae* and lactic acid bacteria were abundant throughout the fermentation process, some of which have been reported as pectinolytic strains (Abbott and Boraston, 2008). In addition, *E. agglomerans* and *Ln. mesenteroides* were predominant species during coffee fermentation. *E. agglomerans* has also been detected as the predominant specie during the semi-dry process of *Coffee arabica* L. (Vilela *et al.,* 2010). *Ln. mesenteroides* was a lactic acid bacterial species frequently found during coffee fermentation by the wet method (Avallone *et al.,* 2001). Other predominant lactic acid bacteria detected in the current study were *L. bervis, L. plantarum* and *E. casseliflavus*. These bacterial strains produce lactic acid resulting in a decreased pH during fermentation, which leads to optimum conditions for yeast to grow. Moreover, other bacteria in *Enterobateriaceae* that have pectinolytic activity similar to those present in the current study include *E. dissolvens*, *E. herbicola* and *K. pneumonia* (Frank *et al.,* 1965; Avallone *et al.,* 2002). Some *Bacillus* species can produce extracellular enzymes that degrade pectin (Ouattara *et al.,* 2008) and some of these species were present in the current study such as *B. subtilis* and *B.cereus*. *Pseudomonas* species were also found in the current study which were able to produce pectinolytic enzyme (Franzetti and Scarpellini, 2007).

The major yeast genera detected in the current study were *Saccharomyces*, *Pichia*, *Kluyveromyces* and *Candida*, which have already been reported in previous studies of coffee fermentation by the wet process (Silva *et al*., 2000; Avallone *et al*., 2001; Masoud *et al*., 2004). Most of these yeasts have the ability to produce pectinolytic enzymes (Agate and Bhat, 1966). *Pichia kluyveri* and *P. anomala* isolated during coffee fermentation by the wet method showed high pectinolytic activity (Masoud and Jespersen, 2006). Some yeast species such as *Debaryomyces hansenii* could promote a decrease in the fungal population (Payne and Bruce, 2001). *S. cerevisiae* and *C. parapsilosis* have been recently reported to be able to produce a special aroma of caramel, herbs and fruits in coffee; however, this was from dry processing (Evangelista *et al*., 2014).

Filamentous fungi were the least detected microorganisms in the wet fermentation process. However, they have been reported to be diverse in coffee cherries and during the natural fermentation method (Silva *et al*., 2000; Silva *et al*., 2008b). In addition, they can grow in coffee beans held in storage (Silva *et al*., 2008a). *Penicillium* was the most common genus in the current study and there have been reports of their pectinolytic activity (Fawole and Odunfa, 1992; Martin *et al*., 2004; Mamma *et al*., 2008). However, some genera detected in the current study were able to produce mycotoxins, which can affect consumer health (Vega *et al.,* 2006; Batista *et al.,* 2009) such as *Penicillium*, *Fusarium* and *Aspergillus*. Therefore, this matter needs to be considered in

coffee production.

There has been less information published on the involvement of each microorganism present in the current study. Nevertheless, it has been suggested that over-fermentation of coffee can cause sour beans which detracts from cup quality (Agresti *et al*., 2008). Furthermore, over-fermentation and bad fermentation with butyric or propionic acids production would be the result of 'alcoholic' and 'stinker' coffee tastes, respectively (Wootton, 1961). In addition, the 'potato taste defect' has been attributed to the growth of some enterobacteria such as *Serratia* and *Cedecea* strains (Gallois and Grimont, 1985). Fortunately, these were not found in this study. Therefore, to limit coffee defects, coffee fermentation has to be controlled.

CONCLUSION

Bacteria, yeasts and filamentous fungi were detected during the fermentation process of coffee by the wet method. The predominant microflora were bacteria, of which *Enterobacteriaceae* and the lactic acid bacteria were the most common. Most strains found in this study have been reported as having the ability to produce pectinolytic enzymes which is interesting for further enzyme study for industrial purposes or may be useful to improve coffee fermentation. However, there were filamentous fungi detected which are able to produce mycotoxins. Furthermore, the involvement of microorganisms and their influence on coffee beverage quality has to be further studied.

ACKNOWLEDGEMENTS

The authors would like to thank the Silpakorn University Research and Development Institute for funding and the Post-Harvesting and Processing Research and Development Office for some financial support and accommodating during the field trips for sample collection. The authors would like to convey special appreciation to the academic committee of the 25th Annual Meeting of the Thai Society for Biotechnology and International Conference (TSB2013) for providing the opportunity for this work to be published in this journal.

LITERATURE CITED

Abbott, D.W. and A.B. Boraston. 2008. Structural biology of pectin degradation by Enterobacteriaceae. **Microbiol. Mol. Biol. R.** 72: 301−316.

Agate, A.D. and J.V. Bhat. 1966. Role of pectinolytic yeasts in the degradation of mucilage layer of *Coffea robusta* cherries. **Appl. Microbiol.** 14: 256−260.

Agresti, P.D.C.M., A.S. Franca, L.S. Oliveira and R. Augusti. 2008. Discrimination between defective and non-defective Brazilian coffee beans by their volatile profile. **Food Chem.** 106: 787−796.

Avallone, S., B. Guyot, J.M. Brillouet, E. Olguin and J.P. Guiraud. 2001. Microbiological and biochemical study of coffee fermentation. **Curr. Microbiol.** 42: 252−256.

Avallone, S., J.M. Brillouet, B. Guyot, E. Olguin and J.P. Guiraud. 2002. Involvement of pectolytic micro-organisms in coffee fermentation. **Int. J. Food Sci. Tech.** 37: 191−198.

Barnett, H.L. and B.B. Hunter. 1987. **Illustrated Genera of Imperfect Fungi**, 4th ed. Macmillan Publishing Company. New York, NY, USA.

Barnett, J.A., R.W. Payne and D. Yarrow. 2000. **Yeasts: Charateristics and Identification**, 3rd ed. Cambridge University Press. Cambridge, United Kingdom.

Batista, L.R., S.M. Chalfoun, C.F. Silva, M. Cirillo, E.A. Varga and R.F. Schwan. 2009. Ochratoxin A in coffee beans (*Coffea arabica* L.) processed by dry and wet methods. **Food Control** 20: 784−790.

Bertrand, B., R. Boulanger, S. Dussert, F. Ribeyre, L. Berthiot, F. Descroix and T. Joet. 2012. Climatic factors directly impact the volatile organic compound fingerprint in green Arabica coffee bean as well as coffee beverage quality. **Food Chem.** 135: 2575−2583.

Chanakya, H.N. and A.A.P. de Alwis. 2004. Environmental issues and management in primary coffee processing. **Trans IChemE.** 82(B4): 291–300.

Clarke, R.J. and R., Macrae. 1987. **Coffee Volume 2: Technology.** 1st ed. Elsevier Science. New York, NY, USA.

Evangelista, S.R., C.F. Silva, M.G.P.d.C. Miguel, C.d.S. Cordeiro, A.C.M. Pinheiro, W.F. Duarte and R.F. Schwan. 2014. Improvement of coffee beverage quality by using selected yeasts strains during the fermentation in dry process. **Food Res. Int.** 61: 189–195.

Farah, A. 2009. Coffee as a speciality and functional beverage, pp 370-395. In P. Paquin (ed.). **Functional and Speciality Beverage Technology.** 1st ed. Woodhead Publishing. Cambridge, UK.

Fawole, O.B. and S.A. Odunfa. 1992. Pectolytic moulds in Nigeria. **Lett. Appl. Microbiol.** 15: 266−268.

Franca, A.S., J.C.F. Mendonca and S.D. Oliveira. 2005. Composition of green and roasted coffees of different cup qualities. **Lebensm. Wiss. Technol.** 38: 709–715.

Frank, H.A., N.A. Lum and A.S.D. Cruz. 1965. Bacteria responsible for mucilage-layer decomposition in Kona coffee cherries. **Appl. Microbiol.** 13: 201−207.

Franzetti, L. and M. Scarpellini. 2007. Characterisation of *Pseudomonas* spp. isolated from foods. **Ann. Microbiol.** 57: 39−47.

Gallois, A. and A.D.P. Grimont. 1985. Pyrazines responsible for the potatolike odor produced by some *Serratia* and *Cedecea* strains. **Appl. Environ. Microbiol.** 50: 1048−1051.

Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams. 1994. **Bergey's Manual of Determinative Bacteriology,** 9th ed. Williams & Wilkins. Baltimore, MD, USA.

Jackels, S.C. and C.F. Jackels. 2005. Characterization of the coffee mucilage fermentation process using chemical indicators: A field study in Nicaragua. **J. Food Sci.** 70: C321−C325.

Joet, T., A. Laffargue, F. Descroix, S. Doulbeau, B. Bertrand, A.D. Kochko and S. Dussert. 2010. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. **Food Chem.** 118: 693−701.

Kurtzman, C.P. and J.W. Fell. 1998. **The yeast, a Taxonomic Study**, 4th ed. Elsevier Science B.U., New York, NY, USA.

Mamma, D., E. Kourtoglou and P. Christakopoulos. 2008. Fungal multienzyme production on industrial by-products of the citrus-processing industry. **Bioresour. Technol.** 99: 2373−2383.

Martin, N., S.R. de Souza, R. da Silva and E. Gomes. 2004. Pectinase production by fungal strains in solid-state fermentation using agro-industrial bioproduct. **Braz. Arch. Biol. Technol.** 47: 813−819.

Masoud, W. and L. Jespersen. 2006. Pectin degrading enzymes in yeasts involved in fermentation of *Coffea arabica* in East Africa. **Int. J. Food Microbiol.** 110: 291−296.

Masoud, W., L.B. Cesar, L. Jespersen and M. Jakobsen. 2004. Yeast involved in fermentation of *Coffea arabica* in East Africa determined by genotyping and by direct denaturating gradient gel electrophoresis. **Yeast.** 21: 549−556.

Ouattara, H.G., B.L. Koffi, G.T. Karou, A. Sangare, S.L. Niamke and J.K. Diopoh. 2008. Implication of *Bacillus* sp. in the production of pectinolytic enzymes during cocoa fermentation. **World J. Microbiol. Biotechnol.** 24: 1753−1760.

Payne, C. and A. Bruce. 2001. The Yeast *Debaryomyces hansenii* as a short-term biological control agent against fungal

spoilage of sawn *Pinus sylvestris* timber. **Biol. Control.** 22: 22−28.

Pitt, J.I. and A.D. Hocking. 1997. **Fungi and Food Spoilage,** 2nd ed. Chapman & Hall. Cambridge, UK.

Silva, C.F., L.R. Batista and R.F. Schwan. 2008a. Incidence and distribution of filamentous fungi during fermentation, drying and storage of coffee (*Coffea Arabica* L.) beans. **Braz. J. Microbiol.** 39: 521−526.

Silva, C.F., L.R. Batista, L.M. Abreu, E.S. Dias and R.F. Schwan. 2008b. Succession of bacterial and fungal communities during natural coffee (*Coffea arabica*) fermentation. **Food Microbiol.** 25: 951−957.

Silva, C.F., R.F. Schwan, E.D. Sousa and A.E. Wheals. 2000. Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. **Int. J. Food Microbiol.** 60: 251−260.

Silva, C.F., D. Vilela, C.D. Cordeiro, W. Duarte, D. Dias and R. Schwan. 2013. Evaluation of a potential starter culture for enhanced quality of coffee fermentation. **World J. Microbiol. Biotechnol.** 29: 235−247.

Vega, F.E., F. Posada, S.W. Peterson, T.J. Gianfagna and F. Chaves. 2006. *Penicillium* species endophytic in coffee plants and ochratoxin A production. **Mycologia.** 98: 31−42.

Vilela, D.M., G.V.D. Pereira, C.F. Silva, L.R. Batista and R.F. Schwan. 2010. Molecular ecology and polyphasic characterization of the microbiota associated with semi-dry processed coffee (*Coffea arabica* L.). **Food Microbiol.** 27: 1128−1135.

Wootton, A.E. 1961. Fermentation and onion flavour. **Kenya Coffee.** 26: 126−129.