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Brief Report

Baseline ABTS Radical Scavenging Capacities of Common Microbial Media and the Impact of Sterilization Methods

Ji-Hyun Kim [1], Chang-Gu Hyun [1,*]

[1] Jeju National University, Jeju, South Korea

[*] ***Corresponding Author:*** Jeju National University, Jeju, South Korea. Email: cghyun@gmail.com

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Abstract

Background: Microbial culture media contain complex nutrient components such as yeast extract, peptides, and carbohydrates, which exhibit inherent redox properties. These baseline antioxidant activities can interfere with assays assessing the radical scavenging ability of microbial metabolites, particularly when media-derived effects are not properly controlled.

Objectives: To quantitatively evaluate the intrinsic 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacities of five commonly used microbial media and to assess how media composition and sterilization methods contribute to assay interference.

Methods: Five commercial liquid media — Tryptic Soy Broth (TSB), de Man-Rogosa-Sharpe (MRS) broth, Luria-Bertani (LB) broth (Miller), Nutrient Broth (NB), and Yeast extract-Malt extract (YM) broth — were tested for ABTS radical scavenging activity. Media were sterilized using either autoclaving (121°C, 20 min) or 0.22 µm syringe filtration. Radical scavenging (%) was calculated from absorbance at 734 nm after 15 min incubation with $ABTS^{+}$ working solution. IC_{50} values were determined by four-parameter logistic regression.

Results: Baseline antioxidant capacity varied significantly depending on media composition, with the following potency order: MRS > TSB ≈ LB > YM > NB. Autoclaving further enhanced radical scavenging activity, particularly in carbohydrate-rich media such as MRS, likely due to the formation of melanoidin-like antioxidant compounds via heat-induced chemical reactions such as the Maillard reaction.

Conclusions: Media-derived radical scavenging effects have substantial potential to confound antioxidant assays. Consideration of both media selection and sterilization method is essential to avoid misinterpretation of microbial functionality. This study provides practical guidance for minimizing assay interference and establishing reliable conditions in antioxidant-related microbiological research.

Keywords: Microbial Media, ABTS Assay, Sterilization, Autoclaving, Maillard Reaction, Radical Scavenging Interference

1. Background

Microbial liquid media serve as essential nutrient reservoirs supporting microbial growth and the production of primary and secondary metabolites. However, these media are composed of complex raw materials, including yeast extract, peptides, and carbohydrates, all of which possess intrinsic redox properties. Consequently, baseline antioxidant activity originating from culture media can significantly influence the assessment of radical scavenging effects attributed to microbial metabolites (1, 2). Recent studies on microbial systems, including lactic acid bacteria, endophytic fungi, and bacterial fermentation, have further highlighted the potential for media-derived interference in ABTS and DPPH assays, emphasizing the need for rigorous uninoculated controls and baseline corrections to accurately distinguish microbial contributions from inherent media effects (3-6).

Despite the widespread use of culture supernatants in antioxidant assays, the contribution of media itself is frequently underestimated. When samples are diluted

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with fresh media or when culture-free controls are insufficiently incorporated, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging signals may be falsely attributed to microbial metabolites rather than media-derived reducing components. This issue becomes particularly problematic in comparative studies using different media formulations, and batch-to-batch variability of raw materials (e.g., yeast extract) further jeopardizes reproducibility and cross-laboratory comparability. Therefore, failure to correct for intrinsic media antioxidant activity can result in misleading conclusions regarding microbial metabolic potential and functional efficacy (7-9).

Furthermore, physicochemical transformations that occur during sterilization processes complicate antioxidant evaluations. High-temperature treatment such as autoclaving can initiate Maillard-type reactions between reducing sugars and amino groups, generating melanoidin-like compounds with radical scavenging capabilities and causing visible browning of media. Thus, both the inherent antioxidant contribution from native media constituents and heat-induced chemical modifications must be considered to avoid experimental artifacts and ensure accurate interpretation (10-12). Among various analytical techniques, the $ABTS^{+}$ radical scavenging assay is widely employed due to its compatibility with both hydrophilic and lipophilic antioxidants (13-15). Nevertheless, systematic characterization of baseline radical scavenging derived solely from microbial media remains limited, and the influence of different sterilization methods — particularly autoclaving versus membrane filtration — on media redox behavior has not been rigorously compared.

To address these knowledge gaps, this study quantitatively evaluated the intrinsic $ABTS^{+}$ radical scavenging capacities of five commonly used microbial media — Tryptic Soy Broth (TSB), de Man-Rogosa-Sharpe (MRS) broth, Luria-Bertani (LB) broth (Miller), Nutrient Broth (NB), and Yeast extract-Malt extract (YM) broth — and investigated how media composition and sterilization method affect baseline antioxidant characteristics. The findings provide essential guidance for selecting suitable media and proper sterilization conditions to minimize confounding antioxidant interference in microbiological research. In this study, the term 'microbiological assays affected by media-derived radical scavenging activity' specifically refers to antioxidant evaluations performed on culture supernatants or fermentation broths to assess the functional properties of microbial metabolites, rather than to classical bacteriological tests based on minimal media for growth or viability assessment. Such antioxidant-related microbiological assays commonly employ complex, nutrient-rich media, in which intrinsic redox-active components and sterilization-induced chemical modifications can substantially influence ABTS or DPPH assay outcomes if not rigorously controlled.

2. Objectives

This study aimed to (1) determine the baseline $ABTS^{+}$ radical scavenging capacities of five widely used microbial liquid media — TSB, MRS, LB, NB, and YM broths; (2) evaluate how intrinsic media composition contributes to the observed antioxidant properties; and (3) compare the impact of two common sterilization methods, autoclaving and membrane filtration, on the redox behavior of these media. By establishing these parameters, we sought to provide essential methodological guidance for minimizing media-derived interference and ensuring accurate interpretation in antioxidant-related microbiological research.

3. Methods

3.1. Culture Media and Reagents

Five commercially available liquid culture media — TSB (Difco™, 4236681), LB Broth (Miller; Difco™, 244620), NB (Difco™, 234000), and YM Broth (Difco™, 271120) — were purchased from BD Biosciences (Sparks, MD, USA). MRS Broth (MBcell™, MB-M1025) was obtained from MBcell (Seoul, Republic of Korea). All media were prepared in distilled water according to the manufacturers' recommended concentrations. The compositional characteristics of the media are summarized as follows: TSB contains pancreatic digest of casein, papaic digest of soybean meal, and dextrose; MRS is enriched with glucose, proteose peptone, meat extract, and yeast extract; LB consists primarily of tryptone, yeast extract, and sodium chloride; NB contains peptone and beef extract; and YM includes yeast extract, malt extract, peptone, and glucose. The

final pH values of the prepared media were as follows: TSB, 7.3 ± 0.2; MRS, 6.5 ± 0.2; LB, 7.0 ± 0.2; NB, 6.8 ± 0.2; and YM, 6.2 ± 0.2.

3.2. Sterilization Procedures

To evaluate the impact of sterilization on intrinsic antioxidant activity, each medium was sterilized using two different methods. For heat sterilization, prepared media were autoclaved at 121°C for 20 min under standard high-pressure steam conditions. In parallel, aliquots of each medium were sterilized by membrane filtration using a 0.22 µm hydrophilic cellulose acetate syringe filter (Nalgene™, Thermo Fisher Scientific, Rochester, NY, USA) to avoid heat-induced chemical modifications. Following sterilization, all media were cooled to room temperature prior to analysis to minimize temperature-dependent effects on $ABTS^+$ radical stability.

3.3. Preparation of $ABTS^+$ Radical Solution

The $ABTS^+$ radical cation was generated by mixing 7.0 mM ABTS (Sigma-Aldrich, St. Louis, MO, USA) with 2.45 mM potassium persulfate at a 1:1 (v/v) ratio. The mixture was incubated at 4°C in the dark for 16 h to allow complete radical formation. Before use, the $ABTS^+$ stock solution was diluted with 95% ethanol to obtain a working solution with an absorbance of 0.760 - 0.800 at 734 nm, measured using a microplate reader (BioTek Instruments, Winooski, VT, USA), according to previously established protocols (14-16).

3.4. $ABTS^+$ Radical Scavenging Assay

The $ABTS^+$ radical scavenging activity of each sterilized medium was evaluated using a microplate-based assay following established procedures (13-16). Briefly, 20 µL of medium sample was mixed with 180 µL of $ABTS^+$ working solution in a 96-well microplate, yielding a total reaction volume of 200 µL. Distilled water served as the negative control, while ascorbic acid (1 mg/mL in distilled water) was used as the positive control. The reaction mixtures were incubated for 15 min at room temperature in the dark, after which absorbance was measured at 734 nm. All measurements were performed in triplicate (n = 3).

3.5. Calculation of Radical Scavenging Activity

Baseline absorbance values originating from reagent blanks and medium-only blanks were subtracted prior to analysis. The $ABTS^+$ radical scavenging activity (%) was calculated using the following equation:

$$\text{Scavenging activity } (\%) = \left[1 - \frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{control}} - A_{\text{control blank}}}\right] \times 100$$

Where A_{sample}, $A_{\text{sample blank}}$, A_{control}, and $A_{\text{control blank}}$ represent absorbance values of the sample, sample blank, control, and control blank, respectively.

3.6. IC_{50} Determination and Statistical Analysis

All $ABTS^+$ radical scavenging experiments were performed in triplicate, and each experiment was independently repeated three times (n = 3). Results are expressed as mean ± standard deviation (SD). Dose-response curves were generated based on $ABTS^+$ radical scavenging percentages measured at increasing concentrations of each culture medium. IC_{50} values, defined as the concentration (% v/v) required to scavenge 50% of $ABTS^+$ radicals, were calculated using non-linear regression analysis fitted to a four-parameter logistic (4PL) model. All non-linear regression analyses were performed using RStudio (version 2024.04.1, Posit Software, Boston, MA, USA), an integrated development environment for the R statistical computing platform. Curve fitting and IC_{50} estimation were conducted using appropriate R packages for dose-response analysis. Given that the primary objective of this study was to characterize baseline antioxidant capacities and derive IC_{50} values rather than to test hypothesis-driven differences between groups, formal inferential statistical comparisons (e.g., ANOVA) were not applied.

4. Results and Discussion

4.1. Baseline $ABTS^+$ Radical Scavenging Values of Common Microbial Media

The $ABTS^+$ radical scavenging assay is widely employed for evaluating antioxidant potential due to its sensitivity and applicability to both hydrophilic and lipophilic antioxidants (16-18). However, when applied to microbial culture systems, baseline antioxidant contributions originating from the culture media itself are often overlooked. To clarify this issue, we first

assessed the intrinsic $ABTS^+$ radical scavenging activities of five commonly used microbial liquid media — TSB, MRS, LB, NB, and YM — in the absence of microbial inoculation.

All tested media exhibited measurable $ABTS^+$ radical scavenging activity, clearly demonstrating that culture broths themselves can act as non-biological sources of antioxidant signals. The magnitude of baseline activity varied markedly depending on media composition (Figure 1). Among the tested formulations, MRS broth showed the strongest intrinsic scavenging capacity, followed by TSB and LB, whereas NB and YM exhibited relatively weaker yet detectable activities. This trend was further supported by IC_{50} analysis (Table 1), in which MRS displayed the lowest IC_{50} value, indicating the highest inherent antioxidant potency. These differences closely correlated with nutrient complexity. Media enriched with yeast extract, carbohydrates, and complex nitrogen sources — such as MRS and TSB — are known to contain reducing compounds capable of directly quenching $ABTS^+$ radicals. In contrast, simpler formulations dominated by peptone hydrolysates, such as NB, showed substantially weaker baseline redox activity. Collectively, these findings confirm that intrinsic antioxidant activity is a fundamental property of microbial media and must be considered when interpreting antioxidant assays using culture supernatants.

Table 1. Baseline $ABTS^+$ Radical Scavenging Activity of Five Commonly Used Culture Media Under Different Sterilization Conditions [a]

Medium	Autoclave, IC_{50} (%)	Filteration, IC_{50} (%)
Tryptic soy broth	0.302	0.288
MRS broth	0.169	0.266
LB broth	0.323	0.331
Nutrient broth	1.660	1.585
YM broth	1.288	1.349

[a] The half-maximal inhibitory concentration (IC_{50}) values (%v/v) were determined for autoclaved and filter-sterilized media. Lower IC_{50} values indicate stronger intrinsic antioxidant capacity.

4.2. Effect of Sterilization Method on Baseline Antioxidant Activity

Beyond inherent media composition, sterilization method exerted a significant influence on baseline $ABTS^+$ radical scavenging behavior (19, 20). To evaluate this effect, all media were sterilized either by autoclaving (121°C, 20 min) or by 0.22 µm membrane filtration, and their antioxidant activities were compared under identical assay conditions. Autoclaving markedly enhanced $ABTS^+$ radical scavenging activity in carbohydrate-rich media, particularly MRS and YM (Figure 1). This increase was accompanied by visible browning of the media following heat treatment, suggesting the formation of melanoidin-like compounds. Such compounds are well-documented products of Maillard-type reactions between reducing sugars and amino groups and possess strong radical scavenging properties. Given the high glucose and amino acid content of MRS, this medium provided an especially favorable chemical environment for thermally induced antioxidant formation, resulting in the most pronounced sterilization-dependent increase in activity (10-12). In contrast, LB and NB, which contain relatively low levels of fermentable carbohydrates, exhibited minimal differences between autoclaved and filter-sterilized conditions. These observations indicate that the impact of sterilization on baseline antioxidant activity is highly substrate-dependent and that heat sterilization is not chemically neutral with respect to redox behavior. Therefore, inconsistent sterilization methods can introduce hidden variability into antioxidant assays and compromise reproducibility.

4.3. Interpretation and Implications for Microbial Antioxidant Assays

The combined effects of media composition and sterilization method have important implications for the interpretation of microbial antioxidant studies. Without proper correction using identically prepared uninoculated controls, media-derived scavenging signals may be mistakenly attributed to microbial metabolites, leading to systematic overestimation of microbial antioxidant potential. This issue is particularly critical in comparative studies employing different culture media or sterilization protocols, where baseline interference may vary substantially. Moreover, the observed differences between autoclaving and membrane filtration highlight the necessity of transparent methodological reporting.

Variations in sterilization conditions across laboratories or experiments can generate chemically distinct media with divergent redox properties, thereby

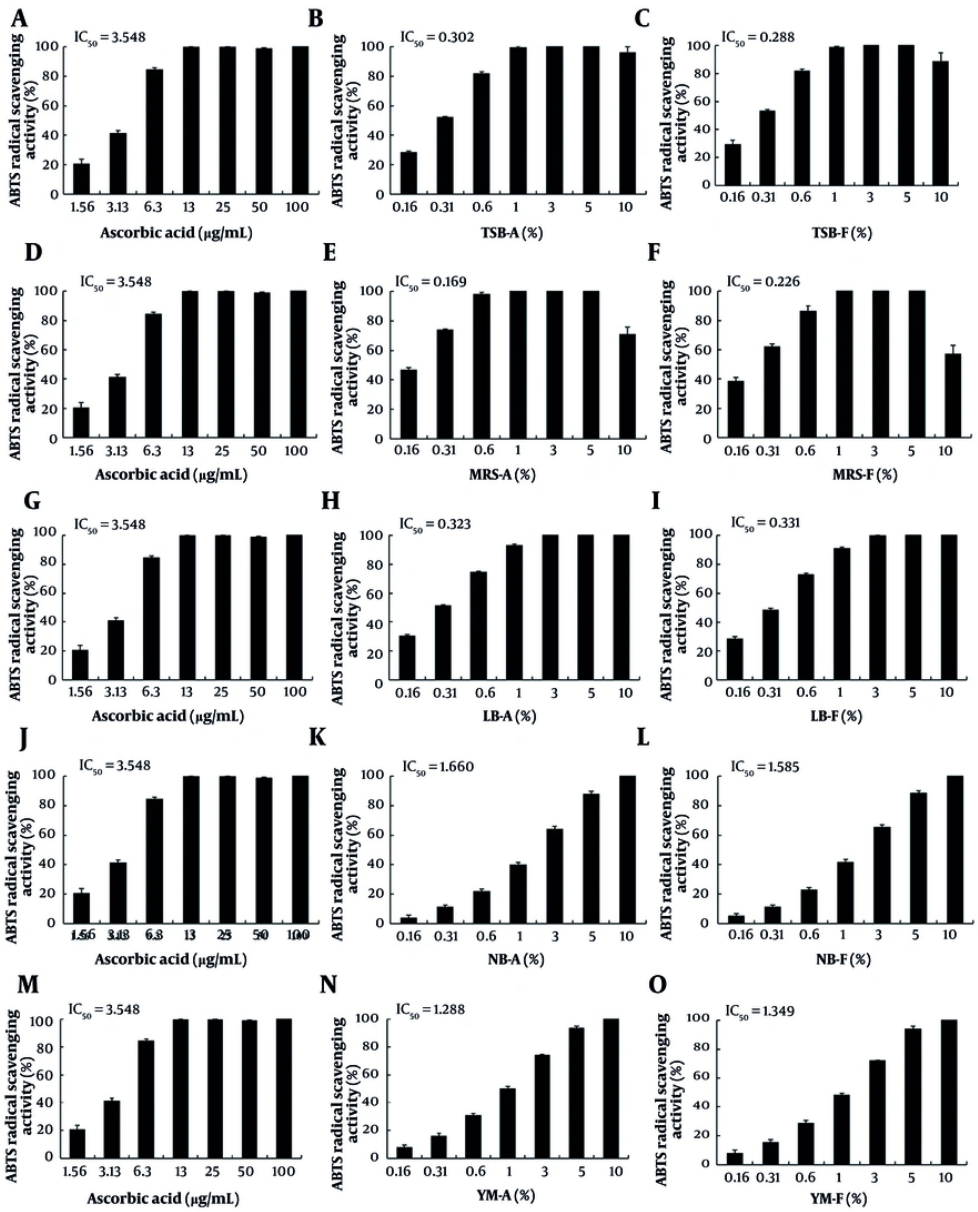

Figure 1. AABTS$^+$ radical scavenging activities of culture media and control antioxidant; ABTS$^+$ radical scavenging activities of ascorbic acid standards and five commonly used microbial culture media prepared by autoclave sterilization (A) or 0.22 μm membrane filtration (F). Ascorbic acid standards are shown in panels (A, D, G, J). Culture media include TSB (B, C), MRS broth (E, F), LB broth (H, I), NB (K, L), and YM broth (M, N, O). Samples were tested at increasing concentrations (%, v/v), and ABTS$^+$ radical scavenging activity was measured at 734 nm after 15 min of incubation in the dark. The half-maximal inhibitory concentration (IC_{50}, % v/v) is indicated in each panel. Data represent mean ± SD (n = 3).

complicating inter-study comparisons. From a methodological standpoint, membrane filtration is preferable when minimizing non-biological antioxidant interference is essential, whereas autoclaving may unintentionally amplify baseline scavenging through heat-induced chemical transformations. Overall, these findings underscore that baseline media redox behavior represents a critical experimental variable rather than a

negligible background effect. Rigorous media-only controls, consistent sterilization procedures, and cautious data interpretation are indispensable for ensuring scientific validity and reproducibility in antioxidant-related microbiological research.

5. Conclusions

This study revealed that commonly used microbial culture media exhibit measurable $ABTS^{+}$ radical scavenging activity even without microbial inoculation. Media enriched with yeast extract and carbohydrates, particularly MRS, showed significantly higher intrinsic antioxidant activity than protein-dominant formulations such as LB and NB. Autoclaving further increased scavenging activity in carbohydrate-rich media due to heat-induced Maillard reactions, emphasizing that sterilization is not chemically neutral. These media-derived effects can lead to overestimation of microbial antioxidant potential if not properly corrected with identically sterilized blank controls. Therefore, careful media selection, consistent sterilization methods, and rigorous baseline correction are essential for accurate and reproducible antioxidant evaluations in microbiological studies.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

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