Fermentation of coffee beans with inoculation of *bacillus subtilis* and its impact on coffee sensory quality

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Abstract. The aim of this research was to study the coffee fermentation with inoculation of *Bacillus subtilis* isolated from civet (*Paradoxorus hermaphroditus*) as starter culture. The quality of coffee was evaluated by sensory quality (cup test). Two treatments were carried out: control/coffee non-inoculated starter culture (treatment F) and coffee inoculated with a *Bacillus subtilis* starter culture (treatment G). The fermentations were conducted for 48 h in triplicate. An inoculation of *Bacillus subtilis* as a starter culture cause a decrease in pH and increase in total titratable acidity of coffee fermentation solution. A panel of five trained coffee testers (Q-grader from Gayo Cupper’s Team) evaluated the samples. Ten sensorial attributes analyzed were fragrance/aroma, flavor, aftertaste, acidity, body, uniformity, balance, sweetness, cleanliness and overall. The result showed that the use of *Bacillus subtilis* starter culture improved quality of coffee fermentation. The aroma, flavour, aftertaste, body, balance and overall were increased. The cupping score total of coffee increased from 82.83 to 84.33. Metabolites analysis should be further investigated to confirm this result, especially organic acids compound.

1. Introduction

Coffee is stated as the most popular beverage worldwide. Coffee is grown mainly by smallholder farmers in over 70 countries, especially where the countries are mostly concentrated in tropical developing countries. The leader of coffee-producing countries is Brazil, followed by Vietnam, Columbia, Indonesia and Ethiopia [1]. On the other hand, coffee consumption is spread all over the globe where the developed countries hold major percentage. Nowadays, coffee is widely consumed due to its stimulant effect and exquisite flavor [2,3]. It is derived from over 1500 chemical substances [4,5]. The chemical substances are reported to be derived from the plant metabolites and the others are products of chemical reactions i.e. fermentation and roasting [6,7].

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There are three different methods of coffee processing: dry, wet and semi-dry processing ones. All methods aim to remove the fruit flesh of coffee cherry. Dry processing implies to the processing that the whole coffee cherry (pulp, parchment and bean) is dried together. As for wet processing, the pulp (exocarp and a part of mesocarp) is removed mechanically where the remaining mesocarp (mucilage) sticks to the parchment is also removed before drying. Semi-dry processing is a combination of both methods above, in which the mucilage is not fully removed after pulping and parchment is dried together with most of all mucilage [8,9,10]. The differences of these three methods impact coffee flavor [11,12,13]. Different applied processing methods induce different metabolic reactions in coffee fruits, which affecting chemical composition of the coffee beans and their cupping quality [3,14].

Coffee fermentation is considered as one of the most important processing steps during wet processing. During fermentation, it is critical for removing mucilage from coffee parchment and reduce water content [10,15]. In common coffee production by farmers, the fermentation is done spontaneously which is carried out by complex microorganisms such as yeast, bacteria and fungi [16,17]. Several researchers claimed that over 50 yeasts and bacterial species have been identified during coffee fermentation. Microorganisms in coffee fermentation contribute to the production of diverse metabolites [18,19,20]. However, this spontaneous fermentation of coffee bean produces variety in quality of coffee products. The use of starter cultures in fermentation industry has shown positive results for several types of fermented food and beverages [20,21,22]. Starter cultures are reduced fermentation time and improve coffee quality [15].

Civet coffee is one of the rarest and the most expensive coffee in the world. In process, civet (Paradoxorus hermaphroditus) eat the coffee berries and pass it through their digestive system. In the digestive tract, biochemical process (fermentation) occurs and produces coffee with distinctive flavor [23,24]. During fermentation in its digestive system, some lactic and non-lactic acid bacteria have isolated and identified from civet [25,26,27,28]. However, it still fewer studies are available about coffee fermentation by bacteria isolated from gastrointestinal tract of civet. Study assisted fermentation by using microorganisms as starter a new trend. Therefore, this research aims to evaluate Bacillus subtilis starter culture performance during coffee fermentation that is isolated from civet in order to enhance quality of coffee fermentation. The performance of Bacillus subtilis starter culture is assessed based on coffee bean condition during fermentation, and the quality of green bean produced by its process.

2. Materials and methods

2.1. Coffee cherries preparation
Freshly harvested coffee cherries (Coffee arabica) were obtained from local farmer in Bener Meriah, Aceh, Indonesia. The cherries were manually unpulped to obtain beans with mucilage.

2.2. Starter culture, fermentation and drying
Bacillus subtilis used in this research was obtained from Microbiological Industry Laboratory, Agricultural Product Technology, Universtas Syiah Kuala. The culture had been previously isolated from gastrointestinal tract of civet and its identification was confirmed by sequencing of the 16S-RNA [28]. Starter culture was prepared by followed procedures of Fauzi and Hidayati [29]. The culture was recultured on MRS agar for 48 h. The culture was then inoculated in 10 ml of MRS broth for 48 h at 37°C. Meanwhile the sterile media was also prepared in the form of coffee fruit skin extract that has been enriched with sugar nutrition (2% of coffee fruit extract). Culture produced from MRS broth was inoculated on sterile media and incubated for 48 h at 37°C. Later, this culture was used as starter in coffee fermentation. Fermentation was conducted in glass jar containing 500 g of unpulped coffee beans. As a control, spontaneous process was allowed to ferment an unpulped coffee bean (without addition of starter culture). In the sample ones, an unpulped coffee bean inoculated with 10% (v/w) starter culture Bacillus subtilis and incubated for 48 h at room temperature. A total of two treatments were carried out:
- F: control (coffee noninoculated)
- G: coffee inoculated with 10% starter culture *Bacillus subtilis*

The all treatment fermentations were conducted in triplicate. After 48 h fermentation, the samples were washed and sun dried until 10% of moisture content was reached.

2.3. Chemical analysis
The liquid of coffee fermentation was collected and measured for pH and total titratable acidity. The pH of coffee liquid was measured by pH meter. The liquid of coffee samples were titrated using a 0.1 N NaOH solution and the acidity was calculated as acetic acid number. Analysis was done in triplicate.

2.4. Coffee cup quality evaluation
Green bean coffee samples were prepared according to The Specialty Coffee Association of America procedure [30]. Samples were roasted in medium degree (65 Agtron scale) in a laboratory roaster with capacity 150 g and was ground in an electrical mill. Five glasses was prepared for each samples. Each glass contained 8.25 g of ground coffee and poured 150 ml of water (93 - 95°C). A panel of three trained coffee testers (Q-grader from Gayo Cupper’s Team) evaluated the samples. Ten sensorial attributes analyzed were fragrance, flavor, aftertaste, acidity, body, uniformity, balance, sweetness, cleanliness and overall.

2.5. Data analysis
Data resulted from analysis are presented as means with corresponding SD and analyzed descriptively.

3. Results and discussion

3.1. Analysis of pH and total titratable acidity
The result obtained in the present study showed that pH and acid total of fermentation solution change during fermentation. The pH and acid total were measured right after the inoculation of starter culture (0 h) and the end of fermentation (48 h) as shown in Figure 1 and 2.

![Figure 1](image-url)

*Figure 1. Change of pH of coffee liquid during coffee fermentation.*

Figure 1 shows the pH of the coffee bean liquid at the beginning of fermentation (0 h) looks higher than fermentation after 48 h. This changing indicates that during 48 h of storage, there has been a fermentation process occurred which produces a number of organic acids since the pH is lower. This fermentation is aided by enzymes that are naturally found in coffee beans and nearby microorganisms.
However, the pH in treatment G which was inoculated with *Bacillus subtilis* was lower than the non-inoculated ones (G). It is suspected that the activity of *Bacillus subtilis* produces greater acid so that the resulting pH is lower. This result is supported by the data of the analysis of total titratable acidity as shown in Figure 2.

![Figure 2](image-url)

**Figure 2.** Change of total titratable acidity coffee solution during coffee fermentation.

Figure 2 shows that the total titratable acidity in the treatment with the addition of *Bacillus subtilis* was greater than the control. The total acid contained in the coffee liquid at the beginning of the fermentation comes from the raw material of coffee beans and its circumstances. The coffee bean and its circumstances contain a number of soluble organic acids. As for the end of fermentation, total titratable acidity tends to increase due to the production of other organic acids as a result of microorganisms activity that metabolize large molecular mass compounds in fermented products [31]. Microbial activity during the fermentation process will cause a decrease in pH along with the increasing of acidity of the product as lactic acid, and other organic acids will accumulate [32]. Some organic acids produced in coffee fermentation include lactic acid, acid acetate, butyric acid, and other carboxylic acids [15,33].

*Bacillus* strains commonly associated with a variety of fermentation products and have been reported to produce a number volatile compounds and organic acids. Some strains of *Bacillus* are reported producing lactic acid [34,35,36,37]. *Bacillus* is considered to be GRAS (generally regarded as safe) status with the Food and Drug Administration for species such as *Bacillus subtilis* and *Bacillus licheniformis* [36,38,39]. These characteristics strongly support the use of *Bacillus subtilis* to become a starter culture in coffee fermentation. However, further research is needed to analyze its metabolites.

### 3.2. Sensory evaluation

Sensory evaluation is an assessment towards sensory properties of food using human senses as measurement tools [40]. Sensory evaluation is commonly done by trained panellists in order to identify the quality of new food product, to determine the product standards or to act as quality control routines [41]. In terms of coffee, its sensory evaluation is usually known as cupping quality. Cupping quality is nowadays classified as one important parameter in world coffee trading (SCAA, 2009). In coffee market, specialty coffee is classified as premium products which is defined by its cupping quality based on SCAA standard. To be named as specialty coffee, the gained score should be exceed 80,00. Based on sensory evaluation by Gayo Cupper Team, both of samples gathered from this treatments are classified as specialty coffee (Table 1). It also could be seen that fermentation with addition of starter culture enabled to produce coffee with better sensory properties (Figure 3).
Furthermore, compared to the score of Gayo civet coffee (84,50) produced by local farmers on farm-site [42], the finding score from inoculated treatment (sample G) is slightly similar (84,33) whereas sample F is lower.

Table 1. Final score (Cupping score) of coffee based on sample treatments.

| Sample                                      | Final Score       |
|----------------------------------------------|-------------------|
| F (Control/non-inoculated with starter culture) | 82.83 ± 0.52     |
| G (Coffee inoculated with starter culture *Bacillus subtilis*) | 84.33 ± 0.52     |

Figure 3 showed that the addition of starter culture in fermentation positively improves the cupping quality of coffee in certain attributes, named as aroma, flavor, body, balance and overall. To be specific, value of aroma, flavor, acidity, and body attributes for sample B are nearly similar with the results of previous research which describes the cupping quality of civet coffee from six coffee plantation areas in Gayo Highland [42], aroma (7,93), flavor (7,85), acidity (7,55) and body (7,97).

Figure 3. Sensory quality attributes of sample based on treatments.

Aroma is perceived in two common ways, before and after poured by hot water. Aroma of civet coffee is reported to be lower than coffee from semi-wash process [43]. Flavor, aftertaste, and acidity assessed by trained panellists in order when the liquor temperature is cooling down [30]. Flavour of coffee is manifestation of the whole process, started from coffee fermentation. Coffee fermentation deforms and decomposes sugars contents and produces volatiles compound. These compounds transform during roasting process and produce favourable flavor for coffee. In civet coffee, flavour and body hold as key quality since its normally have higher score than semi-wash coffee. Figure 3 shows flavor and body attributes of sample are higher than coffee from spontaneous fermentation. Therefore, there is an evidence that ability to place the suitable microrganisms improved the cupping quality of produced coffee. Microbial activity and the extent of fermentation determine the concentrations of free sugars and amino acids that continue to surround the bean and subsequently contribute for production of Maillard and volatiles compounds during roasting process [44]. Therefore it can be state that *Bacillus subtilis* improved coffee quality in this study.

4. Conclusions
The using of *Bacillus subtilis* as starter culture in coffee processing plays a rule in fermentation performance. During the fermentation, inoculated of the starter culture cause a decrease in pH and an
increase in total titratable acidity of coffee fermentation liquid. *Bacillus subtilis* improved coffee sensory quality. The aroma, flavour, aftertaste, body, balance and overall increased. The cupping score total of coffee increased from 82.83 to 84.33. However, more investigations are required with the aim of studying the metabolites of *Bacillus subtilis* on coffee fermentation.

References

[1] International Coffeee Organization 2017. http://www.ico.org/Market-Report-17-18-e.asp
[2] Farah A 2012 *Coffee Constituent*. In: Emerging Health Effect and Diseases Prevention Chu Yi-Fang Editor. John Willey and Sons Inc. (Blackwell Publishing)
[3] Sunarharum W B, Williams D J and Smith H E 2014 *Food Res. Int.* **62** 315–325
[4] Padmapriya R, Tharian J A and Thirunalasundari T 2013 *Int. J. Curr. Sci.* **9** 83-91
[5] Blinova L, Sirotiak M, Bartosova A and Soldan A 2017 *Research Paper* **25** (40) 1-14
[6] Flament I and Bessiere-Thomas Y 2002 *Coffee Flavor Chemistry* John Wiley and Sons Ltd, Baffins Lane, Chichester Schwan and G. Fleet, Eds. (CRC Press Boca Raton)
[7] Poltronieri P and Rossi F 2016 *Challenges* **7** (19):1-22
[8] Boot W J 2007 *Roast magazine* **1** 31–49
[9] Brando C H J and Brando M F 2014 *Methods of coffee fermentation and drying* In Schwan R F, Fleet G H (ed), Cocoa and coffee fermentations 1st ed (CRC Press Boca Raton)
[10] Haile M and Kang W H 2019 *J. Food Quality* 1-6
[11] Duarte G, Pereira A and Farah A 2008 *Proceedings The 22nd International Conference on Coffee Science Campinas Brazil*
[12] Selmar D, Kleinwachter M and Bytof G 2015 *Metabolic responses of coffee beans during processing and their impact on coffee flavor* In: Schwan R F and Fleet G H Editors. Cocoa and coffee fermentations (CRC Press Boca Raton)
[13] Ghosh P and Venkatachalapathy N 2014 *Int. J. Eng. Res. Tech.* **3** 784-794
[14] Bytof G, Knopp S E, Schieberle P, Teutsch I and Selmar D 2005 *Euro. Food Res. Tech.* **220** (3-4): 245–250
[15] Silva S C F, Vilela D M, C de Souza Cordeiro, Duarte W F, Dias D R and Schwan R F 2013 *J. Microbiol. Biotechnol.* **29** (2) 235–247
[16] Silva C F, Batista L R, Abreu L M, Dias E S and Schwan R F 2008 *Food Microbiol.* **25** 951–957
[17] Schwan R F and Fleet G H 2014 *Cocoa and Coffee Fermentations* (CRC Press Boca Raton)
[18] Pereira G V M, Soccol V T and Pandey A 2014 *Int. J. Food and Microbiol.* **188** 60–66
[19] Silva C F, Schwan R F, Dias E S and Wheels A E 2000 *Int. J. Food and Microbiol.* **60** 251–260
[20] Sun S Y, Gong H S, Jiang X M and Zhao Y P 2014 *Food Microbiol.* **44** 15–23
[21] Visintin S, Ramos C L, Batista N, Dolci P, Schwan R F, Cocolin L 2017 *Int. J. Food Microbiol.* **257** 31–40
[22] Martinez S J, Bressani A P P, Miguel M G C P, Dias D R and Schwan R F 2017 *Res. Int. J.* **102** 333-340
[23] Marcone M F 2004 *Food Res. Int.* **37** 901–912
[24] Smith I B 2014 *Kopi luwak coffee-world’s most expensive coffee beans from civet poop or an urban myth.?* (IBS Publishing USA)
[25] Muzaffa M, Patria A, Abubakar A, Rahmi F and Hasni D 2016 *Kopi Luwak: Produksi, mutu dan permasalahannya* (Syiah Kuala University Press Banda Aceh)
[26] Fauzi M 2008. *Isolasi dan karakterisasi bakteri asam laktat biji kopi luwak (civet coffe)* Fakultas Teknologi Pertanian (Universitas Jember)
[27] Suhandono S, Setiadi H, Kristianti T, Kusuma A B, Wedanringtyas W, Djajadi D T and Aryantha I N P 2016 *Microbiol. Indonesia* **10** (2) 65-70
[28] Muzaffa M, Hasni D,Yunita D 2019 *Produksi kopi spesialti berbasis mikroorganisme indigenous luwak* (LPPM Universitas Syiah Kuala Banda Aceh)
[29] Fauzi M, Hidayati N W 2016 *Prosiding Seminar Nasional Hasil Penelitian dan Pengabdian Masyarakat Indonesia*
[30] SCAA (Specialty Coffee Association of America) 2009 Coffe facts and statistics [Online] Available: www.scaa.org
[31] Shukla S, Choi T B, Park H K, Kim M, Lee I K and Kim J K 2010 Food Chem. Toxicol. 48 2005-2010
[32] Charalampopoulos D, Wang R, Pandiella S S, Webb C Isolation and Characterization of Lactic Acid Bacteria from “Ting” in The Northern Province of South Africa Thesis University of Pretoria
[33] Pereira G V M, Neto D V C, Mediros A B P, Soccol V T, Neto E, Woiciechowski A L and Soccol C R 2016 Int. J. Food sci. Tech. 51 1689-1695
[34] Maas R H, Bakker R. R, Jansen M L, Visser D, de Jong E and Eggink G 2008 Appl. Microbiol. Biotechnol. 78 (5) 751-758
[35] Ohara H and Yahata M 1996 J. Ferment. Bioeng. 81 272- 274
[36] Gao T, Wong Y, Ng C and Ho K 2012 Bioresour. Technol. 121 105-110
[37] Qin J, Zhao B, Wang X, Wang L, Yu B, Ma Y, Xu P, 2009 PLoS One 4 (2) 43-59
[38] Lei Z H 2011 Liquor making 38 24-28
[39] Yan Z, Xiao-Wei Z, Jing-Yu C, Jian-Shu H and Bei-Zong H 2013 J. Inst. Brew 119 78-83
[40] Meilgaard, M C, Civille G V and Carr B T Sensory Evaluation Techniques (CRC Press, Boca Raton)
[41] Fuller G W 2011 New Product Development. From Concept to Marketplace third edition (Taylor and Francis Group LLC)
[42] Muzaifa M, Hasni D, Yunita D, Febriani, Patria A, Abubakar A 2019 2019 IOP Conf. Ser.: Mater. Sci. Eng. 523 011002
[43] Abubakar Y, Hasni D, Muzaifa M, Sulaiman, Mahdi and Widayat H P 2019 IOP Conf. Ser.: Mater. Sci. Eng. 523 011002
[44] De Maria C A B, Trugo L C, Aquino Neto F R, Moreira R F A and Alviano C S 1996 Food Chemistry 55 (3) 203-207

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