Vol. 15, No. 1 (2026): 213 - 222 http://dx.doi.org/10.23960/jtepl.v15i1.213-222

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Verification of Alternative Agar Plate Method for Quantitative Analysis of Yeasts and Molds in Cocoa Products

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Article History:

Received : 01 July 2025
Revised : 09 August 2025
Accepted : 22 August 2025

Keywords:

Cocoa cake,
Cocoa liquor,
Cocoa powder,
Rapid microbiology method.

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ABSTRACT

Cocoa beans are vital raw materials in the food industry and are processed into various cocoa products. These products must meet quality and food safety standards, as contamination by yeasts and molds can reduce product quality and shelf life. Conventional microbiological methods often require long incubation times, delaying quality control. This study aimed to verify Symphony Agar as a rapid method for enumerating yeasts and molds in cocoa products following ISO 16140-3:2021 guidelines and to compare it with conventional methods. Verification consisted of two stages: (1) implementation verification, assessing standard deviation of intralaboratory reproducibility (S_{IR}), and (2) food item verification, assessing estimated bias (eBias). Implementation verification on cocoa powder yielded SIR values of 0.121 $\log_{10}$ CFU (pour plate) and 0.171 $\log_{10}$ CFU (spread plat), both below the acceptable threshold, indicating good reproducibility. Food item verification using cocoa powder, cocoa cake, and cocoa liquor showed eBias values under 0.5 $\log_{10}$, meeting the requirements. A comparative study using an independent t-test found no significant difference between Symphony Agar and DG18 Agar. The results indicate that Symphony Agar is suitable as an alternative medium for yeasts and molds analysis in cocoa products, offering the benefit of a shorter incubation time without compromising accuracy.

1. INTRODUCTION

Cocoa is a high-value commodity renowned for distinctive flavor and aroma. Cocoa beans (*Theobroma cacao*) serve as essential raw materials in various industries. Before being utilized in food production, cocoa beans undergo several processing stages, including fermentation, drying, and roasting (Eshun, 2020). These processes contribute to the development of desirable sensory and physicochemical characteristics. Cocoa-derived products such as cocoa liquor, cocoa butter, cocoa cake, and cocoa powder are widely used in the manufacture of chocolate and in a broad range of food, cosmetic, and pharmaceutical applications (Beg *et al*., 2017).

Cocoa processing starts with roasting and grinding dried or fermented cocoa beans to produce cocoa liquor (Bagnulo *et al*., 2023). Cocoa butter is then extracted from the liquor by pressing, followed by steam deodorization under vacuum (Kamphuis, 2017). The leftover solid, called cocoa cake, is pulverized into cocoa powder (BSN, 2009a; Kamphuis, 2017). Among cocoa products, only cocoa powder is required to have SNI certification per Ministry of Industry Regulation No. 60/M-IND/PER/6/2010. Yeast and mold contamination is highest during post-fermentation and powder processing stages, where exposure to air, moisture, and handling create favorable conditions for microbial growth. This contamination significantly reduces product quality and shelf life.

Yeasts and molds are contributors to food spoilage. Yeasts thrive in liquids and are non-toxic, while molds grow in dry foods with low water activity and can produce harmful mycotoxins. Putrefactive molds are a growing concern due to their economic impact on food and beverages (Rawat, 2015; Lorenzo *et al*., 2018). Molds are capable of growing

under a wide range of conditions including low water activity, low pH, and the presence of ethanol or common food preservatives. A study by Iacumin *et al*. (2022) showed that molds of environmental origin specifically *Penicillium* and *Cladosporium* were detected in chocolate bars in Italian supermarkets. This contamination is suspected to be caused by insufficient heat treatment during bean roasting and chocolate conching, which are not enough to achieve commercial sterility.

Conventional methods for analyzing yeasts and molds conducting according to standards like ISO 21527-1:2008, ISO 21527-2:2008 and BAM Chapter 18 (2001). These methods mainly use the spread plate technique, though pour plating is allowed with proper validation (ISO, 2008a; 2008b). The spread plate method is preferred for its flexibility, temperature tolerance, and ease of colony identification (de Souza *et al*., 2021). ISO 21527-1:2008 applies to foods with $a_w \geq 0.95$ using Dichloran Rose Bengal Chloramphenicol (DRBC) agar for the analysis, while ISO 21527-2:2008 is for foods with $a_w \leq 0.95$ using DG18 agar for the analysis; both require 5-day incubation at 25 ± 1 °C. BAM Chapter 18 uses similar conditions with both media and plating techniques. A major limitation of these methods is the long incubation time, prompting the development of faster, more efficient alternatives (Nemati *et al*., 2016).

Advancements in technology have given rise to the development of alternative methods, such as "Symphony Agar" (Biokar, France). This medium has been validated by Association Française de Normalization (AFNOR) with certificate number BKR 23/11-12/18, based on ISO 21527-1:2008 and ISO 21527-2:2008 standards. This medium has been tested on six different product categories, including cocoa powder and chocolate. The analysis time is 54-72 hours, excluding the addition of other ingredients or supplements. Both of pour plate and spread plate method can be used for the analysis (ADRIA Food Expertise, 2022).

It is imperative to validate and verify methods prior to the implementation of alternative methods (ISO, 2021). Validation tests the novel method against the reference method (Ayunina *et al*., 2016). Verification assesses the performance of a validated method before its use in a single laboratory, including factors that may affect results such as storage, technicians, and the working environment (Yusuf *et al*., 2024). According to ISO 16140-3:2021, verification includes implementation verification and food item verification. Implementation verification determines the standard deviation of intralaboratory reproducibility (S_{IR}), which must be ≤ 2 times the standard deviation of interlaboratory reproducibility (S_R). Food item verification measures estimated bias (eBias), which should not exceed 0.5 $\log_{10}$ (ISO, 2021). Prioritizing implementation verification is essential to ensure the laboratory can properly apply the method as validated (Nagur *et al*., 2023). This study aims to verify the yeast and mold enumeration method for cocoa products using Symphony Agar to be used in a single laboratory and conduct a comparative study between Symphony Agar and DG18 Agar.

2. MATERIALS AND METHODS

2.1. Materials

This study utilized a commercial rapid culture medium Symphony Agar (Biokar, France) alongside various cocoa-derived products, including cocoa powder, cocoa cake, and cocoa liquor. Implementation verification was conducted using cocoa powder samples exhibiting natural contamination of yeasts and molds within the range of 30–300 CFU/g. Food item verification was carried out on cocoa powder, cocoa cake, and cocoa liquor samples subjected to artificial contamination at low, medium, and high levels. For artificial contamination (spiking), *Aspergillus brasiliensis* WDCM 00053 and *Saccharomyces cerevisiae* WDCM 00058 were utilized. A comparative analysis of yeast and mold counts was performed between Symphony Agar and Dichloran 18% Glycerol Agar (DG18), using naturally contaminated samples of yeasts and molds at low (10–100 CFU/g), medium (100–200 CFU/g), and high (200–300 CFU/g) contamination levels.

2.2. Symphony Agar Method Protocol

Sample analysis was conducted using two types of inoculation methods: pour plate inoculation and surface inoculation each performed in two replicates. All procedures were carried out under aseptic conditions. For pour plate inoculation, 1 mL of the sample and its serial dilutions were transferred into empty, sterile petri dishes. Approximately 15 mL of molten Symphony Agar medium was poured into each plate. The contents were mixed thoroughly by gentle swirling

and allowed to solidify on flat surface. Incubate the plates aerobically, lids uppermost, in an upright position in the incubator at 25 °C for 3 days. For surface inoculation, in order to estimate low microbial counts, 1 mL (0.4 mL, 0.3 mL and 0.3 mL) of the initial suspension was inoculated onto the surface of three separate petri dishes (Ø 90 mm). The inoculum was then evenly spread over the surface using a sterile spreader (sterile triangle or "hockey stick"). The inoculated plates were incubated in an upright position at 25 °C for 3 days (Biokar Diagnostics, 2022).

2.3. Standard Deviation of Intralaboratory Reproducibility (S_{IR})

The determination of standard deviation of intralaboratory reproducibility (S_{IR}) was performed using 12 cocoa powder samples from different lots with natural contamination levels of 30-300 CFU/g. The selection of cocoa powder as the sample for implementation verification because this product is included in the scope of validation by AFNOR (ADRIA Food Expertise, 2022). Each sample was subjected to a thorough homogenisation process, followed by division into two test portions of 10 g each. These test portions were then meticulously mixed with 90 mL of Buffered Peptone Water (BPW). Each test portion was analysed with different analyst, batch media and equipment, including micropipettes (100 µL – 1000 µL, Eppendorf, Germany) and incubators (IC 404 ECO, Incucell, Germany; INE 600, Memmert, Germany), following the technical data sheet protocol, incubated at at 25 °C for 3 days (Biokar Diagnostics, 2022). The standard deviation of intralaboratory reproducibility (S_{IR}) was determined for the pour plate and spread plate techniques. The calculation of S_{IR} was performed utilising the following formula:

$$S_{IR} = \sqrt{\sum_{i=1}^{n} \frac{(y_{iA} - y_{iB})^2/2}{n}} \quad (1)$$

where S_{IR} is standard deviation of intralaboratory reproducibility. n is number of samples (i.e., 1 to 10). y_{iA} and y_{iB} is data transformed into log10 (CFU/g) from conditions a and b, respectively (ISO, 2021).

2.4. Determination of eBias in Food Item Verification

Determination of eBias was conducted using pour plate and spread plate techniques. The food items utilised in this study were cocoa powder, cocoa cake, and cocoa liquor. Inoculum suspensions were prepared by culturing *A. brasiliensis* WDCM 00053 and *S. cerevisiae* WDCM 00058 on DG18 Agar incubated at 25 °C for 5 days. Three levels of inoculum suspension were utilized, comprising a high level of approximately 4×10^4 CFU/mL, a medium level of approximately 4×10^3 CFU/mL, and a low level of approximately 4×10^2 CFU/mL Food items with different lots were used for each inoculum suspension level. A mixture of 10 g of the sample and 90 mL of BPW was spiked with 1 mL of inoculum suspension for each level, and the test was performed in duplicate. The inoculum suspension was then subjected to serial dilution (1:9).

The artificially contaminated food items and the inoculum suspension used to inoculate the food items were enumerated following the technical data sheet protocol, incubated at 25 °C for 3 days (Biokar Diagnostics, 2022). The analysis was carried out under replicability conditions by one technician at the same time (Nagur *et al*., 2023). The mean values were calculated as log CFU/g for each suspension level. These results were then converted to log cfu/test portion (10 g). The eBias was calculated as the absolute difference between the contaminated test portion and the inoculum suspension. If eBias is found to be less than 0.5 log10, the method can be used in the laboratory (ISO, 2021).

2.5. Comparative Study of Cocoa Product Samples

Six samples of each cocoa powder, cocoa cake, and cocoa liquor with natural contamination (30–300 CFU/g) were analysed for yeast and mold using a commercial rapid method with Symphony Agar, incubated for 3 days at 25 °C (Biokar Diagnostics, 2022), and for conventional method using DG18 agar medium, incubated for 5 days at 25 °C (ISO, 2008b; Tournas *et al*., 2001). The analysis was conducted using both pour plate (PP) and spread plate (SP) techniques.

2.6. Data Analysis

The S_{IR} and eBias calculations were performed using Microsoft Excel for ISO 16140-3:2021-Version 20210319 (Ohn *et al*., 2024). Comparative study of Symphony Agar and DG18 Agar was conducted using Microsoft Excel using independent sample *t*-test (Kim, 2015).

3. RESULT AND DISCUSSION

3.1. Colony Characteristics and Composition of Symphony Agar Media

The characteristics of colonies on Symphony Agar observed showed clear morphological differences as shown in Figure 1. The colonies, which were pink in colour, were identified as yeast due to their round shape, smooth surface, and absence of hyphae formation. Rose bengal is assimilated by the yeast which facilitates counting by colouring it pink. The molds present on this media manifest characteristic yellowish-white colonies that evolve in a filamentous or fibrous configuration. This difference in characteristics suggests that Symphony Agar can be used to distinguish between yeasts and molds based on their colony morphology (Biokar Diagnostics, 2022). In contrast, on DG18, both yeasts and molds appear whitish and can only be differentiated by the presence of spores in molds (Figure 2).

Figure 1. Yeast and mold colonies without cocoa product samples (spread plate) (left), yeast and mold colonies with cocoa samples (spread plate) (centre) and yeast and mold colonies with cocoa samples (pour plate) in Symphony Agar (right)

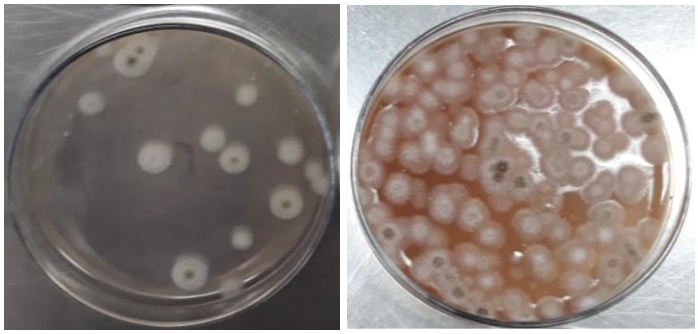

Figure 2. Yeast and mold colonies without cocoa product samples (spread plate) (left), yeast and mold colonies with cocoa samples (pour plate) in DG18 Agar (right)

Figure 3. Mold colonies in Symphony Agar (left), Mold colonies DG18 Agar (right)

Symphony Agar has been structured in a way that reduces the spread of thallus of mold such as *Mucor*, which allows colony counting after 54 hours of incubation. The media is also well adapted for counting mold spores (Biokar Diagnostics, 2022). This is demonstrated through the analysis of yeasts and molds in cocoa powder sample tested using Symphony Agar and DG18 Agar, as shown in Figure 3. Mold spore growth on Symphony Agar does not cover the entire surface of the petri dish, making colony counting easier compared to DG18 Agar. *Mucor* is a type of mold that can be found anywhere. *Mucor* species are fast-growing mold, which have highly developed mycelium and branched hyphae. Hyphae in *Mucor* are generally coenocytic (Rajguru *et al*., 2021).

3.2. Standard Deviation of Intralaboratory Reproducibility (SIR) in Implementation Verification

Implementation verification is a process that ensures a laboratory is correctly implementing a microbiological test method following a reference method and its validation results. The determination of the standard deviation of intralaboratory reproducibility (S_{IR}) is used in implementation verification (ISO, 2021). According to ISO 16140-3:2021, the selection of food items for implementation verification can be based on validation report of the method. The cocoa-based food matrices used in the validation of Symphony Agar were unsweetened cocoa powder and brown cocoa powder (ADRIA Food Expertise, 2022). The use of food items with natural contamination is preferable, if possible (ISO, 2021).

The food items tested in implementation verification were naturally contaminated cocoa powders with a concentration range of 10–300 CFU/g, representing typical levels of yeasts and molds found in the laboratory of user. The cocoa powder has a brown color with a balanced cocoa flavor, a fat content of 10–12%, and a pH ranging from 5.8 to 6.6. The verification process involves different technicians, instruments, tools, and media batches in one laboratory (Nagur *et al*., 2023). In addition to using different media batches, in this study the treatment of the media was also differentiated. One batch of media was taken directly from the autoclave in a fresh condition. The other batch of media was prepared a day in advance and allowed to solidify; it was later re-melted using a water bath at 85 °C until completely liquefied.

Based on the implementation verification results shown in Table 1 and Table 2, most of the yeast and mold colonies observed were higher than the expected number but still in the range of implementation verification design. This higher colony count of is attributed to the dynamic growth of yeasts and molds during storage, as the samples were produced in July 2024, while the implementation verification was conducted in December 2024. During this period, natural contaminants in the samples may have continued to grow, which could have affected the observed colony counts.

Table 1. Calculation of standard deviation of intralaboratory reproducibility (S_{IR}) (pour plate)

Sample Code	x_{iA}	x_{iB}	y_{iA}	y_{iB}	$\lvert y_{iA} - y_{iB} \rvert$	$\lvert y_{iA} - y_{iB} \rvert^2$
PB1	75	35	1.875	1.544	0.331	0.110
PB2	50	135	1.699	2.130	0.431	0.186
PB3	70	90	1.845	1.954	0.109	0.012
PB4	150	120	2.176	2.079	0.097	0.009
PB5	130	135	2.114	2.130	0.016	0.000
PB6	85	115	1.929	2.061	0.131	0.017
PB7	130	140	2.114	2.146	0.032	0.001
PB8	200	170	2.301	2.230	0.071	0.005
PB9	260	235	2.415	2.371	0.044	0.002
PB10	295	340	2.470	2.531	0.062	0.004
PB11	305	270	2.484	2.431	0.053	0.003
PB12	410	380	2.613	2.580	0.033	0.001
					Sum	0.35
					Sum/(2 × 12)	0.015
					S_{IR}	0.121

Table 2. Calculation of standard deviation of intralaboratory reproducibility (S_{IR}) (spread plate)

Sample Code	x_{iA}	x_{iB}	y_{iA}	y_{iB}	$\lvert y_{iA} - y_{iB} \rvert$	$\lvert y_{iA} - y_{iB} \rvert^2$
PB1	30	30	1.477	1.477	0	0
PB2	120	90	2.079	1.954	0.125	0.016
PB3	110	105	2.041	2.021	0.02	0
PB4	140	80	2.146	1.903	0.243	0.059
PB5	95	40	1.978	1.602	0.376	0.141
PB6	80	80	1.903	1.903	0	0
PB7	70	20	1.845	1.301	0.544	0.296
PB8	140	135	2.146	2.13	0.016	0
PB9	140	300	2.146	2.477	0.331	0.11
PB10	335	230	2.525	2.362	0.163	0.027
PB11	300	185	2.477	2.267	0.21	0.044
PB12	300	355	2.477	2.55	0.073	0.005
					Sum	0.698
					Sum/(2 × 12)	0.029
					S_{IR}	0.171

Note: x_{iA} = result by analyst A (CFU/g), x_{iB} = result by analyst B (CFU/g), y_{iA} = log10 (x_{iA}), y_{iB} = log10 (x_{iB}), |…..| = absolute value

The S_{IR} results obtained using the pour plate technique were 0.121 $\log_{10}$ CFU and 0.171 $\log_{10}$ CFU using the spread plate technique. These results were below 2 × the lowest average S_{IR} value for the sample matrices in the validation study (2 × 0.094) or 0.188 $\log_{10}$ CFU (ISO, 2021; ADRIA Food Expertise, 2022). The slightly higher S_{IR} value observed in the spread plate method may be attributed to analyst unfamiliarity, as conventional testing in the laboratory of user typically uses the pour plate method following BAM Chapter 18 with DG18 Agar. Nevertheless, both S_{IR} values remain below the acceptance threshold, indicating that the variability is not practically significant. This indicates that the implementation verification was successful and can be proceeded to food item verification. While some samples exhibited higher contamination levels than initially designed, the implementation verification remains valid according to the requirements of ISO 16140-3:2021. These findings are consistent with the study results of Melanda (2022), who conducted implementation verification using the spread plate technique for yeasts and molds on sliced tomato samples. An S_{IR} value of 0.11 $\log_{10}$ CFU was obtained, complying with the SR threshold specified in the Symphony Agar validation documentation.

3.3. Estimated Bias (eBias) in Food Item Verification

Determination of the estimated bias (eBias) is an important step in food item verification. This verification compares artificially contaminated samples and inoculum controls. The food items used include cocoa powder, cocoa cake, and cocoa liquor. Different food items may cause unique challenges to the testing method. For example, the high fat content of some foods can interfere with microbial detection (Putri *et al*., 2024). Cocoa liquor is a particularly challenging food item as it contains approximately 48% fat (BSN, 2009b). During preparation of cocoa liquor, samples and enrichment media need to be pre-warmed at 45º C following sample preparation based on SNI 3749:2009 (BSN, 2009b).

Verification was conducted using artificial contamination with a mixed culture of *Saccharomyces cerevisiae* and *Aspergillus brasiliensis* at high, medium, and low levels of contamination. *S. cerevisiae* was selected because it was used in the interlaboratory study for the Symphony Agar validation report and serves as a positive control in the the laboratory of user. Similarly, *A. brasiliensis* is a common mold found in proficiency test samples and is also used as a positive control in the the laboratory of user. Inoculum preparation is a critical step in the verification process. A preliminary test of the inoculum is necessary to determine the appropriate contamination level (Nagur *et al*., 2023).

The analysis included artificially contaminated samples, uncontaminated samples (negative control), and the mixed culture used for the contamination (positive control). The procedure followed the Symphony Agar method protocol (Biokar Diagnostics, 2022). The eBias values were determined according to ISO 16140-3, and the results showed that both the pour plate and spread plate methods (Table 3) met the acceptance limit of not exceeding 0.5 $\log_{10}$

(ISO, 2021). Based on the results of implementation and food item verification, the laboratory of user can use Symphony Agar for yeast and mold analysis in cocoa powder, cocoa cake, and cocoa liquor. However, it should be noted that Symphony Agar is a newly developed medium for yeast and mold analysis.

Table 3. eBias determination result of food item verification

Food Items	Microbial Plating Techniques	eBias Values		
		High	Medium	Low
Cocoa Powder	Pour Plate	0.230	0.209	0.081
Cocoa Cake	Pour Plate	0.085	0.207	0.166
Cocoa Liquor	Pour Plate	0.082	0.159	0.007
Cocoa Powder	Spread Plate	0.266	0.199	0.140
Cocoa Cake	Spread Plate	0.200	0.082	0.283
Cocoa Liquor	Spread Plate	0.258	0.026	0.221

To date, there are no documented studies in the scientific literature specifically evaluating the effectiveness or verification of Symphony Agar for cocoa products. Nevertheless, a study by Grandhay *et al*. (2024) suggests that Symphony Agar is a promising medium for the isolation of yeasts from water samples. The study found that Symphony Agar was comparable to DRBC medium in supporting the growth and identification of diverse yeast species, with both media enabling the isolation of 97 yeast species. In addition, Melanda (2022) has reported the verification of Symphony Agar for yeasts and molds analysis in various product categories, including composite foods, processed fruits and vegetables, dairy products, non-alcoholic beverages, and raw meat. Verification of food items for challenging food products, such as onion strips, pork loin, pomegranate juice, elderberry, and broad beans, showed results ≤ 0.5 $\log_{10}$, although some of them were close to the threshold value.

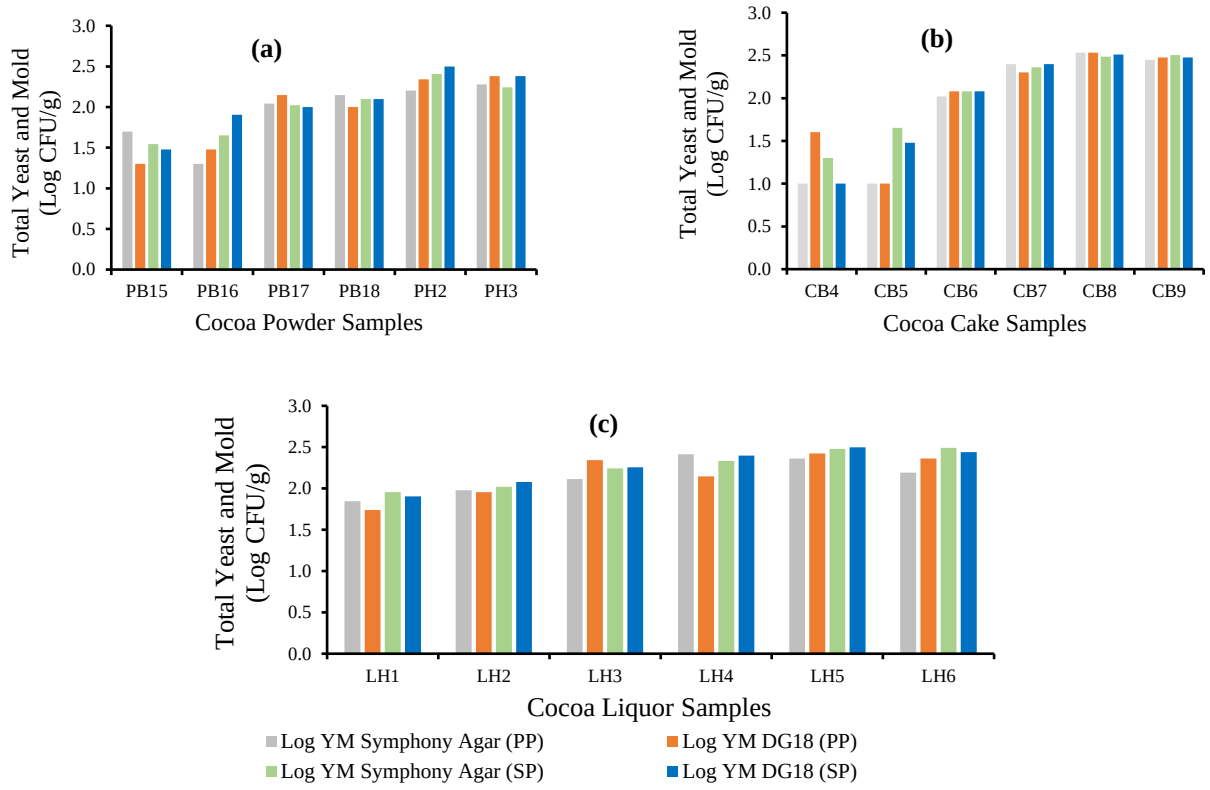

Figure 3. Comparative study of total yeast and mold (log CFU/g) based on food items (Symphony Agar and DG18 Agar) and Microbial Plating Techniques (PP and SP) for: (a) cocoa powder (A), (b) cocoa cake (B), and (c) cocoa liquor (C)

3.4. Comparative Study of Cocoa Product Samples

The comparative study between a commercial rapid method (Symphony Agar) and conventional methods (ISO and BAM) aimed to evaluate whether Symphony Agar could replace DG18 Agar. The results of the comparative study of the two media are presented as a graph in Figure 3 and as shown in Table 4. This evaluation was made by considering the difference in incubation time, Symphony Agar requiring only three days incubation while DG18 Agar needs five to seven days incubation. This comparative study used independent t-test statistical analysis. It is used when the two groups being compared are independent. The *t*-test was used on experimental subjects divided into two independent groups, with one group receiving treatment A and the other group receiving treatment B. A *p*-value greater than 0.05 indicates no difference between the two groups (Kim, 2015).

Table 4. Results of t-test (independent) Symphony Agar and DG18 Agar

Sample Matrices	Media test	Microbial Plating Techniques	Mean ± Standard Deviation (log CFU/g)	p-value
Cocoa Powder	Symphony Agar	Pour Plate	1.95 ± 0.38	0.987
	DG18 Agar	Pour Plate	1.94 ± 0.45	
Cocoa Cake	Symphony Agar	Pour Plate	1.90 ± 0,72	0.800
	DG18 Agar	Pour Plate	2.00 ± 0,59	
Cocoa Liquor	Symphony Agar	Pour Plate	2.15 ± 0,22	0.941
	DG18 Agar	Pour Plate	2.16 ± 0,27	
Cocoa Powder	Symphony Agar	Spread Plate	1.99 ± 0.34	0.754
	DG18 Agar	Spread Plate	2.06 ± 0.36	
Cocoa Cake	Symphony Agar	Spread Plate	2.06 ± 0,49	0.825
	DG18 Agar	Spread Plate	1.99 ± 0,62	
Cocoa Liquor	Symphony Agar	Spread Plate	2.25 ± 0.23	0.947
	DG18 Agar	Spread Plate	2.26 ± 0.23	

Comparative study using independent *t*-test presented in a way that could be easily understood by non-technical teams such as sales, production, and management. It is important to demonstrate that Symphony Agar has undergone a scientific evaluation aligned with ISO 16140-3:2021 standards. For context, Waluyo *et al*. (2021) used a paired t-test to compare the physical changes of cocoa beans before and after drying. Although it is a different form of the t-test, both approaches rely on *p*-values to determine the significance of the observed differences.

Independent t-tests were conducted for each cocoa product (cocoa powder, cocoa cake, and cocoa liquor), comparing Symphony Agar and DG18 Agar with 6 samples per group. No significant differences were observed across all products, media and plating techniques ($p > 0.05$), indicating comparable performance between the two media. According to Kim & Park (2019), t-tests are valid for small sample sizes when the assumptions of normality and homogeneity of variances are met, as indicated by *p*-values greater than 0.05.

4. CONCLUSION

The verification of Symphony Agar according to ISO 16140-3:2021 demonstrated that this method is suitable as an alternative media for analyzing yeasts and molds in cocoa powder, cocoa cake, and cocoa liquor. Comparative analysis with DG18 Agar using an independent t-test showed no significant difference between the two methods. This confirms that Symphony Agar can effectively replace DG18 Agar, with the added advantage of a shorter incubation time of three days. Furthermore, the inclusion of rose bengal in Symphony Agar enhances the distinction between yeast and mold colonies, facilitating more accurate microorganism identification.

ACKNOWLEDGEMENTS

The authors would like to thank all colleagues who supported this study. Due to the confidential nature of the project, specific details and affiliations are not disclosed.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
CFA	✓	✓			✓	✓	✓	✓	✓		✓		✓	✓
UH		✓		✓				✓		✓	✓	✓		
HDK	✓	✓		✓				✓		✓	✓	✓	✓	

C: Conceptualization
M: Methodology
So: Software
Va: Validation
Fo: Formal Analysis
I: Investigation
D: Data Curation
R: Resources
O: Writing - Original Draft
E: Writing - Review & Editing
Vi: Visualization
Su: Supervision
Fu: Funding Acquisition
P: Project Administration

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