FERMENTATION OF COCOA (Theobroma cacao L.) BEANS USING Candida sp. AND Blastobotrys sp

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FERMENTATION OF COCOA (*Theobroma cacao* L.) BEANS USING *Candida* sp. AND *Blastobotrys* sp.

By

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Chairman : Assoc. Prof. Yaya Rukayadi
Faculty : Food Science and Technology

Spontaneous fermentation often produces a variety of end products. Starter culture has been widely used in many fermented food in order to control fermentation process and produce consistent products. The objective of this study was to evaluate the effect of *Candida* sp. and *Blastobotrys* sp. as starters in cocoa bean fermentation in term of optimal media culture, survivability of the cultures and fermentation products of cocoa bean fermentation (pH, temperature, microbial count, proximate analyses, bioactive compounds and volatile compounds). Optimum formulation of molasses yeast extract (MYE) media for the growth of *Candida* sp. and *Blastobotrys* sp. was determined using response surface methodology (RSM). Survivability of the cultures was measured by counting of colony-forming units (cfu) on yeast peptone dextrose (YPD) media. Sugar utilization by both types of yeasts in the optimum MYE media were determined using high performance liquid chromatography (HPLC) analysis. The yeasts were then used as a starter culture in cocoa bean fermentation and the products were evaluated for pH, temperature, proximate content, microbial safety and volatile compounds. The results showed that optimal concentrations of MYE medium were determined as follows: *Candida* sp. (2 g/100 ml, yeast extract; and 10 g/100 ml, molasses) and *Blastobotrys* sp. (2 g/100 ml, yeast extract; and 1.92 g/100 ml, molasses). The optimum MYE media was able to support the growth of *Candida* sp. and *Blastobotrys* sp. up to 140 and 70 days at room temperature, respectively. During survivability of yeasts, both of yeasts rapidly consumed glucose and fructose compared to sucrose at the beginning of the fermentation. Cocoa beans in spontaneous fermentation, *Blastobotrys* sp.-fermentation and *Candida* sp.-fermentation raised in temperature from 32°C (day 0) to maximum temperature valued 40°C, 42°C and 43°C after 3 days fermentation, respectively. The pH of cocoa for spontaneous fermentation, *Blastobotrys* sp.-fermentation and *Candida*sp.-fermentation at beginning of process were pH 4.07, 4.11 and 4.19, respectively, then the pHs of end products were 5.15, 5.44 and 4.99, respectively. Microbial safety of all type of fermentation showed that the number of *E. coli* and *Salmonella* sp. were decreased from 10^3 - 10^5 cfu/ml to <10 cfu/ml after 3 and 5 days of fermentation. No growth of *B. cereus*, *S. aureus* and *Pseudomona*s sp. were detected during fermentations. Proximate content of fermented beans from spontaneous fermentation, *Blastobotrys* sp.-fermentation and *Candida* sp.-fermentation showed a

significant ($p<0.05$) decreases of crude protein (13.86 - 9.83%), (14.50 - 11.99%) and (14.89 - 13.04%) after 7 days fermentation. Significant decrease in carbohydrate content (32.01% - 12.98%) was observed in spontaneous fermentation only. However, *Blastobotrys* sp.-fermentation and *Candida* sp.-fermentation showed increase of carbohydrate value from 9.84 to 19.98% and 22.53 to 22.64%. Spontaneous fermentation showed significant increase in fat (41.51 - 60.32%) and crude fibre content (3.83 - 8.79%) of fermented beans. Significant decreased in ash content for spontaneous fermentation and *Blastobotrys* sp.-fermentation were (4.44 - 3.06%) and (3.88 - 3.15%), respectively. Significant decreased in moisture content was observed in and *Blastobotrys* sp.-fermentation (6.84 - 4.71%) and *Candida* sp.-fermentation (8.20 - 5.69%) but significant increase was observed from spontaneous fermentation (4.34 - 5.02%). *Blastobotrys* sp.-fermentation product contained low in caffeine (30.59%) while *Candida* sp.-fermentation product contained low in theobromine (16.53%) but high in stigmasterol (1.68%), beta-sitosterol (3.03%) and tocopherol (5.38%). Fermentation showed a total of 20 volatile compounds related to the desirable notes and off-flavour. High individual compounds of alcohol (5) and ester (6) were detected from *Blastobotrys* sp.-fermentation compared to spontaneous fermentation; alcohol (3) and ester (4), and *Candida* sp.-fermentation: alcohol (4) and ester (2). Based on these findings, *Candida* sp. and *Blastobotrys* sp. could be used as potential starter cultures for cocoa beans fermentation.

Keywords: *Blastobotrys* sp., *Candida* sp., cocoa bean, fermentation, starter culture

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

FERMENTASI BIJI KOKO (*Theobroma cacao* L.) MENGGUNAKAN *Candida* sp. DAN *Blastobotrys* sp.

Oleh

NURUL HUSNA BINTI MAHAZAR

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Fermentasi secara spontan sering menghasilkan pelbagai produk akhir. Kultur pemula banyak digunakan secara meluas dalam makanan yang ditapai dengan tujuan pengawalan proses dan penghasilan produk yang konsisten. Objektif kajian ini adalah untuk menganalisa kesan penggunaan *Candida* sp. dan*Blastobotrys* sp. sebagai kultur pemula dari segi optimum kultur media, ketahanan kultur dan produk fermentasi biji koko (perubahan fisikal, keselamatan mikrob, analisis proksimat dan sebatian meruap). Kaedah respon permukaan (RSM) digunakan bagi menghasilkan medium molasses yeast extract (MYE) yang optimum untuk pertumbuhan *Candida* sp. dan *Blastobotrys* sp.. Ketahanan kultur ditentukan menggunakan unit pembentukan koloni (cfu) di atas medium yeast peptone dextrose. Penggunaan gula bagi kedua-dua yis dalam MYE media telah diukur menggunakan kromatografi cecair prestasi tinggi (HPLC). Kultur kemudian digunakan sebagai kultur pemula di dalam fermentasi biji koko dan produk fermentasi dinilai pada pH, suhu, kandungan proksimat, keselamatan mikrob dan sebatian meruap. Hasil kajian menunjukkan kepekatan optima media kultur adalah seperti berikut; *Candida* sp. (2 g/100 ml, yeast extract; dan 10 g/100 ml, molasses) dan *Blastobotrys* sp. (2 g/100 ml, yeast extract; dan 1.92 g/100 ml, molasses). Kultur media yang optimum mampu menampung pertumbuhan *Candida* sp. dan *Blastobotrys* sp. sehingga 140 dan 70 hari masing-masing pada suhu bilik.Semasa fermentasi yis dalam MYE media, yis menggunakan glukosa dan fruktosa lebih cepat berbanding sukrosa masa awal fermentasi. Suhu yang dicatat semasa fermentasi secara spontan, fermentasi-*Blastobotrys* sp. dan fermentasi-*Candida* sp. meningkat dari 32°C kepada suhu maksimum iaitu 40°C, 42°C and 43°C masing-masing selepas 3 hari fermentasi. pH biji cocoa pada awal fermentasi; fermentasi secara spontan, fermentasi-*Blastobotrys* sp. dan fermentasi-*Candida* sp. ialah pH 4.07, 4.11 dan 4.19 masing-masing. Produk fermentasi bagi ketiga-tiga jenis fermentasi mencatatkan pH 5.15, 5.44 dan 4.99 masing-masing. Keselamatan mikrob bagi semua jenis produk fermentasi; fermentasi secara spontan, *Blastobotrys* sp.-fermentasi dan *Candida* sp.-fermentasi menunjukkan bilangan *Escherichia coli* dan *Salmonella* sp. menurun daripada (10^3 - 10^5) cfu/ml kepada <10 cfu/ml selepas 3 dan 5 hari fermentasi. Tiada pertumbuhan *Bacillus cereus*, *Staphylococcus aureus* dan *Pseudomonas* sp. semasa fermentasi ini. Kandungan proksimat biji koko yang di tapai fermentasi secara spontan, fermentasi-*Blastobotrys* sp.

danfermentasi-*Candida* sp. menunjukkan penurunan protin yang signifikan ($p<0.05$) iaitu (13.86 - 9.83%), (14.50 - 11.99%) and (14.89 - 13.04%) selepas 7 hari fermentasi. Penurunan karbohidrat (32.01% - 12.98%) yang signifikan pada fermentasi spontan. Walau bagaimanapun, fermentasi-*Blastobotrys* sp. danfermentasi-*Candida* sp. menunjukkan peningkatan karbohidrat dari 9.84 kepada 19.98% dan 22.53 kepada 22.64%. Fermentasi spontan menunjukkan kenaikan yang signifikan kandungan lemak (41.51 - 60.32%) dan serat (3.83 - 8.79%) biji koko yang ditapai. Penurunan kandungan abu bagi fermentasi spontan dan fermentasi-*Blastobotrys* sp. adalah (4.44 - 3.06%) dan (3.88 - 3.15%) masing-masing. Kandungan kelembapan mencatatkan penurunan yang signifikan bagi fermentasi-*Blastobotrys* sp. (6.84 - 4.71%) dan fermentasi-*Candida* sp. (8.20 - 5.69%) tetapi kenaikan yang signifikan didapati pada fermentasi spontan (4.34 - 5.02%). Produk fermentasi-*Blastobotrys* sp. menunjukkan kandungan kafein (16.53%) yang rendah, manakala produk fermentasi-*Candida* sp. menunjukkan kandungan theobromine (16.53%) yang rendah tetapi tinggi dengan kandungan stigmasterol (1.68%), beta-sitosterol (3.03%) dan tocopherol (5.38%). Sebanyak 20 sebatian meruap dikesan semasa fermentasi biji koko yang menyumbang kepada rasa koko yang diingini dan juga sebaliknya. Sebatian meruap tunggal alkohol (5) dan ester (6) terhasil dari fermentasi-*Blastobotrys* sp. berbanding fermentasi spontan; alkohol (3) dan ester (4), dan fermentasi-*Candida*sp.; alkohol (4) dan ester (2). Berdasarkan hasil yang diperoleh, *Candida* sp. dan*Blastobotrys* sp. berpotensi sebagai kultur pemula untuk fermentasi biji koko.

Kata kunci: *Blastobotrys* sp., *Candida* sp., biji koko, fermentasi, kultur pemula

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I certify that a Thesis Examination Committee has met on 30 November 2015 to conduct the final examination of Nurul Husna binti Mahazar on her thesis entitled "Fermentation of Cocoa (*Theobroma cacao* L.) Beans using *Candida* sp. and *Blastobotrys* sp." in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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%	Percent
°C	Degree Celsius
AAB	Acetic acid bacteria
B. cereus	*Bacillus cereus*
cfu	Colony forming unit
E. coli	*Escherichia coli*
g	Gram
kg	Kilo gram
LAB	Lactic acid bacteria
ml	Millilitre
MRS	de Man, Rogosa and Sharpe
MYE	Molasses-yeast extract
PDA	Potato-dextrose agar
rpm	Rotation per minute
RSM	Response surface methodology
S. aureus	*Staphylococcus aureus*
v/v	Volume per volume
XLD	Xylose lysine deoxycholate
YPD	Yeast-peptone-dextrose

CHAPTER 1

INTRODUCTION

1.1 Introduction

Cocoa or *Theobroma cacao* L. has valuable products derived from it. Seeds of cocoa or cocoa beans are the raw material for chocolate production (Bartley, 2005). Cocoa beans will go through many process steps before a final product is produced. The processes include post-harvest treatments in plantation and manufacturing steps in a chocolate processing. Post-harvest treatments include breaking of cocoa pod, fermentation, drying, packing and storage of cocoa beans (Lima et al., 2011). Fermentation is a key process step for chocolate production because there will be no chocolate flavour without fermentation. During fermentation, cocoa flavour precursors are produced and the flavour is fully developed during roasting and further steps in cocoa processing (de Brito et al., 2001; Saltini et al., 2013).

Fermentation involves keeping the cocoa bean mass (beans and mucilage pulp) well insulated followed by aeration up to seven day. The microbiology of such fermentation is rather complex and variable. Various types of microorganisms include yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) are present during fermentation (Ardhana and Fleet, 2003; Schwan and Wheals, 2004; Camu et al., 2007). Yeasts are predominate microorganisms at early fermentation as they degrade fermentable sugars of mucilaginous pulp surrounding the seeds (Ho et al., 2014). Different genera of yeast were reported in previous studies include *Candida, Hanseniaspora, Kluyveromyces, Kloeckera, Pichia, Rhodotorula, Saccharomyces and Torulaspora* (Ardhana and Fleet, 2003; Meersman et al., 2013). However, some of the genera or species were differ in different countries (Schwan and Wheals, 2004).

The diversity of lactic acid bacteria and acetic acid bacteria during cocoa bean fermentation is much restricted compared to yeast. Genera of lactic acid bacteria isolated from cocoa bean fermentation are *Lactobacillus, Fructobacillus* and *Leuconostoc* but *Lactobacillus* is the one commonly isolated (Schwan et al., 2014). *Acetobacter* is a genus of acetic acid bacteria where it species, *Actobacter pasteurianus* common isolated from studies reported by Papalexandratou et al. (2011), Meersman et al. (2013) and Papalexandratou et al. (2013). The role of lactic acid bacteria is to ferment sugar and utilizing citric acid of cocoa pulp while acetic acid bacteria oxidize ethanol into acetic acid (Rodriguez-Campos et al., 2011).

Unfermented cocoa beans are characterized with bitter and astringent taste (de Melo Pereira et al., 2012). During fermentation, microbial activity took place in mucilaginous cocoa pulp producing various metabolites and generates heat that kills embryo of the bean. Metabolites produced are then diffuse into the cotyledon and trigger the enzymatic reaction. Enzymatic reaction in the cotyledon results in formation of flavour precursor

(Afoakwa et al., 2008). Flavour precursor is essential for the formation of chocolate flavour (Ramli et al., 2006).

A suitable cultivation condition is important in producing mass culture of microorganisms. Carbon, nitrogen and vitamin sources are the major components that should be in the formulation of a growth medium. Selection of carbon source from crude material such as agricultural waste is much preferable by the industries due to its low cost. Oxygen, temperature and pH play important roles in determining growth of a selected starter culture (Egli, 2015). Response surface methodology (RSM) is a statistical approach to determine an optimum condition of factors for desired responses (Li et al., 2002) Optimization of culture medium using RSM has been reported in previous study for cultivation of microorganisms such as *Candida intermedia* Y-1981 (Yönten and Aktaş, 2014) and *Tetraselmis suecica* (Azma et al., 2011).

Cultivated culture used as a starter culture in cocoa bean fermentation might have a better control of the process resulting consistent and improved of quality end products (Lefeber et al., 2012). This is because a spontaneous fermentation involves diverse species of microorganisms that might produce variety of end products and quality. Fermentation of cocoa bean is a complex process comprises microbial, interaction and biochemical reactions. Thus, the present study was conducted to evaluate fermentation of cocoa beans using *Candida* sp. and *Blastobotrys* sp. as starter cultures. Further, the effect of these starter cultures on fermentation and fermented products were evaluated.

1.2 Problems Statements

Spontaneous fermentation is an uncontrolled process, involving succession of different types of microorganisms that results in variety of end product of fermented beans. A potential use of starter culture is preferred to control the fermentation process producing relatively better quality of a fermented product. This project was funded by Barry Callebaut Malaysia. The selected yeasts (*Candida* sp. and *Blastobotrys* sp.) were given by Barry Callebaut to evaluate the effect of these starter cultures on fermentation of cocoa beans.

1.3 Objectives

The objectives of this study were:

1. To optimize medium composition for the growth of *Candida* sp. and *Blastobotrys* sp. using response surface methodology.

2. To determine the survival of *Candida* sp. and *Blastobotrys* sp. in the optimized molasses- yeast extract (MYE) media.

3. To evaluate the effect of starter culture on quality of fermented cacao beans.

REFERENCES

Abadias, M., Teixidó, N., Usall, J. and Viñas, I. (2003). Optimization of growth conditions of the postharvest biocontrol agent *Candida sake* CPA-1 in a lab-scale fermenter. *Journal of Applied Microbiology*, 95(2): 301-309.

Abe, M., Takaoka, N., Idemoto, Y., Takagi, C., Imai, T. and Nakasaki, K. (2008). Characteristic fungi observed in the fermentation process for Puer tea. *International Journal of Food Microbiology*, 124(2): 199–203.

Aculey, P. C., Snitkjaer, P., Owusu, M., Bassompiere, M., Takrama, J., Nørgaard, L., Petersen, M. A. and Nielsen, D. S. (2010). Ghanaian cocoa bean fermentation characterized by spectroscopic and chromatographic methods and chemometrics. *Journal of Food Science*, 75(6): S300-S307.

Adamafio, N. A., Kyeremeh, K., Matey, A. T., Mingle, C. and Kolawole, O. M. (2013). *In situ* degradation of cocoa (*Theobroma cacao*) pod husk theobromine by *Candida krusei*. *International Journal of Biotechnology and Biochemistry*, 9(1): 135-144.

Afoakwa, E. O. (2014). Cocoa Production and Processing Technology. Boca Raton, FL: CRC Press.

Afoakwa, E. O., Paterson, A., Fowler, M. and Ryan, A. (2008). Flavour formation and character in cocoa and chocolate: a critical review. *Critical Reviews in Food Science and Nutrition*, 48: 840–857.

Afoakwa, E. O., Paterson, A., Fowler, M. and Ryan, A. (2009).Matrix effects on flavor volatiles release in dark chocolates varying in particle size distribution and fat content using GC–mass spectrometry and GC–olfactometry. *Food Chemistry*, 113(1): 208–215.

Afoakwa, E. O., Quao, J., Takrama, F. S., Budu, A. S. and Saalia, F. K. (2012).Changes in total polyphenols, o-diphenols and anthocyanin concentrations during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans.*International Food Research Journal*, 19(3): 1071-1077.

Afoakwa, E. O., Quao, J., Takrama, J., Budu, A. S. and Saalia, F. K. (2013). Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. *Journal of Food Science and Technology*,50(6): 1097–105.

Amin, I., Jinap, S., Jamilah, B., Harikrisna, K. and Biehl, B. (2002). Oligopeptide patterns produced from *Theobroma cacao* L. of various genetic origins. *Journal of the Science of Food and Agriculture*,82(7): 733-737.

Anane, E., van Rensburg, E. and Görgens, J. F. (2013).Optimisation and scale-up of α-glucuronidase production by recombinant *Saccharomyces cerevisiae* in aerobic

fed-batch culture with constant growth rate.*Biochemical Engineering Journal*,81:1–7.

Andersson, S. C., Rumpunen, K., Johansson, E. and Olsson, M. E. (2008). Tocopherols and tocotrienols in sea buckthorn (*Hippophae rhamnoides* L.) berries during ripening. *Journal of Agricultural and Food Chemistry*,56(15): 6701-6706.

AOAC (2005).Official Methods of Analysis, 19th Ed., Association official Analytical Chemists. Washington DC, USA.

Aprotosoaie, A. C., Luca, S. V. and Miron, A. (2016).Flavor chemistry of cocoa and cocoa products—an overview.*Comprehensive Reviews in Food Science and Food Safety*, 15(1): 73-91.

Ardhana, M. M. and Fleet, G. H. (2003).The microbial ecology of cocoa bean fermentations in Indonesia.*International Journal of Food Microbiology*, 86(1): 87-99.

Augustin, M. A. and Khor, K. L. (1986).Determination of sugars in soft drinks by high performance liquid chromatography.*Pertanika*, 9(1): 119-123.

Ayad, E. H. (2009). Starter culture development for improving safety and quality of Domiati cheese.*Food Microbiology*, 26(5): 533-541.

Ayeni, L. S., Adetunji, M. T., Ojeniyi, S. O., Ewulo, B. S. and Adeyemo, A. J. (2008). Comparative and cumulative effect of cocoa pod husk ash and poultry manure on soil and maize nutrient contents and yield.*American-Eurasian Journal of Sustainable Agriculture*, 2(1): 92-97.

Azma, M., Mohamed, M. S., Mohamad, R., Rahim, R. A. and Ariff, A. B. (2011).Improvement of medium composition for heterotrophic cultivation of green microalgae, *Tetraselmis suecica*, using response surface methodology.*Biochemical Engineering Journal*, 53(2): 187-195.

Badrie, N., Bekele, F., Sikora, E. and Sikora, M. (2015).Cocoa agronomy, quality, nutritional, and health aspects.*Critical Reviews in Food Science and Nutrition*, 55(5): 620-659.

Bamforth, C. W. (2005). *Food, Fermentation and Micro-organisms*. Oxford, UK: Blackwell Science.

Bartley, B. G. D. (2005). *The Genetic Diversity of Cacao and Its Utilization*. Oxford: CABI Publishing.

Batista, N. N., Ramos, C. L., Dias, D. R., Pinheiro, A. C. M. and Schwan, R. F. (2015). The impact of yeast starter cultures on the microbial communities and volatile compounds in cocoa fermentation and the resulting sensory attributes of chocolate. *Journal of Food Science and Technology*, 1-10.

Bekatorou, A., Psarianos, C. and Koutinas, A. (2006).Production of food grade yeasts. *Food Technology and Biotechnology,* 44(3): 407–415.

Bera, D., Lahiri, D. and Nag, A. (2006).Studies on a natural antioxidant for stabilization of edible oil and comparison with synthetic antioxidants.*Journal of Food Engineering,*74(4): 542-545.

Böer, E., Steinborn, G., Florschütz, K., Körner, M., Gellissen, G. and Kunze, G. (2009).*Arxula adeninivorans* (*Blastobotrys adeninivorans*)—A Dimorphic Yeast of Great Biotechnological Potential. In T. Satyanarayana and Kunze G. (Eds.), *Yeast Biotechnology: Diversity and Applications* (pp. 615-634). Milan: Springer.

Burtner, C. R., Murakami, C. J., Kennedy, B. K. and Kaeberlein, M. (2009).A molecular mechanism of chronological aging in yeast.*Cell Cycle*, 8(8): 1256–1270.

Camu, N., De Winter, T., Addo, S. K., Takrama, J. S., Bernaert, H. and Vuyst, L. (2008). Fermentation of cocoa beans: influence of microbial activities and polyphenol concentrations on the flavour of chocolate. *Journal of the Science of Food and Agriculture,* 88(13): 2288-2297.

Camu, N., De Winter, T., Verbrugghe, K., Cleenwerck, I., Vandamme, P., Takrama, J. S., Vancanneyt, M. and De Vuyst, L. (2007). Dynamics and biodiversity of populations of lactic acid bacteria and acetic acid bacteria involved in spontaneous heap fermentation of cocoa beans in Ghana. *Applied and Environmental Microbiology*, 73(6): 1809-1824.

Cappucino J. G. and Sherman N. (2011).*Microbiology A Laboratory Manual.* San Francisco, CA: Pearson/Benjamin-Cummings.

Carlson, A., Signs, M., Liermann, L., Boor, R. and Jem, K. J. (1995).Mechanical disruption of *Escherichia coli* for plasmid recovery.*Biotechnology and Bioengineering*, 48(4): 303-315.

Carpenter, D. R., Hammerstone Jr, J. F., Romanczyk Jr, L. J. and Aitken, W. M. (1994). Lipid composition of *Herrania* and *Theobroma* seeds. *Journal of the American Oil Chemists' Society,* 71(8): 845-851.

Cayol, J. L., Ollivier, B., Alazard, D., Amils, R., Godfroy, A., Piette, F. and Prieur, D. (2015).The Extreme Conditions of Life on the Planet and Exobiology. In Bertrand, J. C., Caumette, P., Lebaron, P., Matheron, R., Normand, P. and Sime-Ngando, T (Eds), *Environmental Microbiology: Fundamentals and Applications* (pp. 353-394). Netherlands: Springer.

Chilton, S. N., Burton, J. P. and Reid, G. (2015). Inclusion of fermented foods in food guides around the world. *Nutrients*, 7(1): 390-404.

Choudhary, D. K. and Johri, B. N. (2009). Basidiomycetous Yeasts: Current Status. In Satyanarayana, T. and Kunze, G. (Eds.), *Yeast Biotechnology: Diversity and Applications* (pp. 19-46). Netherlands: Springer.

Cletus, P. K. and Christie, J. R. (2007). Multigene phylogenetic analysis of the *Trichomonascus*, *Wickerhamiella* and *Zygoascus* yeast clades, and the proposal of *Sugiyamaella* gen. nov. and 14 new species combinations. *FEMS Yeast Research*, 7: 141–151.

Conrad, M., Schothorst, J., Kankipati, H. N., Van Zeebroeck, G., Rubio-Texeira, M. and Thevelein, J. M. (2014).Nutrient sensing and signaling in the yeast *Saccharomyces cerevisiae*.*FEMS Microbiology Reviews*, 38(2): 254-299.

Copetti, M. V., Iamanaka, B. T., Pitt, J. I. and Taniwaki, M. H. (2014). Fungi and mycotoxins in cocoa: from farm to chocolate. *International Journal of Food Microbiology*, 178: 13–20.

Crafack, M., Keul, H., Eskildsen, C. E., Petersen, M. A., Saerens, S., Blennow, Skovmand-Larsena, M., Swiegersb, J. H., Petersend, G. B., Heimdald, H. and Nielsen, D. S. (2014). Impact of starter cultures and fermentation techniques on the volatile aroma and sensory profile of chocolate.*Food Research International*, 63: 306-316.

Crafack, M., Mikkelsen, M. B., Saerens, S., Knudsen, M., Blennow, A., Lowor, S., Takrama, J., Swiegers, J. H., Petersen, G. B., Heimdal, H. and Nielsen, D. S. (2013). Influencing cocoa flavour using *Pichia kluyveri* and *Kluyveromyces* marxianus in a defined mixed starter culture for cocoa fermentation. *International Journal of Food Microbiology*, 167(1): 103-116.

Cruz, S. H., Cilli, E. M. and Ernandes, J. R. (2002).Structural complexity of the nitrogen source and influence on yeast growth and fermentation.*Journal of the Institute of Brewing*, 108(1): 54-61.

D'Amore, T., Russell, I. and Stewart, G. G. (1989).Sugar utilization by yeast during fermentation.*Journal of Industrial Microbiology*, 4(4): 315-323.

Day, M. P., Schmidt, S. A., Smith, P. A. and Wilkes, E. N. (2015).Use and impact of oxygen during winemaking.*Australian Journal of Grape and Wine Research*, 21(S1): 693-704.

de Brito, E. S., García, N. H. P., Gallão, M. I., Cortelazzo, A. L., Fevereiro, P. S. and Braga, M. R. (2001). Structural and chemical changes in cocoa (*Theobroma cacao* L.) during fermentation, drying and roasting.*Journal of the Science of Food and Agriculture*, 81(2): 281-288.

de Melo Pereira, G. V., Miguel, M. G. D. C. P., Ramos, C. L. and Schwan, R. F. (2012). Microbiological and physicochemical characterization of small-scale cocoa fermentations and screening of yeast and bacterial strains to develop a defined starter culture. *Applied and Environmental Microbiology*, 78(15): 5395-5405.

de Melo Pereira, G. V., Soccol, V. T., Pandey, A., Medeiros, A. B. P., Lara, J. M. R. A., Gollo, A. L. and Soccol, C. R. (2014). Isolation, selection and evaluation of yeasts for use in fermentation of coffee beans by the wet process. *International Journal of Food Microbiology*, 188: 60-66.

De Vuyst, L., Lefeber, T., Papalexandratou, Z. and Camu, N. (2010).The functional role of lactic acid bacteria in cocoa bean fermentation. *Biotechnology of Lactic Acid Bacteria: Novel Applications*, 301-325.

Dias, D. R (2014).Agro-Industrial Uses of Cocoa By-Products.In Schwan, R. F. and Fleet, G. H. (Eds.), *Cocoa and Coffee Fermentations*. (pp 309-340). Boca Raton, FL: CRC Press.

do Nascimento, M. D. S., Pena, P. O., Brum, D. M., Imazaki, F. T., Tucci, M. L. S. and Efraim, P. (2013). Behavior of *Salmonella* during fermentation, drying and storage of cocoa beans. *International Journal of Food Microbiology*, 167(3): 363-368.

Dorta, C., Cruz, R., de Oliva-Netto, P. and Jose, D. (2006).Sugar cane molasses and yeast powder used in the fructooligosaccharides production by *Aspergillus japonicas*-FCL 119T and *Aspergillus niger* ATCC 20611. *Journal of Industrial Microbiology and Biotechnology*, 33: 1003-1009.

Duarte, W. F., Dias, D. R., Oliveira, J. M., Teixeira, J. A., e Silva, J. B. D. A. and Schwan, R. F. (2010). Characterization of different fruit wines made from cacao, cupuassu, gabiroba, jaboticaba and umbu. *LWT-Food Science and Technology*, 43(10): 1564-1572.

Ducki, S., Miralles-Garcia, J., Zumbé, A., Tornero, A. and Storey, D. M. (2008). Evaluation of solid-phase micro-extraction coupled to gas chromatography–mass spectrometry for the headspace analysis of volatile compounds in cocoa products. *Talanta*, 74(5): 1166-1174.

Egli, T. (2015). Microbial growth and physiology: a call for better craftsmanship. *Frontiers in Microbiology*, 6.

El-Gendy, N. S., Madian, H. R. and Amr, S. S. A. (2013). Design and optimization of a process for sugarcane molasses fermentation by *Saccharomyces cerevisiae* using response surface methodology. *International Journal of Microbiology*, 2013.

El-Helow, E. R., Elbahloul, Y., El-Sharouny, E. E., Ali, S. R. and Ali, A. A. M. (2015). Economic production of baker's yeast using a new *Saccharomyces cerevisiae* isolate. *Biotechnology and Biotechnological Equipment*, 29(4): 705-713.

Elias, M., Wieczorek, G., Rosenne, S. and Tawfik, D. S. (2014).The universality of enzymatic rate–temperature dependency. *Trends in Biochemical Sciences*, 39(1): 1-7.

Eskes, A. B. and Lanaud, C. (2001). Cocoa. In Charrier, A., Jacquot, M., Hamon, S., Nicolas, D. (Eds), *Tropical Plant Breeding*. (pp. 78–105). Enfield, NH: Science Publishers.

Ferrari, M. D., Bianco, R., Froche, C. and Loperena, M. L. (2001).Baker's yeast production from molasses/cheese whey mixtures. *Biotechnology Letters*, 23(1): 1-4.

Ferreira, I. M. P. L. V. O., Pinho, O., Vieira, E. and Tavarela, J. G. (2010). Brewer's *Saccharomyces* yeast biomass: characteristics and potential applications. *Trends in Food Science and Technology*, 21(2): 77-84.

Finn, S., Condell, O., McClure, P., Amézquita, A. and Fanning, S. (2013). Mechanisms of survival, responses and sources of *Salmonella* in low-moisture environments. *Frontiers in Microbiology*, 4.

Flores, C. L., Rodríguez, C., Petit, T. and Gancedo, C. (2000).Carbohydrate and energy-yielding metabolism in non-conventional yeasts.*FEMS Microbiology Reviews*, 24(4): 507-529.

Franco, R., Oñatibia-Astibia, A. and Martínez-Pinilla, E. (2013). Health benefits of methylxanthines in cacao and chocolate. *Nutrients*, 5(10): 4159-4173.

Frauendorfer, F. and Schieberle, P. (2006). Identification of the key aroma compounds in cocoa powder based on molecular sensory correlations. *Journal of Agricultural and Food Chemistry*, 54(15): 5521-5529.

Frauendorfer, F. and Schieberle, P. (2008).Changes in key aroma compounds of Criollo cocoa beans during roasting. *Journal of Agricultural and Food Chemistry*, 56(21): 10244-10251.

Freire, E. S., Schwan, R. F. and Mororo, R. C. (1996). The cocoa-pulp agroindustry and the uses of its residues in Bahia: Progress achieved in the last ten years. In Proceedings of the 12th International Cocoa Research Conference, Salvador, Bahia, Brazil (pp. 1013-1020).

Gálvez, S. L., Loiseau, G., Paredes, J. L., Barel, M. and Guiraud, J. P. (2007). Study on the microflora and biochemistry of cocoa fermentation in the Dominican Republic. *International Journal of Food Microbiology*, 114(1): 124-130.

Garcia, F. A. P. (2010). Cell wall disruption. In M.C. Flickinger (Ed.), Encyclopedia of *Industrial Biotechnology, Bioprocess, Bioseparation, and Cell Technology* (pp. 1481–1491) New York: John Wiley and Sons Inc.

Gargaud, M. (2011).*Encyclopedia of Astrobiology*. Berlin, Heidelberg: Springer Science & Business Media.

Garjonyte, R., Melvydas, V. and Malinauskas, A. (2016). Mediated amperometry reveals different modes of yeast responses to sugars. *Bioelectrochemistry*, 107: 45-49.

Giersberg, M., Florschütz, K., Baronian, K. and Kunze, G. (2012).*Arxula adeninivorans* (*Blastobotrys adeninivorans*)–An Imperfect Dimorphic Yeast of Biotechnological Potential.InSatyanarayana, T., Johri, B.N. and Prakash, A. (Eds), *Microorganisms in Sustainable Agriculture and Biotechnology*. (pp. 453-468). Milan: Springer.

Gomez, F. (2011).Aerobe. In Gargaud, M. (Ed.), *Encyclopedia of Astrobiology* (pp. 21-21). Berlin, Heidelberg: Springer Science & Business Media.

Gopal, A. R. and Kammen, D. M. (2009). Molasses for ethanol: the economic and environmental impacts of a new pathway for the lifecycle greenhouse gas analysis of sugarcane ethanol. *Environmental Research Letters*, 4(4): 044005

Greaves, R. B. and Warwicker, J. (2007).Mechanisms for stabilisation and the maintenance of solubility in proteins from thermophiles.*BMC Structural Biology*, 7(1): 18.

Hakobyan, L., Gabrielyan, L. and Trchounian, A. (2012). Yeast extract as an effective nitrogen source stimulating cell growth and enhancing hydrogen photoproduction by *Rhodobacter sphaeroides* strains from mineral springs. *International Journal of Hydrogen Energy*, 37(8): 6519-6526.

Hashim, P., Jinap, S., Muhammad, S. K. S. and Ali, A. (1999).Effect of drying time bean depth and temperature on free amino acid, peptide-N, sugar and pyrazine concentrations of Malaysian cocoa beans.*Journal of the Science of Food and Agriculture*, 79: 987–994.

Herker, E., Jungwirth, H., Lehmann, K. A., Maldener, C., Fröhlich, K. U., Wissing, S. and Madeo, F. (2004). Chronological aging leads to apoptosis in yeast. *The Journal of Cell Biology*,164(4): 501–7.

Herman, P. K. (2002). Stationary phase in yeast.*Current Opinion in Microbiology*, 5(6): 602-607.

Ho, V. T. T., Zhao, J. and Fleet, G. (2014). Yeasts are essential for cocoa bean fermentation. *International Journal of Food Microbiology*, 174: 72–87.

Ho, V. T. T., Zhao, J., Srzednicki, G. and Fleet, G. (Eds.). (2013). Proceeding from AFHW '13: *International Symposium on Agri-Foods for Health and Wealth*. Bangkok, Thailand

Horák, J. (2013). Regulations of sugar transporters: insights from yeast. *Current Genetics*, 59(1-2): 1-31.

Huang, S. L., Wu, L. C., Liang, H. K., Pan, K. T., Horng, J. T. and Ko, M. T. (2004). PGTdb: a database providing growth temperatures of prokaryotes. *Bioinformatics*, 20(2): 276–278.

Ibarz, A. and Augusto, P. E. (2015).An autocatalytic kinetic model for describing microbial growth during fermentation.*Bioprocess and Biosystems Engineering*, 38(1): 199-205.

Igbinadolor, R. O. (2009). Other Tropical Fruit Vinegars.In Solieri, L. and Giudici, P. (Eds.), *Vinegars of the World* (pp. 261-271). Milan: Springer.

International Cocoa organisation (ICCO). (2013). Growing cocoa. Retrieve 9 January 2016 from International Cocoa Organisation. http://www.icco.org/about-cocoa/growing-cocoa.html.

Iwaro, A. D., Bekele, F. L. and Butler, D. R. (2003).Evaluation and utilisation of cacao (*Theobroma cacao* L.) germplasm at the International Cocoa Genebank, Trinidad. *Euphytica,* 130(2): 207-221.

Jabeen, G., Farooq, R., Khan, A. U. and Khan, A. A. (2015).Acetyl-coa pathway for biosynthesis of organics.*Asian Journal of Chemistry*, 27(1): 1–8.

Jinap, S. and Thien, J. (1994).Effect of drying on acidity and volatile fatty acids content of cocoa beans.*Journal of the Science of Food and Agriculture*, 65: 67–75.

Kalil, M. S., Alshiyab, H. S. and Yusoff, W. M. W. (2008).Effect of nitrogen source and carbon to nitrogen ratio on hydrogen production using *C. acetobutylicum*. *American Journal of Biochemistry and Biotechnology*, 4(4): 393-401.

Kalil, M. S., Alshiyab, H. S. S. and Yusoff, W. M. W. (2009).Media improvement for hydrogen production using *C. acetobutylicum* NCIMB 13357. *American Journal of Applied Sciences*, 6(6): 1158-1168.

Kim, J., Kim, J., Shim, J., Lee, C. Y., Lee, K. W. and Lee, H. J. (2014). Cocoa phytochemicals: recent advances in molecular mechanisms on health. *Critical Reviews in Food Science And Nutrition*, 54(11): 1458-1472.

Koga, Y. (2012). Thermal adaptation of the archaeal and bacterial lipid membranes. *Archaea*, 2012.

Kojic, E. M. and Darouiche, R. O. (2004).*Candida* infections of medical devices.*Clinical Microbiology Reviews,*17(2): 255-267.

Konings, W. N., Albers, S. V., Koning, S. and Driessen, A. J. (2002). The cell membrane plays a crucial role in survival of bacteria and archaea in extreme environments. *Antonie Van Leeuwenhoek*, *81*(1-4): 61-72.

Kothe, E. and Köhler, J. M. (2015).Changing growth behavior of heavy-metal tolerant bacteria: media optimization using droplet-based microfluidics.*Engineering in Life Sciences,* 15(3): 327–332.

Kotzamanidis, C. H., Roukas, T. and Skaracis, G. (2002). Optimization of lactic acid production from beet molasses by *Lactobacillus delbrueckii* NCIMB 8130.*World Journal of Microbiology and Biotechnology,* 18(5): 441-448.

Kris-Etherton, P. M. and Keen, C. L. (2002).Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. *Current Opinion in Lipidology*, 13(1): 41-49.

Kusch, H., Engelmann, S., Bode, R., Albrecht, D., Morschhäuser, J. and Hecker, M. (2008). A proteomic view of *Candida albicans* yeast cell metabolism in exponential and stationary growth phases.*International Journal of Medical Microbiology*, 298(3): 291-318.

Lee, K. W., Kim, Y. J., Lee, H. J. and Lee, C. Y. (2003). Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *Journal of Agricultural and Food Chemistry*, 51(25): 7292-7295.

Lefeber, T., Papalexandratou, Z., Gobert, W., Camu, N. and De Vuyst, L. (2012). On-farm implementation of a starter culture for improved cocoa bean fermentation and its influence on the flavour of chocolates produced thereof. *Food microbiology*, *30*(2): 379-392.

Lemmer, A. (2012). Mineral substances and macronutrients in the anaerobic conversion of biomass : An impact evaluation. *Engineering in Life Sciences*, 12(3): 287-294.

Li, C., Bai, J., Cai, Z. and Ouyang, F. (2002). Optimization of a cultural medium for bacteriocin production by *Lactococcus lactis* using response surface methodology. *Journal of Biotechnology*, 93(1): 27-34.

Li, H., Wang, H., D'Aoust, J.Y. and Maurer, J. (2013).*Salmonella* species, In Doyle, M.P.,Buchanan, R.L. (Eds.), *Food Microbiology: Fundamentals and Frontiers* (pp. 225–262). Washington, DC: ASMPress

Lima, L. J. R., Almeida, M. H., Nout, M. J .R. and Zwietering, M. H. (2011).*Theobroma cacao* L., "The food of the Gods": quality determinants of commercial cocoa beans, with particular reference to the impact of fermentation. *Critical Reviews in Food Science and Nutrition,* 51(8): 731–61.

Liu, J., Huang, J., Sun, Z., Zhong, Y., Jiang, Y. and Chen, F. (2011). Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: assessment of algal oils for biodiesel production. *Bioresource Technology*, 102(1): 106-110.

Liu, X., Jia, B., Sun, X., Ai, J., Wang, L., Wang, C., Zhao, F., Zhan, J. and Huang, W. (2015).Effect of initial PH on growth characteristics and fermentation properties of *Saccharomyces cerevisiae*.*Journal of Food Science*,80(4): M800-M808.

Lopes, U. V. and Pires, J. L. (2014).Botany and Production of Cocoa.In Schwan, R. F. and Fleet, G. H. (Eds.), *Cocoa and Coffee Fermentations* (pp.129-192). Boca Raton, FL: .CRC Press.

Maqueda, M., Pérez-Nevado, F., Regodón, J. A., Zamora, E., Álvarez, M. L., Rebollo, J. E. and Ramírez, M. (2011).A low-cost procedure for production of fresh autochthonous wine yeast.*Journal of Industrial Microbiology and Biotechnology*, 38(3): 459-469.

Martínez-Pinilla, E., Oñatibia-Astibia, A. and Franco, R. (2015).The relevance of theobromine for the beneficial effects of cocoa consumption. *Frontiers in Pharmacology*, 6.

Meersman, E., Steensels, J., Mathawan, M., Wittocx, P. J., Saels V., Struyf, N. and Verstrepen, K. J. (2013). Detailed analysis of the microbial population in

Malaysian spontaneous cocoa pulp fermentations reveals a core and variable microbiota. *PLoS ONE*, 8(12).

Middelhoven, W. J., Hoogkamer-Te Niet, M. C. and Kreger-Van Rij, N. J. W. (1984).*Trichosporon adeninovorans* sp. nov., a yeast species utilizing adenine, xanthine, uric acid, putrescine and primary n-alkylamines as the sole source of carbon, nitrogen and energy. *Antonie Van Leeuwenhoek*, 50(4): 369-378.

Mills, D. A., Johannsen, E. and Cocolin, L. (2002). Yeast diversity and persistence in *Botrytis*-affected wine fermentations. *Applied and Environmental Microbiology*, 68(10): 4884–4893.

Mira, N. P., Teixeira, M. C. and S´a-Correia, I. (2010). Adaptive response and tolerance to weak acids in *Saccharomyces cerevisiae*: a genome-wide view. *Omics*, 14(5): 525–40.

Motamayor, J. C., Risterucci, A. M., Heath, M. and Lanaud, C. (2003). Cacao domestication II: progenitor germplasm of the Trinitario cacao cultivar. *Heredity*, 91(3): 322-330.

Murakami, C. J., Wall, V., Basisty, N. and Kaeberlein, M. (2011). Composition and acidification of the culture medium influences chronological aging similarly in vineyard and laboratory yeast.*PloS ONE*,6(9).

Myers, R. H., Montgomery, D. C. and Anderson-Cook, C. M. (2009).*Response Surface Methodology: Process And Product Optimization Using Designed Experiments*. Hoboken, NJ: John Wiley and Sons.

Narendranath NV and Power R. 2005. Relationship between pH and medium dissolved solids in terms of growth and metabolism of *Lactobacilli* and *Saccharomyces cerevisiae* during ethanol production.*Applied and Environmental Microbiology*, 71(5): 2239–43.

Nazaruddin, R., Seng, L. K., Hassan, O. and Said, M. (2006).Effect of pulp preconditioning on the content of polyphenols in cocoa beans (*Theobroma cacao*) during fermentation.*Industrial Crops and Products*, 24(1): 87-94.

Oren, A. (2012). Diversity of Anaerobic Prokaryotes And Eukaryotes: Breaking Long Established Dogmas. In Altenbach, A., Bernhard, J. M. and Seckbach, J. (Eds.), *Anoxia: Evidence For Eukaryote Survival And Paleontological Strategies*(pp. 39-47). Netherlands: Springer.

Orij, R., Brul, S. and Smits, G. J. (2011). Intracellular pH is a tightly controlled signal in yeast. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1810(10): 933-944.

Owusu, M. (2010).*Influence of Raw Material and Processing on Aroma in Chocolate*.Ph.D Thesis.Department of Food Science, University of Copenhagen.

Owusu, M., Petersen, M. A. and Heimdal, H. (2011).Effect of fermentation method, roasting and conching conditions on the aroma volatiles of dark chocolate.*Journal of Food Processing and Preservation*, 36(5): 446-456.

Papalexandratou, Z. and De Vuyst, L. (2011).Assessment of the yeast species composition of cocoa bean fermentations in different cocoa-producing regions using denaturing gradient gel electrophoresis.*FEMS Yeast Research*, 11: 564–74.

Papalexandratou, Z., Lefeber, T., Bahrim, B., Lee, O. S., Daniel, H. M. and Vuyst, L. (2013).*Hanseniaspora opuntiae*, *Saccharomyces cerevisiae*, *Lactobacillus fermentum*, and *Acetobacter pasteurianus* predominate during well-performed Malaysian cocoa bean box fermentations, underlining the importance of these microbial species for a successful cocoa. *Food Microbiology*,*35*(2): 73–85.

Papalexandratou, Z., Vrancken, G., De Bruyne, K., Vandamme, P. and De Vuyst, L. (2011). Spontaneous organic cocoa bean box fermentations in Brazil are characterized by a restricted species diversity of lactic acid bacteria and acetic acid bacteria. *Food Microbiology*, 28(7): 1326–38.

Polagruto, J. A., Wang-Polagruto, J. F., Braun, M. M., Lee, L., Kwik-Uribe, C. and Keen, C. L. (2006). Cocoa flavanol-enriched snack bars containing phytosterols effectively lower total and low-density lipoprotein cholesterol levels. *Journal of the American Dietetic Association*, 106(11): 1804-1813.

Pretorius, I. S. (2000). Tailoring wine yeast for the new millennium: Novel approaches to the ancient art of winemaking. *Yeast*, 16: 675–729.

Quilez, J., Garcia-Lorda, P. and Salas-Salvado, J. (2003).Potential uses and benefits of phytosterols in diet: present situation and future directions. *Clinical Nutrition*, 22(4): 343-351.

Raines-Casselman, M. B. (2005). *Yeast Propagation and Maintenance Principles and Practices*. California: The Maltose Falcons.

Ramli, N., Hassan, O., Said, M., Samsudin, W. and Idris, N. A. (2006). Influence of roasting conditions on volatile flavor of roasted Malaysian cocoa beans. *Journal of Food Processing and Preservation*, 30(3): 280-298.

Ramos, C. L., Dias, D. R., Miguel, M. G. D. C. P. and Schwan, R. F. (2014).Impact of different cocoa hybrids (*Theobroma cacao* L.) and *S. cerevisiae* UFLA CA11 inoculation on microbial communities and volatile compounds of cocoa fermentation. *Food Research International*, 64: 908-918.

Rastogi, G., Bhalla, A., Adhikari, A., Bischoff, K. M., Hughes, S. R., Christopher, L. P. and Sani, R. K. (2010). Characterization of thermostable cellulases produced by *Bacillus* and *Geobacillus* strains. *Bioresource Technology*, 101(22): 8798-8806.

Revillion, J. P., Brandelli, A. and Ayub, M. A. Z. (2003). Production of yeast extract from whey using *Kluyveromyces marxianus*. *Brazilian Archives of Biology and Technology*, 46(1): 121-128.

Rodriguez-Campos, J., Escalona-Buendía, H. B., Contreras-Ramos, S. M., Orozco-Avila, I., Jaramillo-Flores, E. and Lugo-Cervantes, E. (2012).Effect of fermentation time and drying temperature on volatile compounds in cocoa.*Food Chemistry*, 132(1): 277-288.

Rodriguez-Campos, J., Escalona-Buendía, H. B., Orozco-Avila, I., Lugo-Cervantes, E. and Jaramillo-Flores, M. E. (2011).Dynamics of volatile and non-volatile compounds in cocoa (*Theobroma cacao* L.) during fermentation and drying processes using principal components analysis.*Food Research International*, 44(1): 250-258.

Rollero, S., Bloem, A., Camarasa, C., Sanchez, I., Ortiz-Julien, A., Sablayrolles, J. M., Dequin, S. and Mouret, J. R. (2015). Combined effects of nutrients and temperature on the production of fermentative aromas by *Saccharomyces cerevisiae* during wine fermentation.*Applied Microbiology and Biotechnology*, 99(5): 2291-2304.

Rozada, R., Antonio, J., Charalampopoulos, D., Thomas, K. and Pandiella, S. S. (2009). Effect of storage temperature and media composition on the survivability of *Bifidobacteriumbreve* NCIMB 702257 in a malt hydrolisate.*International Journal of Food Microbiology*, 133(1): 14–21.

Rusconi, M. and Conti, A. (2010).*Theobroma cacao* L., the Food of the Gods: a scientific approach beyond myths and claims. *Pharmacological Research*, 61(1): 5-13.

Saltini, R., Akkerman, R. and Frosch, S. (2013). Optimizing chocolate production through traceability: A review of the influence of farming practices on cocoa bean quality. *Food Control*, 29(1): 167–187.

Samagaci, L., Ouattara, H. G., Goualle, B. G. and Niamke, S. L. (2014). Growth capacity of yeasts potential starter strains under cocoa fermentation stress conditions in Ivory Coast. *Emirates Journal of Food and Agriculture*, 26(10): 861.

Sardi, J. C. O., Scorzoni, L., Bernardi, T., Fusco-Almeida, A. M. and Mendes Giannini, M. J. S. (2013).*Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of Medical Microbiology*,62(Pt 1): 10–24.

Sarka, E., Bubnik, Z., Hinkova, A., Gebler, J. and Kadlec, P. (2012).Molasses as a by-product of sugar crystallization and a perspective raw material.*Procedia Engineering*, 42: 1219-1228.

Sarlin, P. J. and Philip, R. (2013). A molasses based fermentation medium for marine yeast biomass production. *International Journal of Research in Marine Sciences*, 2(2): 39-44.

Sayed, E. T., Barakat, N. A., Abdelkareem, M. A., Fouad, H. and Nakagawa, N. (2015). Yeast extract as an effective and safe mediator for the baker's-yeast-based microbial fuel cell. *Industrial and Engineering Chemistry Research*, 54(12): 3116-3122.

Schmitz, R. A., Daniel, R., Deppenmeier, U. and Gottschalk, G. (2013).The Anaerobic Way of Life.In Rosenberg, E. (Ed.).*The Prokaryotes* (pp. 259-273). Berlin, Heidelberg: Springer.

Schönheit, P., Buckel, W. and Martin, W. F. (2016).On the origin of heterotrophy.*Trends in Microbiology*, 24(1): 12–25.

Schwan, R. F. and Wheals, A. E. (2004).The microbiology of cocoa fermentation and its role in chocolate quality.*Critical Reviews in Food Science and Nutrition*,44(4): 205-221.

Schwan, R. F., de Meilo Pereira, G. V. and Fleet G. H. (2014). Microbial Activities during Cocoa Fermentation.In Schwan, R. F. and Fleet, G. H. (Eds.),*Cocoa and Coffee Fermentations*(pp.129-192). Boca Raton, FL: CRC Press.

Selamat, J., Dimick, P. S. and Hollender, R. (1995). Flavour evaluation of chocolate formulated from cocoa beans from different countries. *Food Control*, 6:105−110.

Serra-Bonvehí, J. (2005). Investigation of aromatic compounds in roasted cocoa powder.*European Food Research and Technology*, 221:19-29.

Sicard, D. and Legras, J. L. (2011). Bread, beer and wine: yeast domestication in the *Saccharomyces sensu stricto* complex. *Comptes Rendus Biologies*, 334(3): 229-236.

Silva, C. F., Schwan, R. F., Dias, S. and Wheals, A. E. (2000).Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil.*International Journal of Food Microbiology*, 60: 251–260.

Singh, I., Turner, A., Sinclair, A., Li, D. and Hawley, J. (2007).Effects of gamma-tocopherol supplementation on thrombotic risk factors.*Asia Pacific Journal of Clinical Nutrition*, 16(3): 422-428.

Skountzou, P., Soupioni, M., Bekatorou, A., Kanellaki, M., Koutinas, A. A., Marchant, R. and Banat, I. M. (2003). Lead (II) uptake during baker's yeast production by aerobic fermentation of molasses. *Process Biochemistry*, 38(10): 1479-1482.

Smit, G., Smit, B. A. and Engels, W. J. M. (2005). Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. *FEMS Microbiology Reviews*, 29: 591-610.

Smit, H. J. (2011). Theobromine and the pharmacology of cocoa.In: Fredholm, B. B. (Ed), *Methylxanthines.* (pp. 201-234). Berlin: Springer.

Smit, H. J. and Blackburn, R. J. (2005). Reinforcing effects of caffeine and theobromine as found in chocolate. *Psychopharmacology (Berl),* 181: 101–106.

Steinberg, F. M., Bearden, M. M. and Keen, C. L. (2003). Cocoa and chocolate flavonoids: implications for cardiovascular health. *Journal of the American Dietetic Association*, 103(2): 215-223.

Swiegers, J. H., Bartowsky, E. J., Henschke, P. A. and Pretorius, I. S. (2005).Yeast and bacterial modulation of wine aroma and flavour. *Australian Journal of Grape and Wine Research*, 11(2): 139.

Tamang, J. P. and Kailasapathy, K. (2010).*Fermented Foods and Beverages of The World*. Florida: CRC Press.

Thommes, K. and Strezov, V. (2015).Fermentation of Biomass.In Strezov, V. and Evan, T. J. (Eds.), *Biomass Processing Technologies* (pp. 257-268). Boca, Raton: CRC Press.

Thompson, S. S., Miller, K. B. and Lopez, A. S. (2001).Cocoa and Coffee.In Doyle M. J., Beuchat, L. R. and Montville, T. J. (Eds), *Food Microbiology—Fundamentals and Frontiers*. (pp 721–733). Washington, DC: ASM Press.

Tortora, G. J., Funke B. R. and Case C. L. (2015). *Microbiology: An Introduction*. San Francisco: Pearson Benjamin Cummings.

Urbieta, M. S., Donati, E. R., Chan, K. G., Shahar, S., Sin, L. L. and Goh, K. M. (2015). Thermophiles in the genomic era: Biodiversity, science, and applications. *Biotechnology Advances,* 33(6): 633–647.

Verna, R. (2013). The history and science of chocolate.*Malaysian Journal of Pathology,* 35(2): 111–121.

Verstrepen, K. J., Iserentant, D., Malcorps, P., Derdelinckx, G., Van Dijck, P., Winderickx, J. and Delvaux, F. R. (2004). Glucose and sucrose: hazardous fast-food for industrial yeast? *Trends in Biotechnology,* 22(10): 531–7.

Voigt, J. and Lieberei, R. (2014).Biochemistry of Cocoa Fermentation. In Schwan, R. F. and Fleet, G. H. (Eds.),*Cocoa and Coffee Fermentations* (pp. 193-226). Boca Raton: CRC Press.

Voigt, J., Biehl, B., Heinrichs, H., Kamaruddin, S., Marsoner, G. G. and Hugi, A. (1994). In-vitro formation of cocoa-specific aroma precursors: aroma-related peptides generated from cocoa-seed protein by co-operation of an aspartic endoprotease and a carboxypeptidase. *Food Chemistry*, 49(2): 173-180.

Vuka, T., Mili, I., Rakin, M. and Iler-marinkovi, S. (2007). Utilization of baker's yeast (*Saccharomyces cerevisiae*) for the production of yeast extract: effects of different enzymatic treatments on solid, protein and carbohydrate recovery. *Journal of the Serbian Chemical Society*, 72(5): 451-457.

Vukašinović-Milić, T., Rakin, M. and Šiler-Marinković, S. (2007). Utilization of baker's yeast (*Saccharomyces cerevisiae*) for the production of yeast extract: effects of different enzymatic treatments on solid, protein and carbohydrate recovery. *Journal of the Serbian Chemical Society*, 72(5): 451-457.

Wartmann, M. T. and Kunze, G. (2000).Genetic transformation and biotechnological application of the yeast *Arxula adeninivorans. Applied Microbiology and Biotechnology*, 619–624.

Wasko, B. M., Carr, D. T., Tung, H., Doan, H., Schurman, N., Neault, J. R., Feng, J., Lee, J., Zipkin, B., Mouser, J., Oudanonh, E., Nguyen, T., Stetina, T., Shemmory, A., Lemma, M. and Kaeberlein, M. (2013). Buffering the pH of the culture medium does not extend yeast replicative lifespan. *F1000Research*, 2.

Watanabe, I., Ando, A. and Nakamura, T. (2012). Characterization of *Candida* sp. NY7122, a novel pentose-fermenting soil yeast. *Journal of Industrial Microbiology and Biotechnology*, 39(2): 307–15.

Weiss, A., Delproposto, J. and Giroux, C. N. (2004).High-throughput phenotypic profiling of gene–environment interactions by quantitative growth curve analysis in *Saccharomyces cerevisiae*.*Analytical Biochemistry*, 327(1): 23-34.

Werber, D., Dreesman, J., Feil, F., Van Treeck, U., Fell, G., Ethelberg, S., Hauri, A. M., Roggentin, P., Prager, R., Fisher, I. S., Behnke, S. C., Bartelt, E., Weise, E., Ellis, A., Siitonen, A., Andersson, Y., Tschape, H., Kramer, M. H. and Ammon, A. (2005). International outbreak of *Salmonella* Oranienburg due to German chocolate. BMC Infectious Diseases, 5(1): 7.

Wiley, J. M., Sherwood, L. M. and Woolverton, C. J. (2008).*Prescott, Harley and Klein'sMicrobiology* (pp. 134-135). New York: McGraw Hill Inc.

Xu, W., Liang, L. and Zhu, M. (2015).Determination of sugars in molasses by HPLC following solid-phase extraction.*International Journal of Food Properties*, 18(3): 547-557.

Yan, D., Lu, Y., Chen, Y. F. and Wu, Q. (2011). Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production. *Bioresource Technology*, 102(11): 6487-6493.

Yang, J. Y., Scascitelli, M., Motilal, L. A., Sveinsson, S., Engels, J. M. M., Kane, N. C. and Cronk, Q. C. B. (2013). Complex origin of Trinitario-type *Theobroma cacao* (Malvaceae) from Trinidad and Tobago revealed using plastid genomics. *Tree Genetics and Genomes*, 9(3): 829–840.

Yönten, V. and Aktaş, N. (2014).Exploring the optimum conditions for maximizing the microbial growth of *Candida intermedia* by response surface methodology. *Preparative Biochemistry and Biotechnology*, 44(1): 26-39.

Yoon, S., Mukerjea, R. and Robyt, J. F. (2003). Specificity of yeast (*Saccharomyces cerevisiae*) in removing carbohydrates by fermentation. *Carbohydrate Research*, 338(23): 1127–1132.

Zakrajšek, T., Raspor, P. and Jamnik, P. (2011).*Saccharomyces cerevisiae* in the stationary phase as a model organism—characterization at cellular and proteome level.*Journal of Proteomics*, 74(12): 2837-2845.

Zamora, F. (2009).Biochemistry of Alcoholic Fermentation.In Moreno-Arribas, M. V. and Polo, M. C. (Eds.), *Wine Chemistry and Biochemistry* (pp. 3-26). New York: Springer.

Zeng, Z., Lin, J. and Gong, D. (2009).Identification of lactic acid bacterial strains with high conjugated linoleic acid-producing ability from natural sauerkraut fermentations.*Journal of Food Science*, 74(4): M154-M158.

Zhang, D., Susilo, A. W., Dinarti, D., Bailey, B., Mischke, S. and Meinhardt, L. W. (2014).Genetic identity, ancestry and parentage in farmer selections of cacao from Aceh, Indonesia revealed by Single Nucleotide Polymorphism (SNP) markers.*Tropical Plant Biology*, 7(3-4): 133–143.

Zhao, Z. J., Tong, H. R., Zhou, L., Wang, E. X. and Liu, Q. J. (2010).Fungal colonization of pu-erh tea in Yunnan.*Journal of Food Safety*, 30(4): 769–784.

Zinder, S. H. and Dworkin, M. (2013). Morphological and Physiological Diversity.In Rosenberg, E. (Ed.).*The Prokaryotes*(pp. 89-122). Berlin, Heidelberg: Springer.