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Fermented Local Food 'Dali Ni Horbo' from North Sumatra: Laboratory Formulation and Development of E-Flipbook for Biotechnology Subject

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ABSTRACT

Biotechnology is a fascinating and essential topic in high school education, requiring hands-on laboratory practices and the development of up-to-date teaching materials. Integrating conventional biotechnology with local wisdom can enhance its relevance and appeal. One traditional fermented product with educational potential is *Dali Ni Horbo* (DNH), a Batak dairy product produced through natural milk coagulation. Its fermentation characteristics make it suitable as a contextual medium for biotechnology learning, yet practical and innovative resources involving local products remain limited. This study aims to formulate DNH using cow's milk and goat's milk combined with natural coagulants, namely pineapple juice (*Ananas comosus* L.), papaya leaf juice (*Carica papaya* L.), and bilimbi juice (*Averrhoa bilimbi* L.), as well as to determine the optimal formulation based on organoleptic tests, evaluate the effect of different formulations on DNH quality, and assess the feasibility of DNH laboratory guidelines in the form of an E-Flipbook through expert validation and student feedback. The study employed an experimental research design integrated with the Research and Development (R&D) methodology. Data were analyzed using descriptive statistics, Two-Way ANOVA, and Welch tests. Organoleptic testing by five panelists indicated that the optimal formulation was the SSN10 treatment, consisting of 200 mL cow's milk and 10 mL pineapple juice, with a favorability score of 3.6%. Further analysis showed that the type of milk and natural coagulant used had no significant effect on the color, aroma, texture, or taste of DNH. The developed E-Flipbook demonstrated strong feasibility, receiving ratings of 95% ("Very Good") from material validators, 93.3% ("Very Good") from media validators, and 78% ("Good") from language validators. Student responses were "Very Positive," with an approval rating of 84.31%. These findings suggest that DNH can function as an effective learning material, providing practical fermentation applications while supporting the preservation of local cultural heritage.

***Key words**: fermentation, laboratory guide, local wisdom, natural coagulants, probiotics.*

ABSTRAK

Bioteknologi merupakan topik yang menarik dan esensial dalam pendidikan sekolah menengah, yang membutuhkan praktik laboratorium langsung dan pengembangan materi pengajaran terkini. Mengintegrasikan bioteknologi konvensional dengan kearifan lokal dapat lebih meningkatkan relevansi dan daya tariknya. Salah satu produk fermentasi tradisional yang memiliki potensi edukatif adalah *Dali Ni Horbo* (DNH), makanan khas masyarakat Batak yang secara tradisional dihasilkan melalui koagulasi susu dan berpotensi digunakan sebagai media pembelajaran bioteknologi. Sumber daya pembelajaran praktis dan inovatif di bidang ini masih terbatas. Penelitian ini bertujuan untuk memformulasikan DNH menggunakan susu sapi dan susu kambing yang dikombinasikan dengan koagulan alami, yaitu sari nanas (*Ananas comosus* L.), sari daun pepaya (*Carica papaya* L.), dan sari belimbing wuluh (*Averrhoa bilimbi* L.), serta menentukan formulasi optimal berdasarkan uji organoleptik, mengevaluasi pengaruh formulasi yang berbeda terhadap kualitas DNH, dan menilai kelayakan panduan

laboratorium DNH dalam bentuk E-Flipbook melalui validasi ahli dan umpan balik siswa. Penelitian ini menggunakan desain penelitian eksperimen yang dikombinasikan dengan metodologi Penelitian dan Pengembangan (R&D). Data dianalisis menggunakan statistik deskriptif, Two-Way ANOVA, dan uji Welch. Uji organoleptik, yang dilakukan oleh lima panelis, mengungkapkan bahwa formulasi optimal adalah perlakuan SSN10, yang terdiri dari 200 mL susu sapi dan 10 mL jus nanas, yang mencapai skor kesukaan sebesar 3,6%. Analisis lebih lanjut menunjukkan bahwa penggunaan dua jenis susu dan tiga koagulan alami tidak berpengaruh signifikan terhadap warna, aroma, tekstur, atau rasa DNH. E-Flipbook dievaluasi kelayakannya, menerima peringkat 95,00% ("Sangat Baik") dari validator materi, 93,3% ("Sangat Baik") dari validator media, dan 78% ("Baik") dari validator bahasa. Tanggapan siswa menunjukkan persepsi "Sangat Positif", dengan tingkat persetujuan 84,31%. Temuan ini menunjukkan bahwa DNH dapat berfungsi sebagai materi pengajaran yang efektif, memberikan siswa aplikasi praktis dari konsep fermentasi sambil melestarikan warisan budaya lokal. E-Flipbook yang dikembangkan lebih mendukung tujuan ini, menawarkan sumber daya yang mudah diakses dan menarik untuk pendidikan bioteknologi.

***Kata kunci**: fermentasi, panduan laboratorium, kearifan lokal, koagulan alami, probiotik.*

INTRODUCTION

Dali Ni Horbo (DNH) is a traditional fermented food of the Batak ethnic group from North Sumatra (Surono, 2015). Traditionally prepared from buffalo milk, DNH undergoes a series of fermentation processes. It is highly nutritious, containing 6.2% protein, 12.17% fat, and 12.15% carbohydrates (Diana, 2021). These nutritional components offer various health benefits, including preventing stunting in toddlers (Simbolon et al., 2024), enhancing the immune system (Hasibuan, 2023), and serving as a therapeutic agent for type-2 diabetes mellitus (Fachrial et al., 2023). Unlike many fermented foods, DNH can be produced using simple ingredients and a straightforward process. The production involves boiling buffalo milk for 10 minutes in a sterilized container, followed by the addition of natural coagulants such as pineapple juice or papaya leaf extract to induce coagulation and reduce the milk's fishy odor (Rakhman et al., 2024). The final product has a soft, elastic texture similar to tofu and a cheese-like flavor. However, unlike conventional cheese, DNH is made using natural coagulants instead of synthetic rennet.

Pardosi (2024) identified several natural coagulants, including pineapple (*Ananas comosus* L.), papaya (*Carica papaya* L.), lime (*Citrus aurantiifolia*), and starfruit (*Averrhoa bilimbi* L.), which contain citric acid and proteolytic enzymes that facilitate milk protein coagulation. These coagulants break down milk proteins, promoting curd formation. While previous studies have predominantly used buffalo milk for DNH production, Chetroiu and Marin (2021) reported a decline in buffalo milk availability, prompting the exploration of alternative milk sources such as cow's milk and goat's milk. Unlike earlier research, this study focused on utilizing cow's and goat's milk as alternative substrates for DNH fermentation. Although buffalo milk has a higher fat content (Boro et al., 2018), cow's and goat's milk possess similar nutritional profiles and are more readily available in both traditional and modern markets, making them more accessible for DNH production. The fermentation process for these alternative milks can be achieved using natural coagulants such as pineapple juice, papaya leaf extract, and starfruit juice. These coagulants act as acidifying agents, inducing milk casein coagulation at its isoelectric point (pH 4.6) (Pratiwi et al., 2019).

Effective biotechnology education should incorporate engaging learning activities to enhance students' cognitive, affective, and psychomotor domains. An effective e-module should guide students in problem-solving and provide interactive digital learning, which offers an advantage over traditional printed materials. The shift to digital books is driven by technological advancements, offering greater accessibility, durability, and engagement through interactive features. In the era of the Fourth Industrial Revolution, digital learning tools enhance student motivation and adaptability while preventing material deterioration over time. Hands-on practical activities enable students to actively participate in learning

and validate theoretical concepts through experimentation. However, preliminary observations at SMA Negeri 71 Jakarta (Indonesia) revealed limited exploration of practical activities and laboratory guidelines in biotechnology education. Biology teacher Melisa Epriani, S.Pd., emphasized the need for practical and accessible laboratory guidelines to support biotechnology instruction. This challenge can be addressed by integrating biotechnology concepts with local wisdom into educational media.

This study aims to formulate DNH using cow's milk and goat's milk combined with natural coagulants, including pineapple juice, papaya leaf juice, and bilimbi juice. Furthermore, the study seeks to determine the optimal formulation through organoleptic testing, examine the effects of different milk types and natural coagulants on the organoleptic quality of DNH, and develop a practical laboratory guideline for DNH production in the form of an interactive E-Flipbook. The study also aims to assess the feasibility of the E-Flipbook through expert validation and student feedback to ensure its effectiveness as a biotechnology learning resource. This interactive resource enhances student comprehension of fermentation-based biotechnology, incorporates local wisdom, and fosters 21st-century skills such as critical thinking and digital literacy.

MATERIALS AND METHODS

Dali ni Horbo Preparation

This study employed an experimental approach using a factorial completely randomized design (CRD). The independent variables included two types of milk, namely cow and goat milk, and three types of natural coagulants, including pineapple juice, papaya leaf extract, and starfruit juice, each applied at two concentrations (10 mL and 50 mL). The three natural coagulants used in this study have different contents and processes in helping milk coagulation. Pineapple extract produces bromelain enzyme to break down proteins into amino acids, papaya leaf extract produces papain enzyme that helps milk coagulation, and citric acid in starfruit extract can lower pH to accelerate coagulation.

The process of making pineapple and starfruit juice is carried out using fresh pineapple and starfruit that are grated separately (not mixed). The grated fruit is squeezed using a clean filter cloth to extract the juice. Natural papaya leaf coagulant is made by pounding fresh papaya leaves using a mortar and pestle. The pounded papaya leaves are squeezed with a filter cloth to extract the juice. Each treatment was carried out by heating 200 mL of milk (cow or goat), 0.5 g of salt, and coagulant according to the composition in an aluminum pan at a constant temperature for 30 minutes. A negative control sample was made without coagulant, while the positive control used a combination of 50 mL of pineapple juice and 10 mL of papaya leaf juice.

Data Analysis

The dependent variables comprised organoleptic characteristics, including color, aroma, texture, and taste, which were evaluated through sensory tests conducted by five trained panelists. The organoleptics data assessment were analyzed statistically using Two-Way ANOVA without interaction to determine the effects of different milk types and coagulants. If the assumptions of normality and homogeneity of variance were not met, Welch's test was applied as an alternative statistical approach.

The Construction Of An Electronic Flipbook (E-Flipbook)

The development of the E-Flipbook was based on the Research and Development (R&D) model proposed by Sugiyono (2019). The initial stage involved identifying potential challenges in biotechnology education and gathering relevant information on integrating local food fermentation into practical learning. This was followed by designing the E-Flipbook to create an interactive and structured digital learning module. The developed product underwent expert validation, with assessments from

material, media, and language validators to ensure scientific accuracy, usability, and linguistic clarity. Revisions were made based on validator feedback to improve the module's quality.

The revised E-Flipbook was tested on 72 high school students to assess its feasibility and effectiveness in supporting biotechnology learning. Research instruments, including validation sheets and questionnaires, were used to collect expert assessments and student responses. The collected data were analyzed using descriptive qualitative methods, with expert validation data interpreted based on qualitative statements and feasibility levels determined through percentage-based categorization. The feasibility of the E-Flipbook was classified into five categories: very feasible (81%–100%), feasible (61%–80%), fairly feasible (41%–60%), less feasible (21%–40%), and not feasible (<20%). The feasibility assessment involved observations, interviews, and questionnaire distribution among high school biology teachers and students. The final data interpretation determined the effectiveness of the E-Flipbook as an innovative biotechnology learning tool and its potential for enhancing digital-based interactive education.

RESULT AND DISCUSSION

Production of *Dali ni Horbo*

This study successfully produced DNH in a school laboratory setting using cow's and goat's milk combined with various natural coagulants, including pineapple juice, papaya leaf extracts, and bilimbi juice. The fermentation process was conducted spontaneously without the addition of a microbial starter, with coagulant concentrations of 10 mL and 50 mL per 200 mL of fresh milk. Coagulants play a critical role in the coagulation process by precipitating casein proteins, leading to the formation of solid curds (Nitu et al., 2021). Pineapple juice contains bromelain, an enzyme that facilitates protein hydrolysis and precipitation under acidic conditions (Komansilan et al., 2023). Similarly, papaya leaves contain papain, which breaks down complex milk proteins into simpler compounds (Tigist et al., 2016). Bilimbi, rich in citric acid, effectively lowers the pH of milk and accelerates protein coagulation (Budaraga et al., 2023). The resulting DNH product is illustrated in Figure 1.

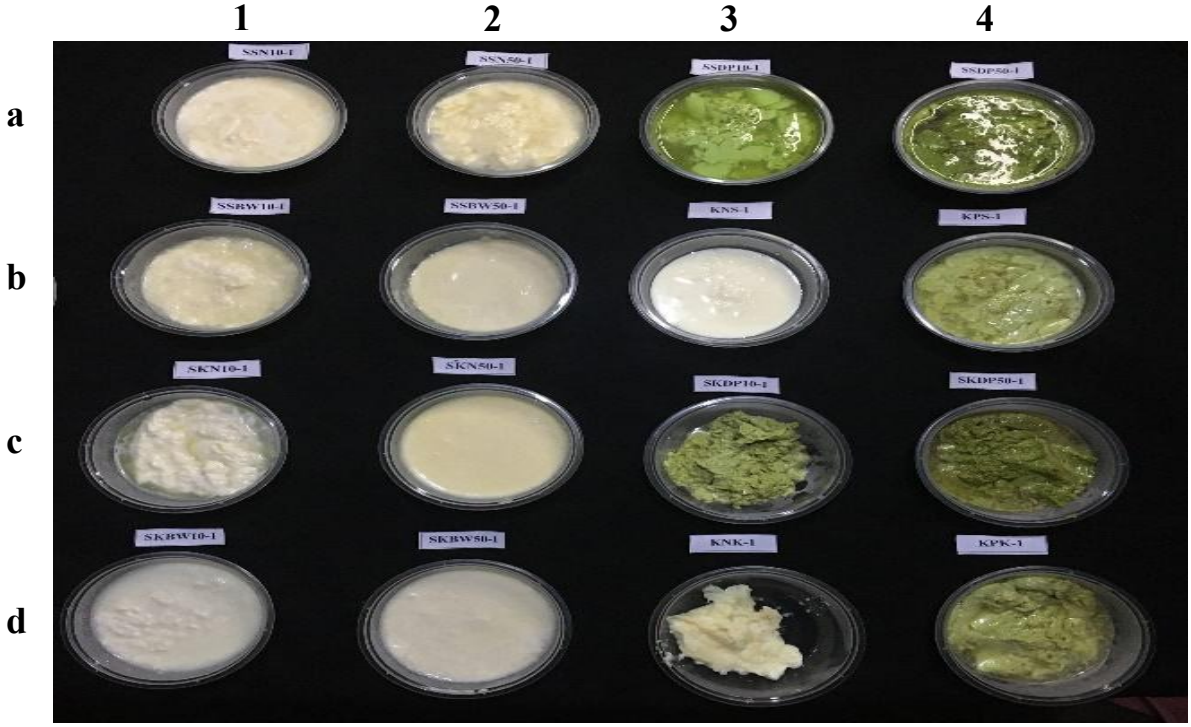

Figure 1. Formulation of 16 *Dali Ni Horbo* products prepared with different natural coagulants and milk types. **(a1)** SSN10-1 = cow's milk + 10 mL pineapple extract; **(a2)** SSN50-1 = cow's milk + 50 mL pineapple extract; **(a3)** SSDP10-1 = cow's milk + 10 mL papaya leaf extract; **(a4)** SSDP50-1 = cow's milk + 50 mL papaya leaf extract; **(b1)** SSBW10-1 = cow's milk + 10 mL bilimbi extract; **(b2)** SSBW50-1 = cow's milk + 50 mL bilimbi extract; **(b3)** KNS-1 = cow's milk control (salt); **(b4)** KPS-1 = cow's milk + pineapple + papaya leaf extract; **(c1)** SKN10-1 = goat's milk + 10 mL pineapple extract; **(c2)** SKN50-1 = goat's milk + 50 mL pineapple extract; **(c3)** SKDP10-1= goat's milk + 10 mL papaya leaf extract; **(c4)** SKDP50-1 = goat's milk + 50 mL papaya leaf extract; **(d1)** SKBW10-1 = goat's milk + 10 mL bilimbi extract; **(d2)** SKBW50- 1= goat's milk + 50 mL bilimbi extract; **(d3)** KNK-1 = goat's milk control (salt); **(d4)** KPK-1 = goat's milk + pineapple + papaya leaf extract.

Spontaneous fermentation relies on naturally occurring microorganisms in milk, primarily lactic acid bacteria (LAB), as described by Luiz et al. (2015). Substrates from the coagulants, such as pectin in bilimbi (Maulida and Maghfiroh 2023) and the acidic conditions provided by pineapple (Rizal et al., 2016), support the growth of LAB, including Lacticaseibacillus casei. The heating process at 75°C for 30 minutes does not entirely eliminate microorganisms, allowing LAB activity to persist and enhance the product's acidity. The production of DNH reflects the application of indigenous knowledge in food processing. Cow's and goat's milk were selected as the base ingredients, and natural coagulants were used to optimize the product's color, aroma, texture, and taste.

The preparation process began with cleaning fresh pineapple, bilimbi, and papaya leaves. Pineapple and bilimbi were grated and filtered using a cloth to extract their juices, while papaya leaves were pounded with a mortar and pestle before filtration. Coagulant concentrations were precisely measured at 10 mL and 50 mL. The coagulation process was initiated by mixing 200 mL of cow's or goat's milk with 0.5 grams of salt, followed by the addition of the designated coagulant. The mixture was stirred evenly and then cooked over a stove for 30 minutes without further agitation to ensure optimal texture formation. The final curdled product was transferred to a plate, resulting in the traditional DNH.

Sensory Evaluation

An organoleptic test was conducted to evaluate the quality of DNH using sensory parameters such as color, aroma, texture, and taste. The assessment was performed by a panel of five chefs from Kadena Glamping Dive Resort, Banten, who specialize in food quality evaluation. A total of 48 DNH samples were assessed across 16 treatment variations, involving two milk sources (cow and goat) and three natural coagulants (pineapple, bilimbi, and papaya leaves) at different concentrations (Figure 2). The highest preference score (3.6%) was recorded for the SSN10 treatment, which combined cow's milk with 10 mL of pineapple juice, yielding an optimal texture, taste, and aroma as perceived by the panelists.

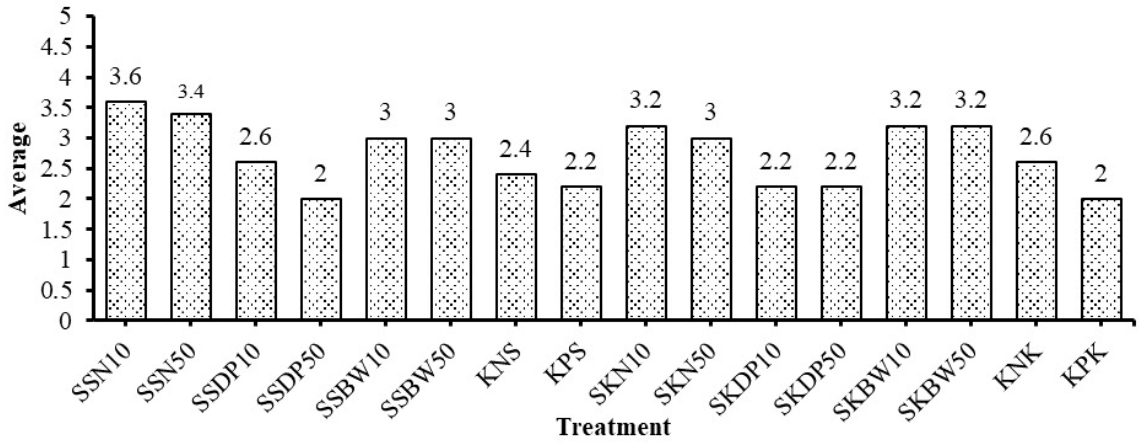

Figure 2. Average organoleptic scores based on sensory evaluations of *Dali Ni Horbo* by panelists

The visual assessment revealed that DNH prepared with cow's milk and pineapple juice (SSN), as depicted in Figure 3, followed by goat's milk and pineapple juice (SKN), cow's milk and bilimbi juice (SSBW), and goat's milk and bilimbi juice (SKBW), exhibited a yellowish-white color. Conversely, samples prepared with papaya leaf extract (SSDP and SKDP) displayed a light to dark green hue. Statistical analysis using Welch's test yielded a significance value of 0.580, indicating no significant effect of milk type or coagulant on product color. This finding aligns with Pardosi (2024), who reported that coagulant concentration did not affect product coloration. The yellowish-white color results from the interaction between milk and fruit juices, whereas papaya leaf extract imparts a green hue (Zulqarnain et al., 2021).

Figure 3. Formulated *Dali Ni Horbo* products with the most preferred score by panelists.

Aroma is a critical parameter in fermented food evaluation. Welch's test produced a significance value of 0.143, suggesting that neither milk type nor coagulant had a significant effect on the aroma of DNH. Treatments SSN10, SSN50, SKN10, SKN50, SSBW10, SSBW50, KPS, and KPK exhibited a strong local cheese-like aroma, consistent with Zhao et al. (2019), who found that proteolytic enzymes in natural coagulants contribute to cheese aroma development. However, SSDP10, SSDP50, SKDP10, and SKDP50 had a weaker aroma due to the distinct grassy smell of papaya leaves (Saloko et al., 2020).

Meanwhile, KNS and KNK treatments retained only the natural milk aroma, without the characteristic cheese scent.

The soft and chewy texture of DNH resulted from the interaction of milk proteins with coagulant enzymes. Statistical analysis indicated a significance value of 0.192, confirming that texture was not significantly affected by milk type or coagulant variation. The uniform texture across all treatments was attributed to the homogeneous protein content in both cow's and goat's milk, coupled with the consistent coagulation effects of the coagulants. Similar findings were reported by Rezaldi and Alhafizi (2023), who described DNH's texture as comparable to tofu.

Taste is a crucial factor in sensory evaluation. Statistical analysis yielded a significance value of 0.75, demonstrating no significant impact of milk type or coagulant variation on taste. The SSN10 and SKN10 treatments exhibited a moderately acidic taste due to lactic acid formation from pineapple juice, whereas SSN50 and SKN50 had a more pronounced bitter taste. According to Elvina et al. (2019), bromelain in pineapple can reduce sweetness and intensify bitterness. Additionally, Zubairi et al. (2023) noted that papaya leaves contain alkaloids contributing to bitterness. Treatments SSBW10, SSBW50, SKBW10, and SKBW50 had a dominant sour taste, while KPS and KPK showed a combined sour and bitter profile. In contrast, KNS and KNK were characterized by a savory taste, as they lacked natural coagulants.

Development of *Dali Ni Horbo* Production in Biotechnology Education

The development of an E-Flipbook as a learning guide for biotechnology practices, integrating DNH formulation, was designed for Grade 10 biotechnology subjects. The development process followed Sugiyono's (2019) framework, consisting of six stages: identifying potential issues, gathering information, designing the product, validating the design, revising the design, and conducting product trials. The E-Flipbook underwent three validation stages: content validation, media validation, and language validation.

Initial observations at SMA Negeri 71 Jakarta revealed challenges in biotechnology education, particularly in conducting practical activities. Interviews with biology teacher Melisa Epriani, S.Pd., indicated that while students demonstrated a strong interest in biotechnology, practical sessions were limited to only twice per semester due to insufficient laboratory resources. Additionally, the existing printed teaching materials were outdated and misaligned with current technological advancements. This study addressed these challenges by developing a practical reference that integrates indigenous knowledge into biotechnology education.

The E-Flipbook was designed using Canva, featuring a color scheme of pastel blue (#4793AF), pastel red (#FC7759), and pastel yellow (#FFC470) to reflect Batak cultural elements. The flipbook incorporates an animated "Batak Cheese" character adorned with ulos and sortali, traditional Batak textiles, and headbands symbolizing prosperity (Prayoga and Situmorang, 2023). The final product was converted into a flipbook format using Heyzine, providing an accessible and interactive digital learning resource.

Product Validation

The E-Flipbook underwent a validation process by three expert validators, covering three aspects: content, media, and language. The content of the E-Flipbook was assessed using a Likert scale and received a feasibility score of 95%, indicating it is "Highly Feasible" (Figure 4). The validation results confirmed that the content aligns with learning outcomes, instructional objectives, and clear, logical practical work procedures.

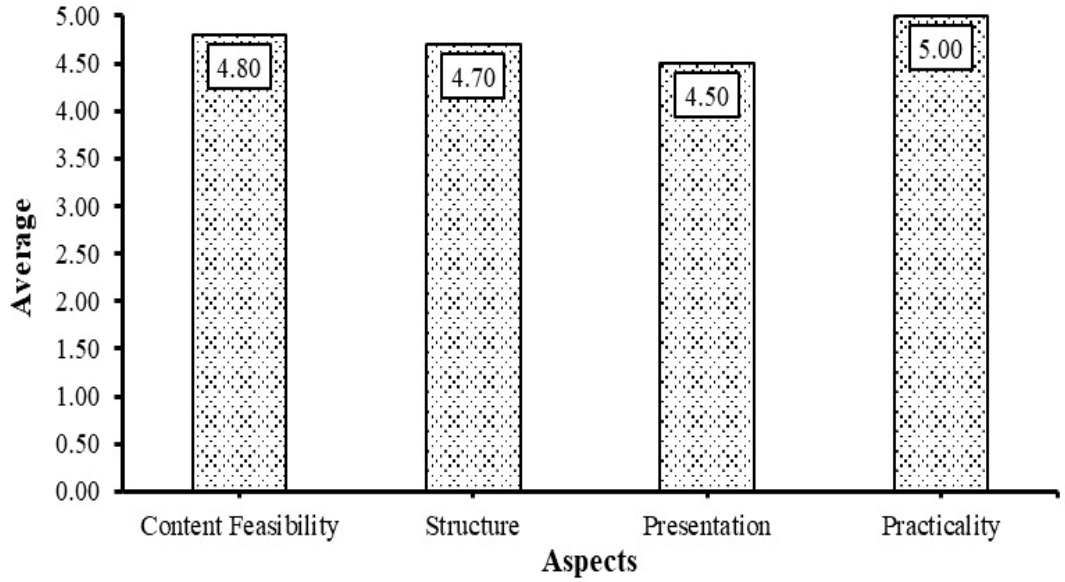

Figure 4. Validation score in terms of subject material presented in the e-flipbook.

Certain aspects, such as material accuracy, observation tables, and quiz questions, received a score of 80%, with suggestions for additional content and questions. This finding is consistent with the study by Hendarto et al. (2019), which also obtained a "Highly Feasible" rating for content validation. Media validation focused on the cover design and internal layout. The cover design received a perfect score (5/5), indicating it is highly attractive and relevant to the theme of local wisdom. The internal design scored 4.5/5, demonstrating that the layout and graphical elements effectively support the learning process (Figure 5). This result aligns with Rosniadi et al. (2022), who also obtained a "Highly Feasible" rating

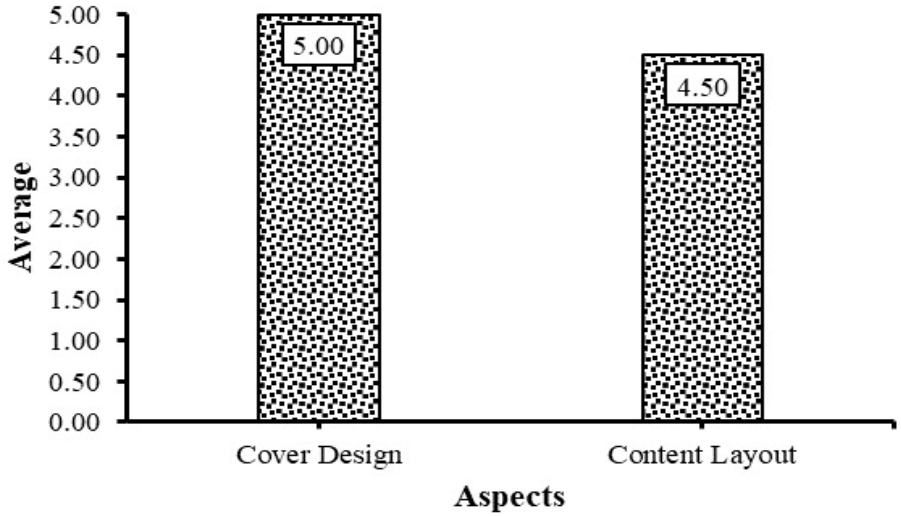

Figure 5. Validation score in terms of appearance and design in the e-flipbook.

Language validation assessed the clarity, conciseness, and appropriateness of the E-flipbook for students' cognitive development. The E-Flipbook achieved a feasibility score of 78%, with most

linguistic aspects scoring above 80% (Figure 6). However, feedback highlighted the need for improvements in spelling (in accordance with the Enhanced Indonesian Spelling System, EYD) and sentence effectiveness to enhance reading fluency. These validation results align with the study by Septiani et al. (2024), which categorized the material as "Feasible."

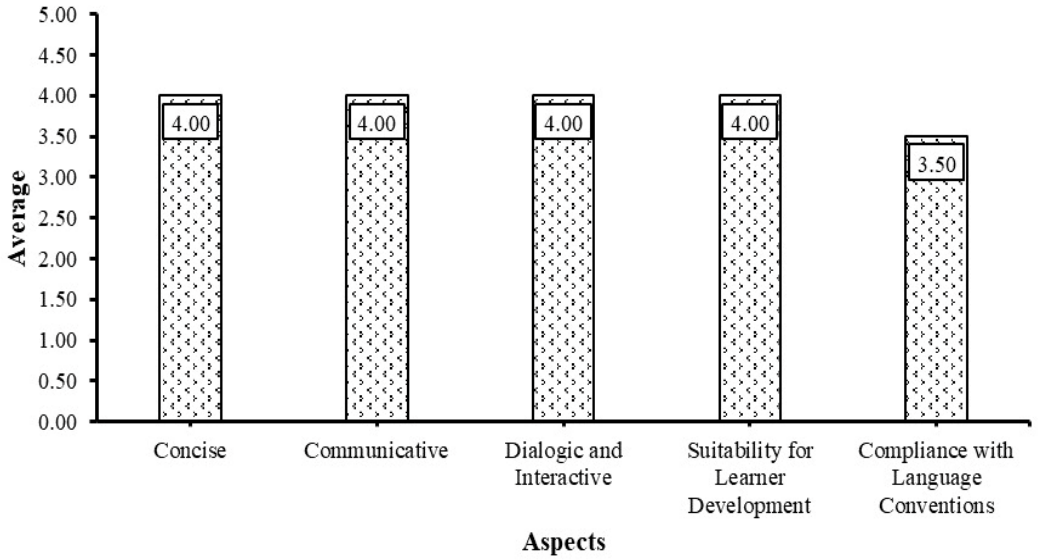

Figure 6. Validation score in terms of language coverage (*Bahasa*) in the e-flipbook.

Revisions were made to the E-Flipbook based on expert feedback to refine its content. The revised version is presented in Table 1. Updates included adding the logos on the cover page. The table of contents was adjusted to improve the formatting of chapter and subchapter headings. Additionally, the term Kegiatan Pembelajaran (Learning Activities) was abbreviated as "KP."

Table 1. Technical revisions on the design of the E-flipbook containing the protocol of *Dali Ni Horbo* production for laboratory practice of biotechnology

Section	Initial Material(s)	Revised Material(s)	Comments and Revisions
Daftar isi (table of contents)			The addition of numerical indicators for chapters and subchapters in the table of contents for better structural clarity.

Capaian pembelajaran (learning outcomes)			Adjustments to the learning outcomes in accordance with the latest *Merdeka Curriculum* standards for the 2023/2024 academic year.
Kegiatan pembelajaran 1 (learning activity 1)			The introduction of the abbreviation KP (*Kegiatan Pembelajaran*) at the top left corner of each section in the E-Flipbook to improve readability and navigation.
Isi (contents)			Modification of the general concept of fermented food products to a focus on traditional Indonesian fermented food products.
			The inclusion of relevant illustrations for each instructional explanation to enhance student engagement and comprehension.
			The addition of interesting facts in each learning activity to improve the E-Flipbook more engaging and enjoyable.

The content validator recommended aligning the learning outcomes with the latest Merdeka Curriculum (2023/2024 academic year), and revisions were made to reflect these updated objectives. Furthermore, the content validator suggested modifying to KP 2 on Fermentation and Its Applications by incorporating examples of traditional Indonesian fermented products that are widely known to the public and used as research materials, such as Dadih (Putri et al., 2025), Dangke (Batrisia et al., 2025), and Naniura (Sibarani et al., 2024), to enhance students' familiarity with local fermentation practices.

The media validator provided feedback on the addition of relevant images and "interesting facts" in each KP to enhance students' comprehension of biotechnology topics. These revisions aim to make the E-Flipbook more engaging and visually appealing. The language validator recommended further refinements in spelling (EYD compliance), word choice (based on the Kamus Besar Bahasa Indonesia, KBBI), and sentence structure to ensure linguistic effectiveness. Student responses to the E-Flipbook were categorized as "Highly Positive" across four aspects: interest (4.24%), content (4.19%), language (4.21%), and integration of local wisdom (4.26%). These results indicated that the E-Flipbook successfully captured students' interest, delivered comprehensive and relevant content, employed accessible language, and effectively incorporated local wisdom into practical learning.

CONCLUSION

The optimal formulation for DNH was achieved in the SSN10 treatment, which received a preference score of 3.6%. This formulation consisted of 200 mL cow's milk, 10 mL pineapple juice, and 0.5 g salt, heated for 30 minutes. The organoleptic evaluation revealed that variations in the use of cow's and goat's milk, as well as natural coagulants (pineapple juice, papaya leaves, and star fruit juice), did not significantly affect the product’s color, aroma, texture, or taste. The formulation of fermented DNH has the potential to serve as a biotechnological practical guide in the form of an E-flipbook, which was rated as "Highly Feasible" by the content validator (95%) and media validator (93.3%), and "Feasible" by the language validator (78%). Additionally, it received a highly positive response from Grade X students at SMA Negeri 71 Jakarta, with an approval score of 84.31%.

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