可可豆发酵过程中的微生物学和生物化学

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【摘要】本研究旨在定量和定性地检测干可可豆的发酵参数，以更有效地确定微生物的作用和功能。三种发酵方法如下：对照组（不接种）、酿酒酵母（FNCC 接种物 3056）、乳酸乳杆菌（FNC0086）和醋酸杆菌（FNCC0016），浓度均为约 108CFU/g，在发酵开始时同时添加（A）；三种接种物分阶段添加（B）。结果表明，在发酵过程中，三种处理均降低了总糖含量、pH 值和总多酚含量。在所有处理中，乙醇、乳酸和乙酸的浓度分别在发酵 24、60 和 108 小时时达到峰值。在发酵 24、48 和 72 小时时，从三种处理中获得了最多的酿酒酵母、乳酸杆菌和醋酸杆菌数量。

【关键词】微生物；酿酒酵母；乳酸杆菌；醋酸杆菌；发酵和干燥可可豆

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During fermentation, microbiology and biochemistry of the cocoa bean

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【**Abstract**】The purpose of this research is to quantitatively and qualitatively examine the fermentation parameters of dry cocoa beans to be more effective in determining the role and function of microorganisms. The three fermentation methods are as follows: control (without inoculums), Saccharomyces cerevisiae (FNCC inoculums 3056), Lactobacillus lactis (FNC 0086), and Acetobacter aceti (FNCC 0016), all at a concentration of approximately 108 CFU/g were added simultaneously at the start of the fermentation (A), and all three inoculums were added in stages (B). The results indicated that during fermentation, all three treatments decreased total sugar content, pH, and total polyphenols. In all treatments, the concentrations of ethanol, lactic acid, and acetic acid peaked at 24, 60, and 108 hours of fermentation, respectively. At 24, 48, and 72 hours of fermentation, the highest populations of Saccharomyces cerevisiae, Lactobacillus lactis, and Acetobacter aceti were obtained from the three treatments.

【**Keywords**】Microbes; Saccharomyces cerevisiae; Lactobacillus lactis; Acetobacter aceti; Fermentation and dried cocoa beans

1 介绍

印度尼西亚主要的种植园产品之一，因为它能创造外汇、创造就业机会，并刺激农业综合企业和农产品加工业的增长[1,2]。一般来说，社区种植园生产的干可可豆不经过自然发酵或添加接种物发酵。通常，可可种植户会将新鲜的可可豆浸泡在水中以去除果肉，然后将其暴露在阳光下[3,4]。出售的干可可豆水分含量未知，质量不一，无论是水分含量还是状况均不理想。这是因为生产可可的农民数量很少，而且发酵过程耗时过长[5,6]。发酵是可可豆加工过程中的关键步骤，特别是对于风味前体化合物的形成。新鲜可可豆的发酵分为两个阶段：第一阶段是去除种子表面的果肉，第二阶段是种子子叶内的水解反应[7,8]。微生物演替是指可可豆发酵过程中微生物种类和数量的变化。发酵的初始阶段以酵母为主，然后是乳酸菌，最后是醋酸菌。

可可，一种种子风味的前体化合物，可以通过发酵形成。发酵分为两个阶段。首先去除果肉，然后

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在种子子叶中进行水解反应。第二步涉及微生物演替过程[9,10]。可可的发酵需要 120 小时或 6 天，并在第 2、3、4 和 5 天进行逆向发酵。在酿酒酵母发酵的早期阶段，微生物菌落在发酵的开始过程中发挥着作用[11,12]。这是因为酿酒酵母。酿酒酵母（Cerevisiae）在乙醇存在下能很好地生长，并且耐受低 pH 值，这是果胶分解活性所必需的。乳酸杆菌（Lactobacillus Lactis）能耐受高氧浓度的酸性环境。在发酵结束时，醋酸杆菌（Acetobacter aceti）占主导地位，因为它能够在 pH3.5 和低乙醇浓度下生长。随后，乙醇被氧化形成乙酸，乙酸随后转化为二氧化碳和水[13]。随着环境温度的升高，酒精和乙酸会扩散到种子中，导致种子死亡[14,15]。

2 方法

果实成熟时长 16 厘米，直径 9 厘米。该方法参考了[16]的研究，并略作修改。发酵过程以三种不同的方式进行。作为对照，I 法进行自发发酵（无接种物）。第二种方法（A）同时添加酿酒酵母纯培养物（FNCC3056）、乳酸杆菌（FNC0086）和醋酸杆菌（FNCC0086）。0016)。第三种技术（B）逐渐加入纯培养物，从发酵开始第 0 小时开始加入酿酒酵母纯培养物（FNCC3056），24 小时后加入乳酸乳杆菌（FNC0086），72 小时后加入醋酸醋杆菌（FNCC0016），总多酚以及乙醇、乳酸和乙酸浓度的变化决定发酵是否成功[10]。

3 结果

结果表明:对照处理II、III的起始温度分别为 28、29、29.5℃,最高发酵温度分别为 38、42、51℃,发酵 120h 时对照、II、III的发酵温度分别为 35、38、39℃;对照、II、III的糖度分别为 3.07±0.67、2.60±0.65、2.70±0.7;对照、II、III的 pH 分别为 4.45±0.17、4.28±0.2、4.20±0.2;对照、Ⅱ、III的还原糖度分别为 10.53±0.55、10.63±0.53 和 10.57±0.52。对照组、II 组和 III 组的酿酒酵母(logcfu/g)分别为 3.55±0.25、2.30±0.5 和 2.22±0.5。对照组、II 组和 III 组的乳酸乳球菌(logcfu/g)分别为 5.42±0.17、6.42±0.6 和 6.23±0.6。对照组、II 组和 III 组的醋酸杆菌(logcfu/g)分别为 4.87±0.47、5.30±0.8 和 9.22±0.8。乙醇(%)对照组、II 组和 III 的乳酸(%)分别为 0.40±0.30、0.33±0.7 和 0.20±0.5a。对照组、II 组和 III 的乳酸(%)分别为 2.00±0.17、1.82±0.9 和 1.70±0.3a。对照组、II 组和 III 的果酸(%)分别为 5.90±0.28、6.32±0.2 和 6.80±0.6。Pholipenol(mgasamgalat/g)对照、II、Ⅲ组分别为 0.079±0.14、0.07±0.05、0.068±0.2（表 1）。

4 讨论

本研究观察到的发酵温度变化与[18]的研究结果

表 1 总糖含量、pH 值、总多酚、乙醇、乳酸、乙酸、S.Cerevisiae（FNCC 3056）、L.lactis（FNC 0086）和 A.aceti（FNCC0016）变化的统计分析结果

范围	治疗		
	控制	二	三
糖含量（%）	3.07 ±0.67a	2.60±0.65a	2.70±0.7a
还原糖（%）	10.53±0.55a	10.63±0.53a	10.57±0.52a
pH	4.45±0.17a	4.28±0.2a	4.20±0.2a
酿酒酵母（log cfu/g）	3.55±0.25a	2.30±0.5a	2.22±0.5a
乳酸乳球菌 (log cfu/g)	5.42±0.17a	6.42±0.6b	6.23±0.6b
醋酸杆菌 (log cfu/g)	4.87±0.47a	5.30±0.8b	9.22±0.8b
乙醇（%）	0.40±0.30a	0.33±0.7a	0.20±0.5a
乳酸（%）	2.00±0.17a	1.82±0.9a	1.70±0.3a
果酸 (%)	5.90±0.28a	6.32±0.2a	6.80±0.6a
菲利潘醇 (mg asam galat/g)	0.079±0.14a	0.07±0.05a	0.068±0.2a

注：后面不同字母表示差异显著 p<0.05。结果为 3 次重复分析的平均值。

一致，他们研究了改善干可可豆发酵过程的方法。在逐渐的处理过程中，酿酒酵母可以重塑更多的果肉糖，增加乙醇的产量，在适当的时候，乙醇被[20]转化为乙酸。在乙酸乳酸菌中，将乙醇转化为乙酸的反应也会产生热量，从而提高发酵温度[20]。当接种物与种子同时加入，并且未达到种子死亡温度时，预计酿酒酵母和乳酸乳酸菌之间会发生竞争。在整个处理过程中，干可可豆的总糖含量下降了长达 120 小时。可可豆中含有蔗糖、果糖和葡萄糖等糖类[21]，其中蔗糖占总糖的 90%，果糖和葡萄糖分别占总糖的 6%（0.9%和 0.7%），其中甘露醇和肌醇的含量低于 0.50 毫克/克[22]。发酵处理开始时，对照组、A 组和 B 组干可可豆的总糖含量分别为 8.45%、8.45%和 8.5%，之后下降到 3.07%、2.60%和 2.70%[22]。

5 结论

发酵后干燥可可豆的品质可以得到提高。处理 B，即在发酵结束时逐渐添加接种物，其品质优于其他处理。

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